Towards better New Zealand adapted industrial hemp (*Cannabis sativa* L.) cultivars: A quantitative genetic analysis of key traits and evaluation of genetic diversity.

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ABSTRACT

Hemp (*Cannabis sativa* L.) is emerging as a promising commercial crop in New Zealand. Breeding programmes developing material specifically adapted to the country's target population of environments are at their early stages of establishment. With 20 approved hemp cultivars, 15 of which are imported, it is vital to prioritize research and development focused on the genetic improvement of hemp to generate cultivars that are well adapted to New Zealand's crop production environments, offering both economic and agronomic benefits. The primary aim of this thesis is to lay the foundation for future hemp breeding in New Zealand by focusing on three critical initial steps: (1) conducting multi-site trials to evaluate the performance of commercial hemp cultivars based on key traits and characterize the effects of Genotype × Environment interaction (G × E interaction), (2) estimating quantitative genetic parameters and predicting genetic gain for key traits to identify breeding strategies that would improve the efficiency of maximizing genetic gain, and (3) assessing genetic diversity using molecular markers and characterizing available genetic resources. These steps are crucial for developing new cultivars that are both climate-resilient and beneficial to New Zealand's hemp industry.

Multi-site trials were conducted for two years over the 2019/2020 and 2020/2021 growing seasons using six hemp cultivars approved to be grown in New Zealand: CFX-2, CRS-1, Ferimon 12, Katani, Futura 75, and Finola. The 2019/2020 trials were conducted in Palmerston North and Wairarapa, while the 2020/2021 trials were conducted at two nearby sites in Palmerston North with different soil characteristics. Across both multi-site trials, biomass yields extend over a broad range from 1.43 to 28.41 t/ha, and seed yields ranged between 0.018 and 3.78 t/ha. The study's findings on G × E interactions showed a complex scenario that mostly differed from prior reported research. Although significant ($P < 0.05$) genotypic variation was observed in most traits, only a few, specifically stand establishment in the 2019/2020 season, along with plant height and stem diameter in the 2020/2021 growing season and biomass yield across both years, showed significant ($P < 0.05$) G × E interactions, suggesting consistent performance across sites. The traits plant height, stem diameter, biomass yield, seed yield, and thousand seed weight have all revealed high cultivar mean broad sense heritability, indicating potential exploitable underlaying genetic variation that could be used to generate breeding pools for cultivar development.

A quantitative genetic analysis was conducted using 50 half-sib families (HS families) focused on six key traits: stem diameter, plant height, number of internodes, seed yield, thousand seed weight, and biomass yield to investigate the magnitude of additive genetic variation within a breeding population. The study also aimed to predict the rate of genetic gain for these traits. Significant ($P < 0.05$) additive genetic variation was observed among the 50 HS families for traits: seed yield, biomass yield, stem diameter, plant height, and the number of internodes, highlighting each trait's additive genetic variation available for breeding. Deterministic simulation of the breeding strategies, among HS family selection (AF-HS) and among- and within-family selection (AWF-HS), was conducted to predict genetic gain for these traits. AWF-HS yielded the highest genetic gains compared to AF-HS. The study estimated moderate to strong positive genetic correlations among all traits, generally higher than the phenotypic estimates. The genetic correlation coefficients suggest that genotypes with higher seed and biomass yields can be developed. There was significant ($P < 0.05$) differences between some HS families and the two commercial check cultivars, Fasamo and Férimon 12. HS families outperforming these commercial checks were identified.

A laboratory experiment examined the genetic diversity and the degree of variability within and among six New Zealand-approved hemp cultivars using seven previously developed microsatellite (SSR) markers. STRUCTURE analysis identified two distinct genetic clusters that align with the plant's reproductive biology (monoecious and dioecious), that further subdivided based on end-use (fibre, seed, fibre and seed, and CBD cultivars). The genetic diversity metrics revealed moderate genetic diversity among the six hemp cultivars. The average observed heterozygosity (H_o) and the expected heterozygosity (H_e) were 0.44 and 0.50, respectively. The Wright's fixation index (F_{IS}) varied from 0.26 (Futura 75) to -0.02 (Finola). The Analysis of Molecular Variance (AMOVA) revealed that a large portion of the total genetic diversity was found within individual cultivars. While only 11% of the molecular variation was attributed to differences among the cultivars, 19% was attributed to variations among individuals within each cultivar. Notably, the genetic variation within individual plants (69%) exceeded the variation observed both among individuals and among different cultivars. Additionally, considerable molecular variation was observed between male and female individuals of dioecious hemp cultivars. The genetic diversity metrics of female and male

groups indicated that female individuals possess greater variation than their male counterparts.

Collectively, results from this Ph.D. study lay the groundwork for future hemp breeding research and development in New Zealand. They provide an initial resource, offering insights into genetic diversity, genotypic variation, and the impact of G × E interactions on key traits evaluated among six introduced offshore cultivars. Furthermore, the estimates of quantitative genetic parameters add value to the existing knowledge base, enhancing the development of hemp cultivars suited to New Zealand crop production environments.

Keywords: *Cannabis sativa* L., industrial hemp, Genotype × Environment interactions, half-sib families, additive genetic variance, genetic gain, genetic diversity, SSR, microsatellites.

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AF-HS	Among family HS family selection
AFLP	Amplified Fragment Length Polymorphisms
AMOVA	Analysis of Molecular Variance
AOAC	The Association of Official Agricultural Chemists
AWF-HS	Among- and within-family selection
BLUE	Best linear unbiased estimates
BLUP	Best Linear Unbiased Predictors
BM	Biomass yield
CBD	Cannabidiol
DNA	Deoxyribonucleic Acid
FC	Field capacity
F_{IS} and F_{ST}	Wright's fixation indices (F-stats)
GE interaction	Genotype-by-environment interaction / Genotype × Environment interaction
H_e	Expected heterozygosity
H_o	Observed heterozygosity
HS families	Half-sib families
IR site	Irrigated site
ISTA	International Seed Testing Association
K	Cluster values
MoH	Ministry of Health
MPI	Ministry for Primary Industries
N_A	Number of alleles
NZ	New Zealand
NZIHA	New Zealand Industrial Hemp Association
OIL	Seed oil content
PCA	Principal Component Analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase chain reaction

PCRU	Pasture and Crop Research Unit
PGU	Plant Growth Unit
PH /HT	Plant height
PRO	Seed protein content
r_A	genetic correlation coefficient
RAPD	Random Amplified Polymorphic DNA
RC	Repeated check
REML	Residual Maximum Likelihood
RF site	Rainfed site
r_p	Pearson correlation coefficient
SD	Stem diameter
Se. EST	Seedling establishment
SNP	Single-Nucleotide Polymorphisms
SSR	Simple sequence repeats (Microsatellite markers)
St. EST	Stand establishment
SY	Seed yield
THC	Δ^9-tetrahydrocannabinol
TSW	Thousand seed weight
WF-HS	Within family selection
ΔG	Genetic gain
$\%\Delta G_s$	Predicted percentage genetic gain relative to the industry standard (s)
$\%\Delta G_{\bar{x}}$	Predicted percentage genetic gain relative to the family population mean ($\bar{x}$)
$\%\Delta G_c$	Predicted genetic gain per cycle
h_b^2	Broad sense heritability
h_n^2	Narrow sense heritability
σ_A^2	Additive genetic variance
σ_g^2	Genotypic variance
σ_{gl}^2	Cultivar × site (G × E) interaction variance

CHAPTER 1

General introduction

Industrial hemp (*Cannabis sativa* L.) is one of the world's most ancient crops (Smith-Heisters, 2008), and is conventionally cultivated for its fibre and seeds (Morin-Crini et al., 2018). Industrial hemp (throughout the remainder of this thesis, the term "hemp" will be used synonymously with "industrial hemp") is an annual crop. It is a member of the *Cannabaceae* family (Welling et al., 2016). Hemp is one of the earliest domesticated crops (Schultes et al., 1974) and has multiple uses that can contribute to both bio-safe agriculture and economy (Docimo et al., 2014; Schluttenhofer & Yuan, 2017; Tang et al., 2017). Hemp fibre has numerous potential innovative applications, and hemp's use in paper production can be dated back to 2000 years ago (Fleming & Clarke, 1998). Hemp fibre can be utilized across multiple industries, including in the bio-building[1] sector, food packaging sector, composite materials production, and sustainable bio-energy production (Ali et al., 2021; Baldini et al., 2020; Dupeyre & Vignon, 1998; Parvez et al., 2021). Since ancient times, hemp seeds have been used in food and traditional medicine (Montero et al., 2023) and as a feed source for birds and fish (Deferne & Pate, 1996). Recent scientific evidence on hemp seed protein and oil characterization has emphasized their great commercial potential in food, animal feed, and cosmetic applications (Bakowska-Barczak et al., 2022).

Botanically, the genus *Cannabis* is comprised of multiple variants. Even though taxonomists disagree on the species classification of these variants, plant breeders typically adopt a biochemical method to classify these variants (West, 1998). *Cannabis* produces a unique class of molecular compounds collectively known as cannabinoids (Pertwee, 2008). Among the 460 known chemicals produced by *Cannabis,* more than 60 are categorized as cannabinoids (Amar, 2006; Moeller et al., 2017), of which two are predominant: Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (Lash, 2002). THC is the psychoactive constituent of *Cannabis,* and CBD is the anti-psychoactive ingredient (Young, 2005). Accordingly, *Cannabis* can be broadly and practically divided into two main categories: the first is marijuana (drug-type), which is primarily bred for high THC

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[1] A building conceived and realized with technologies, materials and performances, that are not harmful to man and the environment over the time (Huovila, 2005). The materials used are bio-based, means that the materials are derived or made from renewable sources (Jones & Brischke, 2017).

and low CBD content; the second, known as "industrial hemp," is characterized by high CBD and low THC levels (West, 1998). Marijuana is cultivated for drug use (Smith-Heisters, 2008), and its intoxicating properties have made it primarily used for "recreational" smoking (Schluttenhofer & Yuan, 2017). Marijuana is one of the oldest[2] psychotropic drugs and has been cultivated for its therapeutic uses since 2737 BC (Li, 1974). Fundamentally, all *Cannabis* strains contain the narcotic compound THC. Varieties categorized as drug-type (marijuana) contain THC concentrations ranging from 3% to 40% (Merfield, 1999). On the other hand, hemp varieties typically contain THC concentrations of less than 0.35%, though they can sometimes range up to 0.5%. (MoH[3], 2020). The upper limit of THC content permitted in hemp differs from country to country, with some countries, such as Switzerland, permitting levels as high as 1% (Sarmento et al., 2015).

For the past 80 years, hemp cultivation has been limited in most Western countries due to a preference for alternative feedstock sources, high labour costs, and legal prohibitions on hemp production imposed by various narcotics drug acts (Salentijn et al., 2015; Vera & Hanks, 2004). However, the hemp industry continued in Eastern Europe, the former Soviet Union and China (de Meijer, 1995). Initiated about two decades ago, the lifting of hemp prohibition has enabled its cultivation in approximately 50 countries today for both commercial and research purposes (Bakowska-Barczak et al., 2022; Schluttenhofer & Yuan, 2017). The major hemp-producing countries are Canada, China, Chile, France and North Korea, whereas the oldest and the most well-established producers are China and Canada. In the European Union, France takes the lead as the largest producer, making up over 60% of the European Union's total production, with Germany contributing 17% and the Netherlands 5%. (European Commission, 2022; New Frontier Data, 2019).

According to Food and Agriculture Organization[4] of the United Nations (2023) data, 11,422 hectares (ha) were harvested for hemp seed, producing 5,566.02 tonnes (t). A

[2] Archaeological evidence and historical records from China indicate that hemp has been cultivated and used in Eastern Asia for roughly 5,000 to 6,000 years (Merlin, 2003).

[3] Ministry of Health

[4] FAO data is not comprehensive and lacks information from key hemp-growing countries such as the USA., Canada, Australia, and some African nations. Some FAO figures are based on estimates (Textile Exchange, 2023).

report by Textile Exchange (2023), which used data from the Food and Agriculture Organization (FAO), US Department of Agriculture (USDA) and the Turkish Statistical Institute (TURKSTAT), states that 79,346 ha were harvested for hemp fibre in 2021, with a total production of 302,451 t. In 2022, the worldwide market for hemp was valued at approximately USD 4.74 billion. Between 2023 and 2030, the market is expected to expand at a 17.1% compound annual growth rate. This growth is primarily attributed to increasing global demand for carbon-based and eco-friendly products as well as the rising demand from industries such as food and beverage, personal care, and animal care across the world (Burton et al., 2022; Grand View Research, 2022; Schluttenhofer & Yuan, 2017). Although the COVID-19 pandemic has constrained the hemp global market's expansion, the pace of growth is anticipated to grow at a faster rate after the COVID-19 pandemic (Grand View Research, 2022; Yano & Fu, 2023).

Although hemp was prohibited in New Zealand for approximately eight decades, recent legislative amendments now allow New Zealanders to produce, trade-in and consume hemp seed food products (as from 12 November 2018) (MoH, 2020). The table presented in Appendix 1 shows the history of hemp in New Zealand from 1883 to 2021. According to Ministry of Health regulations, possessing hemp products[5] without a licence in New Zealand is legal. This includes all products made from the stem (excluding leaves), balms[6] and foods made from the seed (MoH, 2020; R. Barge, personal communication, November 6, 2019). The whole seed is classified as a class C drug, under the Misuse of Drugs Amendment Act 2006 and needs a licence to grow, trade, or process hemp as an agricultural crop. To possess hemp, an individual, corporate body, or partnership needs to be licensed under the Misuse of Drugs (Industrial Hemp) Regulations 2006 (MoH, 2020). The Ministry of Health issues two types of licenses: a general license and a research and breeding license. A general license entitles the holder to undertake procurement within New Zealand, cultivation, supply within New Zealand, and processing of hemp into specified hemp products (MoH, 2020). A research and breeding licence authorizes the licence holder to undertake cultivation and breeding for research purposes only and allows research into the suitability of cultivars (MoH, 2020). The hemp regulations in New Zealand set the allowable limits of THC (given as a % of the dry weight of the plant)

[5] A product of a kind that is derived, in whole or in part, from industrial hemp and a hemp seed food product (MoH, 2020).

[6] A fragrant cream or liquid used to heal or soothe the skin.

at generally below 0.35% and not more than 0.50%. The Food (Safety) Regulations 2002 authorized the production and sale of hemp seed oil for human consumption starting in 2002. In December 2018, hemp seed foods were legalized for human consumption; prior to that, they were only permitted for animal consumption (MoH, 2020).

In 2022, the New Zealand Ministry of Health issued 111 licenses for hemp cultivation, and 278 ha of hemp was grown in 2022 (R. Barge, personal communication, August 29, 2023). Since New Zealand's Hemp Regulations were amended in late 2018, the country's hemp industry has rapidly expanded, despite initial research starting in 2001 (Marsh, 2020; Moore et al., 2021). In a brief period, a substantial industry focused on hemp seeds and oil has emerged, together with growing consumer awareness of hemp's health advantages in food and other products. The industry presents a significant opportunity for New Zealand, as indicated by rising demand and a projected market value of 16.82 billion (NZD 27.10 billion) by 2030 (Grand View Research, 2023). The growing hemp industry in New Zealand has received significant attention from the agricultural and industrial community for its potential to offer a wide range of products. A few studies or reports have investigated the industry's potential for new markets, farmer profitability, socio-economic benefits, and positive environmental impact. These reports consistently stress the importance of developing local cultivars through research and development, aiming to identify hemp cultivars that maximize yield and value (Marsh, 2020; McIntosh et al., 1998; McPartland et al., 2004; Moore et al., 2021; Venture Taranaki, 2022). This is because, among the 20 approved hemp cultivars, 15 are introduced cultivars. Meeting the demands for introduced cultivars better adapted to changing conditions can be challenging due to factors such as variations in climate, soil composition, biotic and abiotic stresses, or other environmental variables that influence the adaptation of the plant species. Developing cultivars with reduced risk of total crop failure is crucial considering these challenges. Therefore, the emerging industry in New Zealand requires in-depth studies focused on developing locally adapted cultivars, as no comprehensive studies have been carried out to date. Even though there are a few breeding programmes conducted to produce New Zealand specific cultivars such as Aotearoa 1, A1Monopurp, A1MACMONO, A1 COMSEED and A1 HCFX to increase productivity (McIntosh, 2021), there remains a need to develop hemp cultivars that are well adapted to New Zealand's cropping systems and environments, offering both economic and agronomic benefits. In this context, to address the necessity for strategic advancements in New

Zealand's hemp breeding efforts, and to establish a foundation for subsequent research and development, this research has formulated the following research questions and objectives.

1.1 Research Questions

The study is focused on the following questions:

1. What is the yield potential of the selected cultivars in different environments in the North Island and will there be a change in their relative performance across the environments?
2. What is the genetic diversity among half-sib families (HS families) representing a breeding pool, and can we predict genetic gain for key traits?
3. How is genetic variation partitioned within and among hemp cultivars – specifically, how different are the levels of genetic variation among cultivars, and how is this useful for future hemp breeding?

1.2 Objectives

This study aims to:

1. compare the expression of key traits, including yield components (biomass and seed), seed quality, and other agronomic traits, within a set of hemp cultivars from Canada, Finland, and France,
2. investigate how genotype and environment affect hemp seed and biomass production by characterising the type and magnitude of Genotype × Environment interaction (G × E interaction) estimated from two-year, multisite hemp trials in the North Island of New Zealand,
3. investigate the magnitude of additive genetic variation within a breeding population for key traits based on half-sib families (HS families) and predict the rate of genetic gain by applying the estimated quantitative genetic parameters in the breeder's equation,
4. characterise the genetic diversity and the degree of variability within and among the set of six commercial hemp cultivars using microsatellite markers.

The overall aim is to generate new information on genotypic variation, genetic diversity, and the impact of G × E interaction on key traits to provide a basis for future hemp breeding research and development programmes in New Zealand. This will enhance the development of hemp cultivars suited to New Zealand environments.

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CHAPTER 2

Literature review

2.1 The hemp plant

Hemp is an annual plant, one of the first crops cultivated as a fibre crop (Salentijn et al., 2015; Shiels et al., 2022; van den Oever et al., 2023). Hemp is naturally dioecious, implying that a plant will have either male or female flowers (Beleggia et al., 2023; Small, 2015) (Figure 2.1 A). Male plants are tall, slender and not as vigorous as females. Male plants senesce after pollen shedding, and female plants continue to grow and develop seeds (Fike, 2016). Monoecious hemp cultivars with both male and female flowers on the same plant have been developed through breeding and selection (Hall et al., 2012). These cultivars are predominantly female and often still produce males and occasional hermaphrodites. (Small & Marcus, 2002). Sex determination of *Cannabis* is very much dependent on environmental and edaphic factors (Amaducci et al., 2008b; Chabbert et al., 2013; Hall et al., 2012; Visković et al., 2023).

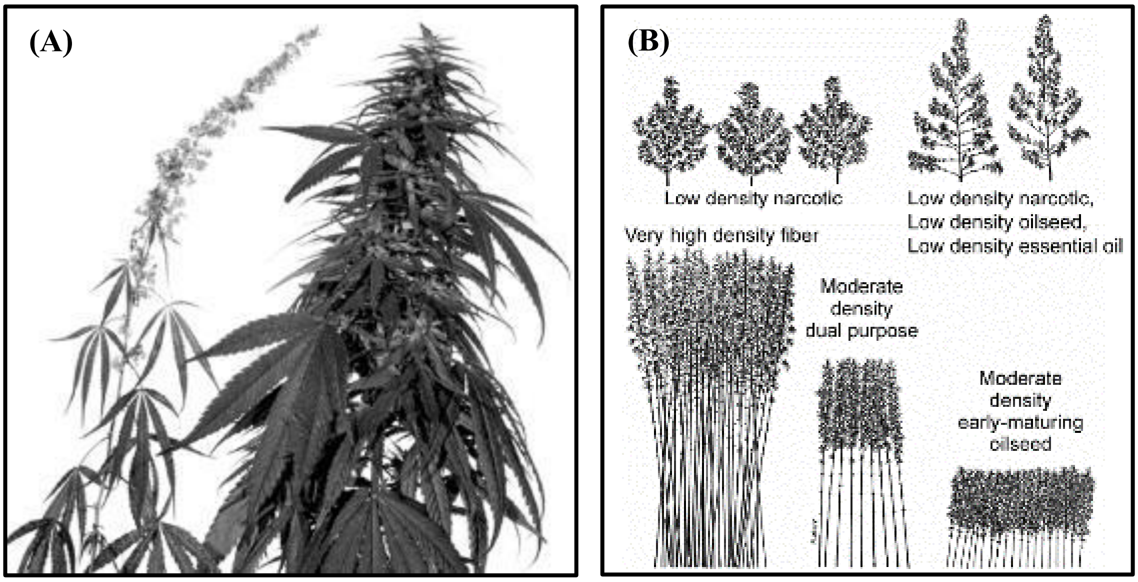

Figure 2.1. Staminate ("male") and pistillate ("female") Cannabis (A) (Left and Right). Typical plant structure of *Cannabis* grown for narcotics, oil seed and fibre in different densities (B). Adapted from Small & Marcus (2002).

In general, the main stem of the *Cannabis* plant (fibre types) grows for 2 to 4 m in height and the diameter of the stem is between 2.5 and 5 centimetres. The stem branches and their characteristics vary depending on the cultivar and growing conditions (Chabbert et al., 2013) (Figure 2.1 B). According to Small & Cronquist (1976) and Small & Marcus

(2002), hemp plants typically exhibit a more robust and taller growth pattern, primarily cultivated for their fiber, while marijuana plants often display a bushier appearance. These distinguishable features can be altered by different agronomic practices depending on the purpose of end-use. Branch number and stem diameter increase with low planting densities, where high seeding rates are likely to develop tall plants with long, erect and unbranched stems (Bócsa & Karus, 1998; Żuk-Gołaszewska & Gołaszewski, 2020). However, Small & Marcus (2002) also reported that hemp and marijuana can appear to be similar.

Hemp stalks have two main components: core fraction, a hollow woody inner core surrounded by the vascular cambium and bark fraction (Figure 2.2); primary outer bast fibres in the phloem tissues consisting of phloem, cortex and epidermal tissue (Ehrensing, 1998; Kaur & Kander, 2023; Rivas-Aybar et al., 2023). The core fraction of the hemp stalk (woody core fibres) is called as shives or hurds (Stevulova et al., 2013). The bast fibres of hemp are categorized as primary bast fibres (long fibres), secondary bast fibres (short fibres) and core fibres (Hoffmann, 1961; Sankari, 2000). The primary bast fibres (coarse fibres) extent nearly the length of the stalk, and the secondary bast fibres (fine fibres) tend to adhere to a woody core (Ehrensing, 1998; Horne, 2020; Zhao et al., 2022). The bast fibres are 3–55 mm long, and the secondary bast fibres are about 2 mm long (Catling & Grayson, 1982; Horne, 2020; Kundu, 1942). The core fraction (hurds or shives) contains much shorter fibres that are about 0.5–0.6 mm long (Bosia, 1976; Sáez-Pérez et al., 2020). The proportion of bark and bast fibre content in hemp stems depend on the genotype, plant height, plant density and developmental stage, and they vary from 14 to 18 % and 17 % to 26 %, respectively (Cromack, 1998; Hoeppner & Menge-Hartmann, 1994; Sankari, 2000).

Figure 2.2. A hemp stem showing the bast fibres, which grow under the “bark” layer. Photo in the public domain.

The root system of the hemp plant comprises a tap root and secondary roots (Chabbert et al., 2013; Clarke & Watson, 2007; Rheay et al., 2021; Żuk-Gołaszewska & Gołaszewski, 2020). The biomass of the hemp root system is relatively low compared to the biomass above the ground. The density of the root system varies with the quality of soil, cultivation practices and sexual phenotype of the plant. Since female plants have a longer vegetative stage than male plants, female plants have a well-developed root system compared to male plants (Chabbert et al., 2013).

After seed germination, a pair of oppositely directed true leaves arise 10 cm or less above the cotyledons. Seven to ten folioles (leaflets) form a leaf of hemp plant (Chabbert et al., 2013). Folioles in a leaf are lanceolate in shape, unequal in size and always odd in number (Clarke, 1999). An opposite leaf pattern can be observed until the plants flower, and the arrangement changes to an alternative pattern at the start of the flowering period (Monthony et al., 2021; Schaffner, 1926).

In dioecious hemp plants, female flowers are more densely clustered at the apex of the stem, with many small leaves surrounding the flowers and these flowers are borne in racemes (Hall et al., 2012; Nelson, 1999) (Figure 2.3 A). However, male flowers are not tightly fixed together with the rachis (not as dense as female flowers) with very few leaves (Hazekamp, 2008; Rana & Choudhary, 2010). (Figure 2.3 B). The female flowers are formed at the apical part of the stem, while male flowers are arranged at the base of the flower head, emerging towards the middle of the stem (Chabbert et al., 2013). In monoecious strains, the plant has both male and female flowers, with male flowers appearing towards the middle of the stem and female inflorescence mainly in the apical part (Bócsa & Karus, 1998; Strzelczyk et al., 2022) (Figure 2.3C).

The sepals are usually green-yellow in male flowers and have five tightly fused stamens (Spitzer-Rimon et al., 2019). The female flowers consist of two pistils emerging from the calyx, two hair like stigmas protruding from each bract, and a single seedpod (Leme et al., 2020; Schilling et al., 2020). Pistils are 3-8 mm in length. Stigmas start out white but progressively darken to amber and brown as the plant matures (Chabbert et al., 2013; McGue et al., 2021). The bracts encapsulate seed pods. Though often confused with the bracts, the calyx is a translucent layer at the flower base and is not visible to the naked eye. The bracts contain a high density of trichomes: glands that secrete the translucent crystal resin called cannabinoids and terpenes (Chabbert et al., 2013; Schilling et al., 2020). These trichomes are epidermal protuberances and can be seen emerging from the surface of the leaves, bracts and stems (Aizpurua-Olaizola et al., 2016; Chabbert et al., 2013; Schilling et al., 2020). The trichomes in hemp plants can secrete secondary metabolites as a defence mechanism. Hemp has various types of trichomes, which can be categorised into two groups, i.e., glandular and non-glandular. The density of trichomes in leaves is low compared to the bracts (Aizpurua-Olaizola et al., 2016; Chabbert et al., 2013).

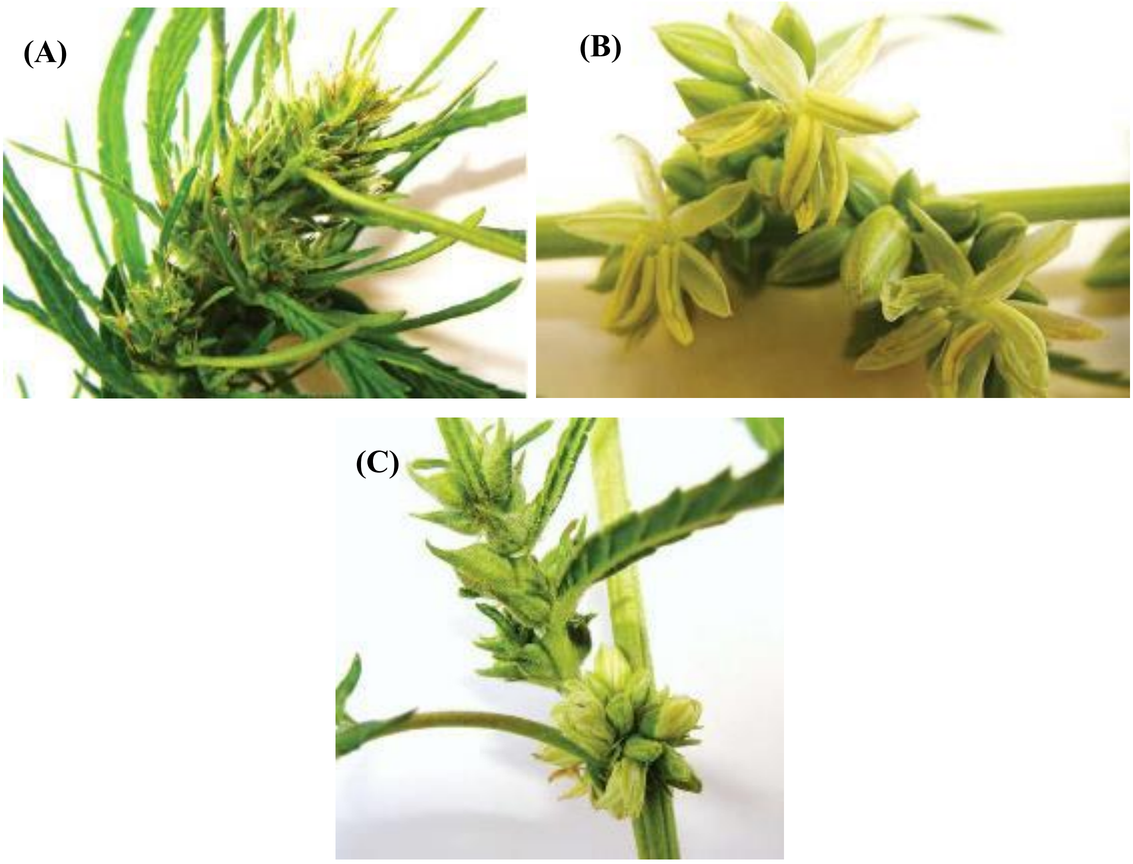

Figure 2.3. Female inflorescence of industrial hemp (A). Male flower bud cluster showing open flowers (B). Monoecious hemp panicle with male flower 'whorl' below female flowers (C). Adapted from Hall et al. (2012).

The hemp seed is an achene[7] in a botanical point of view (Small & Marcus, 2002). The ovule develops into a compact fruit known as an indehiscent achene, with the seed (embryo) occupying its entire volume (Chabbert et al., 2013). A leathery husk containing

[7] A small hard indehiscent fruit formed from one carpel (Evans, 2009).

its testa[8] and pericarp[9] envelops the fruit (Chabbert et al., 2013; Schultz et al., 2020). Seeds are ovoid, and the surface of the seed is greenish-grey mottled in appearance (Nelson, 1999). The seed size may vary depending on the variety and is 3-5 mm long (Dunford, 2020). The seed has two cotyledons rich in oil and protein (Chabbert et al., 2013; Dunford, 2020; Small, 2015).

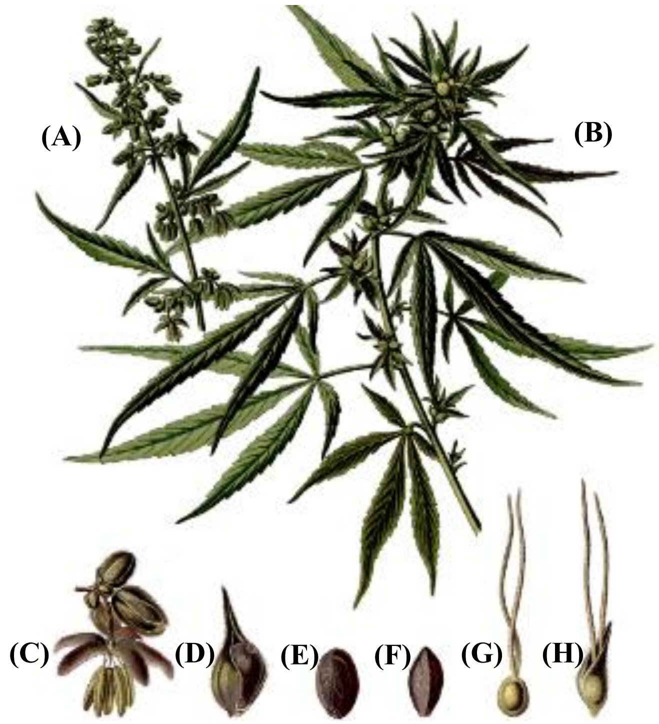

Figure 2.4. Painting of *Cannabis sativa*. Flowering male branch (A). Fruiting female branch (B). Cluster of male flowers (C). Fruit (achene) surrounded by perigonal bract (D). View of the wide (flat) side of an achene (E). View of the narrow side of achene (F). Pistil, showing ovary and two stigmatic branches (G). Pistil surrounded by a young perigonal bract (H). Adapted from Small (2015).

There are several systems to determine the growth stage of the hemp plant. According to the system of Mediavilla et al. (1998), the growth stages of *Cannabis sativa* L. are determined as (1) germination and emergence, (2) vegetative stages, (3) flowering and seed formation and (4) senescence.

[8] The outer, usually hard, integument or coat of a seed.

[9] The pericarp may be dry or fleshy and is composed of three layers not easily distinguishable in dry fruits, but if the pericarp is thick and fleshy these layers may be easily differentiated into epicarp (outermost layer), mesocarp (middle layer), and endocarp (inner layer surrounding the seeds) (Yahia & Carrillo-López, 2018).

Hemp can be grown for fibre, seeds, cannabinol (CBD) or as a dual purpose and is adaptable to various geographic zones and climates (Salentijn et al., 2015). Selective breeding has produced hemp varieties for specific end-use, including fibre, seed, medicinal, and seed and fibre (dual-purpose) (Rupasinghe et al., 2020; van Bakel et al., 2011).

Some of these cultivars can be adapted to different end-uses based on their agronomic performance because it is a well-known fact that in breeding for fibre, food or medicinal uses, the selection is applied for several characters simultaneously and not just to one since multiple characteristics contribute the economic value.

2.2 Hemp's use for industrial purposes

2.2.1 Hemp fibre

Hemp is the oldest example of industrial applications (Crini et al., 2020a; Zatta et al., 2012). Hemp was traditionally a fibre crop grown for its long and strong fibre (bast fibres), which was often used for clothing and paper making (Crini et al., 2020a; Rehman et al., 2021; Stevulova et al., 2014). Hemp paper-making dates back over 2,000 years (Fleming & Clarke, 1998). With the arrival of economically feasible base materials such as cotton and synthetic fibres, higher labour costs as well as the prohibition of *Cannabis* due to narcotic issues, the commercial importance of hemp dropped significantly (Chandra et al., 2017; Crini et al., 2020b; Milanovic et al., 2012; Zatta et al., 2012). However, hemp's potential has revived interest and facilitated a remarkable comeback.

Beyond the potential of hemp fibre to replace other plant fibres and wool, it can compete with glass fibre and steel (Colomer-Romero et al., 2020; Small & Marcus, 2002).

Hemp fibre has numerous innovative applications. It is used in:

1. production of high-quality special paper (Ahmed et al., 2022; Brunet & Lalanne, 2000).
2. production of textiles (Dhondt & Muthu, 2021; Rehman et al., 2021) and geotextiles or agricultural textiles: ground covers designed to reduce weeds and biodegradable matting designed to prevent soil erosion (Hasan, 2020; Small & Marcus, 2002).
3. production of thermal insulation products, thermoplastics (Small & Marcus, 2002) and car interior panels (Holbery & Houston, 2006; Shaker et al., 2020).

4. production of composite materials, including plastic components for automobiles (Dupeyre & Vignon, 1998).
5. The food packaging sector to strengthen expanded starch foams (Ali et al., 2021; Bénézet et al., 2012).
6. the bio-building construction sector: hemp hurds alone (Baldini et al., 2020; Jarabo et al., 2012; Li et al., 2006; Elfordy et al., 2008) or hurds together with bast fibres (de Bruijn et al., 2009) chemically combined with binding agents are used to form light panels, high-quality plaster, stone-like material and hemp concrete (Small & Marcus, 2002). Hemp concrete ("hempcrete") is made of a mixture of lime and hemp hurds (Elfordy et al., 2008).
7. sustainable bioenergy production (Finnan & Styles, 2013; Parvez et al., 2021; Rehman et al., 2013) including use of hemp for the production of ethanol (Prade et al., 2011; Sipos et al., 2010; Viswanathan et al., 2021), biogas (Kreuger et al., 2011; Michal et al., 2023) and biomass for combustion (Aluru et al., 2013; Frankowski & Sieracka, 2021; Prade et al., 2011; Rice, 2008).

Increasing the extent of annual plants as a fibre source is becoming more relevant as deforestation (use of trees) causes forests to be lost (Nabuurs et al., 2007; Parvez et al., 2021). Large-scale production, low production and processing prices, quality of fibre for diverse end-uses and appropriate technology for processing raw material will increase the potential of hemp fibre to compete with world-leading fibre sources (Blandinières & Amaducci, 2022a; Small & Marcus, 2002). However, except for the quality of the hemp fibre, other criteria mentioned above need to be adequately met for hemp in New Zealand.

2.2.2 Hemp seeds

Hemp seeds are an excellent source of high-quality proteins and oils (Baldino et al., 2022; Park et al., 2012). Rodriguez-Leyva & Pierce (2010) and Xu et al. (2022) reported that hemp seed contains more digestible protein (88 – 91%) than soybean (71%). Hemp has the ability to produce abundant seed yields with high protein concentrations, making it a valuable source of protein without gluten and legume allergens (House et al., 2010). Hemp seed contains all the essential amino acids required by the human body (Callaway, 2004; Deferne & Pate, 1996; Galasso et al., 2016; Russo & Reggiani, 2015) and provides a balanced amino acid profile (Xu et al., 2022). The major seed proteins of hemp seed are albumin, a globular protein and edestin, a legumin, and both are rich in essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Callaway, 2004; Potin & Saurel, 2020). The albumin fraction is about 25 % of the hemp seed storage proteins (Wang & Xiong, 2019). Edestin accounts for 60 – 80 % of the total protein content (Odani & Odani, 1998; Tang et al., 2006). Hemp proteins also possess valuable techno-functional properties such as gelling, emulsifying, and foaming properties, which can be effectively utilized in the food industry and for creating innovative plant-based food products (Potin & Saurel, 2020).

Table 1.1. Typical nutritional content (%) of hemp seeds[a].

	Whole seeds	Seed meal
Oil (%)	35.5	11.1
Protein	24.8	33.5
Carbohydrates	27.6	42.6
Moisture	6.5	5.6
Ash	5.6	7.2
Energy (kJ/100 g)	2200	1700
Total dietary fibre (%)	27.6	42.6
Digestible fibre	5.4	16.4
Non-digestible fibre	22.2	26.2

[a] The nutritional values presented in Table 2.1 have been derived experimentally from the whole seed of the Finola hemp cultivar and its seed meal after cold (45°C) oil pressing (Callaway, 2004).

Hemp seeds contain about 30 – 35 % of oil with a valuable fatty acid composition (Bócsa et al., 2005; Calzolari et al., 2021). It contains two dietary essential fatty acids, linoleic acid and α-linolenic acid, in the ratio of 2.5-3:1, which has been claimed as ideal for human nutrition (Anwar et al., 2006; Galasso et al., 2016; Irakli et al., 2019). According to Erasmus (1993) and Devi & Khanam (2019), only hemp seeds contain these two fatty acids in such an ideal nutritious ratio, making it a valuable essential oil for promoting cell and body growth and strengthening the immune system. Present-day awareness about the nutritional and functional role of fats in human nourishment has made a great deal of interest in hemp seed oil as it has a good taste and offers various advantages over other vegetable oils (Galasso et al., 2016; Xu et al., 2021). The seeds also contain 20 – 30 % of carbohydrates, 10 – 15 % of insoluble fibre and various minerals vital for human nutrition (Matthäus et al., 2006; Strzelczyk et al., 2023). Table 2.1 shows the typical nutritional content of hemp seed. Even though the demand for hemp seed as human food has not yet in the world-wide mass-commercial markets, the nutritional quality of hemp seeds has long been recognized and regarded highly in Asia, India, Russia and Eastern Europe (Burton et al., 2022; Callaway, 2004).

Hemp seeds are known to be used in the following applications:

1. Hemp flour to produce bakery products (Korus et al., 2017; Lukin & Bitiutskikh, 2017)
2. Hemp powder to produce extruded products, like energy bars (Jozinović et al., 2017; Norajit et al., 2011)
3. Hemp protein powder to produce protein-rich beverages (Grace et al., 2014; Pihlanto et al., 2020; Plundrich et al., 2013)
4. Hemp protein to produce dairy replacements and infant formula such as hemp milk (Dabija et al., 2018; Yano & Fu, 2023). Hemp milk is produced by homogenizing ground whole seeds in water (1:5 w/v) and filtering the milk, generally containing 4% protein and 5% fat (Wang et al., 2018).
5. Hemp flour as an alternative to soy ingredients for preparing meat products (processed meat products) (Naumova et al., 2017; Zając et al., 2019)
6. Hemp seed oil can be used in the production of face creams, body lotions, soaps, hair conditioners and even in the production of perfumes (Baral et al., 2020; Bócsa et al., 2005).

2.2.3 Cannabinoids

Cannabinoids represent a unique class of terpenoid-phenolic compounds found in *Cannabis*, around 144 distinct cannabinoids identified to date, including Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) (Pertwee, 2014; Specchio et al., 2020; Yang et al., 2020). Although *Cannabis* plants exhibit similar taxonomic and morphological characteristics, they can be categorized into two groups, namely "industrial hemp" and "marijuana", based on their phytochemical profiles (Yang et al., 2020). Specifically, *Cannabis* plants with a total THC concentration of less than or equal to 0.35% on a dry weight basis are legally defined as "industrial hemp" in New Zealand (MoH, 2020). "Industrial hemp" typically contains non-psychoactive cannabinoids, including CBD and CBG, as their primary constituents.

CBD, one of the most extensively researched cannabinoids, is primarily used for pharmaceutical and medicinal applications due to its potent antioxidative and anti-inflammatory properties, which may offer neuroprotection in both acute and chronic neurodegenerative conditions (Hampson et al., 1998; Lastres-Becker et al., 2005). It has also been reported as a promising antiepileptic agent for treating intractable pediatric epilepsy (Devinsky et al., 2014). Furthermore, CBD products have been associated with numerous medical and therapeutic benefits, including the potential treatment of anxiety (Crippa et al., 2011; Shannon et al., 2019), insomnia (Tang et al., 2019), joint pain and inflammation (Dhondt et al., 2021), arthritis (Blake et al., 2006), depression (Oberbarnscheidt & Miller, 2020), muscle tension (Dhondt et al., 2021), migraines (Dhondt et al., 2021), chronic pain (Dhondt et al., 2021), nausea (Parker et al., 2011), and inflammatory pain (Wade et al., 2003). Notably, more controlled clinical trials are needed to validate the efficacy of CBD in these applications (Dhondt et al., 2021; Shannon et al., 2019).

In addition to CBD and THC, hemp also contains other cannabinoids such as CBG, a relatively unknown non-psychoactive phytocannabinoid with analgesic, anti-proliferative, and antibacterial properties (Moreno et al., 2020; Russo & Marcu, 2017), and cannabinol (CBN), a minor constituent in fresh plant material that results from the oxidation of THC (Izzo et al., 2009; Moreno et al., 2020). CBN is typically considered a chemical marker for poor or lengthy storage conditions (Hartsel et al., 2016; McPartland & Russo, 2001; Moreno et al., 2020). Other non-psychoactive cannabinoids reported in hemp include cannabichromene (CBC), cannabicyclol, cannabielsoin, cannabitriol, among others (Cerino et al., 2021; Hartsel et al., 2019).

2.3 Centre of origin and species designation

Despite Central Asia being proposed as the centre of origin of *Cannabis sativa* (Ansari et al., 2018; McPartland, 2018), some botanists considered Europe (Keppen, 1886; Thiébaut de Berneaud, 1835, as cited in Zhang et al., 2018) or a region spanning Asia and Europe (Herder, 1892; Vavilov, 1926) as the centre of origin. Abel (1980) has reported that *Cannabis* has been originated in Central Asia around 8000 BC, in the territory that now comprises Mongolia and Southern Siberia. Fike (2016) mentioned Eurasia as the centre of origin for *Cannabis* with an introduction of fibre lines from East to China and West to Europe.

A molecular analysis conducted by Zhang et al. (2018) has revealed that this species possibly originated from low-latitude areas of China. This finding shows a contradiction with the postulate of the Central Asia-origin of *Cannabis* but partly supports the speculation of Linnaeus (1737) (As cited in Zhang et al., 2018), who reported the native range of *Cannabis sativa* was India Orientali (the Indian subcontinent, southeastern Asia, and the Malay Archipelago), Japonia (Japan), and Malabaria (the region of Malabar, which is a part of the southwestern coast of India). Small (2015) added value to this by indicating that "the seeds of some wild-growing populations in India are remarkably small, unlike those collected from any other area of the old world, but whether this is indicative of an ancient wild form is unclear".

Conflicting taxonomic classification information on *Cannabis sativa* exists in scientific literature (Lightfoot et al., 2016; McPartland, 2018), and the ongoing debate about its potential subdivision into distinct subspecies continues. While hemp and marijuana are generally considered members of *Cannabis sativa* L., some researchers, such as Hillig (2005), have proposed that these two should be classified as distinct species (Fike, 2016). Carl Linnaeus' original classification (1753) recognized a single species as *Cannabis sativa* (as cited in Flores-Sanchez & Verpoorte, 2008), but Jean-Baptiste Lamarck (1785) proposed a new species called *Cannabis indica* Lam. due to morphological differences observed between European hemp and Indian strains (as cited in Compendium, 2020). However, J. Lindley rejected Lamarck's taxonomic approach in 1838, considering *Cannabis* a monospecific genus, following Linnaeus' classification (as cited in Pollio, 2016). Nonetheless, Schultes et al. (1974) advocated reinstating *Cannabis indica* as a separate species and introduced a new species, *Cannabis ruderalis*. The taxonomy of sativa, indica, and ruderalis as distinct species or subspecies within *Cannabis sativa* lacks consensus among researchers.

In 2018, McPartland proposed classifying the genetic variations within *Cannabis sativa* as subspecies, namely *Cannabis sativa* subsp. *sativa* and *Cannabis sativa* subsp. *indica*, by employing quantitative criteria based on key molecular DNA sequences (DNA barcode). This classification is further supported by Barcaccia et al. (2020) and Kovalchuck et al. (2020). As a result, *Cannabis* is currently acknowledged as a monospecific genus originating in Europe and Asia, respectively (Rull, 2022). Small (2015) pointed out that "no other species has generated so much misunderstanding, argument and contradictory literature". Genomic studies on *Cannabis* varieties of different origins propose that the concept of polytypic nature of this species cannot be excluded. However, arguments will be continued until the genetic background of these plants is completely figured out (Clarke & Merlin, 2016; Lynch et al., 2016; Vergara et al., 2016).

2.4 Agronomy of industrial hemp

2.4.1 Soil requirements and fertilization

A well-prepared seedbed with fine and homogeneous soil is important to achieve the expected plant density and for better crop establishment (Struik et al., 2000; Visković et al., 2023). Hemp grows best in soil rich in nutrients, capillary and aeration, with a pH between 6.0 and 7.5 and a good water-holding capacity (Adesina et al., 2020; Gusovius & Lühr, 2017). Since hemp is sensitive to waterlogging, it needs well-drained soil (Ostapczuk et al., 2021; van der Werf, 1991). Hemp grows well on sandy loam, followed by clay loam and is poorly adapted to heavy clay or sandy soils (Adesina et al., 2020; Li, 1982). Waterlogging in clay soils and poor water-holding capacity in sandy soils can severely affect seedling emergence (Adesina et al., 2020; Struik et al., 2000).

Nitrogen, potassium and phosphorous are the most vital nutrients for high hemp production (Tang et al., 2022; Young, 2005). In a study by Cockson et al. (2019), it was found that hemp experienced significant mineral deficiencies in nitrogen (N), potassium (K), boron (B), and copper (Cu). Among these, nitrogen is the most crucial mineral for hemp growth, development, and production, playing an essential role in the synthesis of secondary metabolites. (Deng et al., 2019; Landi et al., 2019). The nitrogen uptake is most intensive for the first six to eight weeks, while potassium and phosphorous are needed more during flowering and seed formation (McIntosh et al., 1998). Struik et al. (2000) found that in Northern Europe, there was a gradual increase in stem dry matter when nitrogen was applied within a range of 100 kg N ha^{-1} to 220 kg N ha^{-1}. However, in nitrogen-rich soils of Northern Italy, nitrogen showed limited effectiveness in the same study. Amaducci et al. (2002) observed that nitrogen fertilization enhanced stem production, while Prade et al. (2011) reported no significant response to applied nitrogen. In a separate study by Finnan & Burke (2013), it was found that yields increased with nitrogen addition, but the effect levelled off at 120 kg ha^{-1}, with no notable difference between the 120 kg ha^{-1} and 150 kg ha^{-1} treatments. In relation to the impact of nitrogen (N) fertilization on hemp seed production, Vera et al. (2010) found that higher N rates resulted in increased grain protein and grain oil content. Adamovics et al. (2016) determined that a N rate of 150 kg N ha^{-1} was sufficient for grain hemp production. Further investigations by Aubin et al. (2016) revealed that N fertilization rates up to 200 kg N ha^{-1} had positive impacts on stem diameter, plant height, seed yields, and biomass yields for dual-purpose hemp cultivars. In contrast, Karche (2019) indicated that 60 kg N ha^{-1} was adequate for dual-purpose hemp production, with no additional increase in seed yield as N rates increased. Additionally, Ascrizzi et al. (2019) found that applying 100 kg N ha^{-1} resulted in the tallest plants and the highest yields of seeds and inflorescences. Application of nitrogen should be decided based on available nitrogen in the soil before planting since studies have shown negligible yield responses for supplemental nitrogen fertilization in nitrogen-rich soils (Prade et al., 2011; Struik et al., 2000), whilst a significant increase in yield was indicated in marginal conditions (Amaducci et al., 2002; Finnan & Burke, 2013; Struik et al., 2000; Vera et al., 2010). Due to variations in environmental conditions and methodologies among these studies, it is difficult to accurately determine the stem and seed yield per unit of nitrogen fertilization using existing literature data, thus highlighting the need for further investigation (Adesina et al., 2020; Tang et al., 2022).

Phosphorus (P) is an essential nutrient for hemp throughout its entire life cycle. It plays a crucial role in promoting disease resistance and maintaining plant health. When hemp plants lack sufficient phosphorus, they experience stunted growth, leading to lower yields of seeds and stems (Adesina et al., 2020; Bruce et al., 2022). For grain crops, it is recommended to have 56 kg ha^{-1} of available P_2O_5 in the soil, while fibre crops require 67 kg ha^{-1} of phosphorus (Kostuik & Williams, 2019; Visković et al., 2023). However, the effects of phosphorus application on hemp biomass and seed yields have been inconsistent and minimal, according to a study by Vera et al. (2004). Potassium requirements for hemp are still not well-established, with inconclusive findings in existing studies (Bruce et al., 2022; Wylie et al., 2021). One study even concluded that potassium had no significant impact on hemp biomass and seed yield (Cockson et al., 2019). On the other hand, Visković et al. (2023) emphasized that hemp has a substantial potassium requirement, citing Jeff & Williams (2019), who recommend 336 kg ha^{-1} of K for both grain/dual-purpose hemp and fibre crops. Comparatively low information is available on the correlation between plant nutrients and plant quality on seed and fibre yield (Gusovius & Lühr, 2017).

The effect of nutrition on the yield and quality of hemp is complex and depends upon specific environmental conditions (de Meijer, 1995). The 5-minute guide to industrial hemp in New Zealand recommended 120 to 150 Kg/ha nitrogen, 50 to 80 Kg/ha phosphate and 60 to 80 Kg/ha potash to achieve an optimum hemp yield (McIntosh et al., 1998).

2.4.2 Climate: photoperiod and temperature

Photoperiod and temperature significantly impact hemp plant growth and development and flower formation (Gusovius & Lühr, 2017). The plant growth rate increases with the increasing temperature within the optimum temperature range and decreasing day length during the photoperiod-sensitive phase (Amaducci et al., 2008a; Lisson et al., 2000b). Hemp is a short-day plant: it often initiates flowering and changes phyllotaxy from an opposite leaf pattern to an alternative leaf pattern when the day lengths start to decrease (Blandinières & Amaducci, 2022a; Hall et al., 2014). The time of flowering in hemp is considered important since it determines the hemp biomass yield in terms of quality and quantity (Amaducci et al., 2008a; Amaducci et al., 2012) by strongly influencing the duration of the vegetative phase (Blandinières & Amaducci, 2022a). Long, inductive dark periods cease vegetative growth, and plant physiology changes to promote reproductive development (Meijer et al., 1995). However, seedling emergence and vegetative growth stages require sufficient sunshine hours (Clarke, 1999). van der Werf et al. (1994) showed that prolonged light periods (artificially) delayed, even stopped flowering and increased the dry matter yield by 2.8 t ha^{-1} in fibre cultivars. The photoperiod for hemp has been reported to be from nine hours (Heslop-Harrison & Heslop-Harrison, 1969) to 14 hours (Borthwich & Scully, 1954; Lisson et al., 2000a; Lisson et al., 2000b; Lisson et al., 2000c). The diversity in flowering precocity among hemp genotypes does not appear to be influenced by variations in the maximum optimum photoperiod, as studies have reported a relatively constant value of approximately 14 hours across different hemp genotypes exhibiting varying levels of flowering precocity (Amaducci et al., 2008b; Blandinières & Amaducci, 2022a; Hall et al., 2014; Lisson et al., 2000b). Despite this, hemp flowering has been reported at daylengths longer and shorter than 14 h (Pahkala et al., 2008; Sengloung et al., 2009). A study conducted by Sengloung et al. (2009) revealed that photoperiod between 11 to 12 hours induced flowering in Thai hemp varieties. According to Hall et al. (2014), cultivation of hemp is limited to regions with seasonal day lengths exceeding a minimum threshold period, generally between 14 -16 h (Hall et al., 2014), where the plants undergo premature floral transition. Hence, it is crucial to select and sow a suitable cultivar at the optimal timing to prevent premature flowering, which can minimize the duration of the vegetative growth phase and biomass production (Blandinières & Amaducci, 2022b; Tang et al., 2016). It should be noted that the effects of flowering and photoperiodism on hemp seed production have been scarcely studied.

The role of temperature is distinct and essential in hemp production during various growth stages (Adesina et al., 2020), and the selection of the sowing date must consider both soil and air temperatures as a critical parameter (Blandinières & Amaducci, 2022a). The recommended soil temperature for germination and growth is typically 8-10 °C, ensuring fast and uniform emergence, canopy closure, weed suppression, and reduced crop heterogeneity (Amaducci & Ranalli, 2020; Desanlis et al., 2013). Seedling emergence in field conditions was fastest at air temperatures between 13.5°C and 18.5 °C but slower at temperatures below 10°C or above 24 °C (Cosentino et al., 2012). Even though hemp plant growth is severely affected by cold temperatures, plants in the vegetative stage (seedlings up to the fifth pair of leaves) can thrive at -5°C for brief periods of time (Desanlis et al., 2013; Merfield, 1999). Studies revealed that hemp flowering time and duration are affected by temperature (Cosentino et al., 2012). Temperature impacts floral transition, lengthening the vegetative phase at low temperatures and causing a delay in flowering. Conversely, high temperatures expedite the transition to flowering and promote floral development (Dowling et al., 2021). Generally, hemp grows well in a range of climatic conditions; however, it is more responsive to environments where the temperature is between 15°C and 27°C (Cole & Zurbo, 2008). As per Merfield (1999), hemp fibre and seed production require 2,000 Growing Degree Days[10] (GDD) and 3,000 GDD, respectively. Hemp crops will reach full ground cover after 400 GDD (Struik et al., 2000).

It is evident that temperature and photoperiod have a large influence on hemp biomass production and seed set; hence these climatic conditions strongly affect the flowering time. This has stimulated many studies on the effect of genotype and environment on hemp.

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[10] Growing Degree Days (GDD) is a method for quantifying heat accumulation over time, crucial for understanding plant growth stages. The values are added together to give an estimate of the amount of seasonal growth your plants have achieved. It is a measure of the heat a plant requires to mature and yield a successful crop (Grigorieva et al., 2010; Miller et al., 2001).

2.4.3 Pests: Insects, Diseases and Weeds

Hemp is often promoted as a "pest tolerant crop," and John McPartland wrote: "*Cannabis* has a reputation for being pest free" (McIntosh et al., 1998). In general, hemp has been renowned for being hardy, tolerant and versatile for pests and diseases, although the degree of resistance has been exaggerated: *Cannabis* is a host to pests and diseases. About 300 pests have been identified in *Cannabis,* but only a few cause economic yield losses (McIntosh et al., 1998). The majority of the *Cannabis* pests are insects, and among them, European corn borers and hemp borers are the most destructive. Non-arthropods such as snails, slugs, and domestic and non-domestic mammals cause damage to the seedlings and flowering tops and are categorized as minor pests (McPartland, 1996). Even though some authors categorised birds as minor pests, birds are one of the most critical when growing hemp for seed (Jankauskienė & Gruzdevienė, 2015). However, hemp has been generally considered that it can be grown without pesticides (Desanlis et al., 2013) and with a low-input technique (Struik et al., 2000).

The common pests of *Cannabis* that cause damage to hemp plants are lepidopterous stem borers, predominately European corn borers (*Ostrinia nubilalis*) and hemp borers (*Grapholita delineana*). Beetle larvae are another group of pests known to tunnel into both the stems and roots of hemp plants. Some examples of these beetles include *Psylliodes attenuata*, *Ceutorhynchus rapae*, *Rhinocus pericarpius*, *Thyestes gebleri*, and various species of *Mordellistena*. In field-grown hemp plants, damage to leaves and flowering tops is typically caused by caterpillars such as hemp borers and budworms (e.g., *Heliothis armigera* and *Heliothis viriplaca*), beetles (e.g, *Psylliodes attenuata*), and bugs such as aphids (e.g., *Phorodon cannabis*, *Myzus persicae*, *Aphis fabae*), whiteflies (e.g., *Trialeurodes vaporariorum*, *Bemisia* spp.) and leafhoppers. Additionally, leafminers contribute to the array of potential threats. The most important non-insect pests are mites (*Tetranychus urticae*, *Aculops cannabicola*). Slugs (e.g. *Deroceras panormitanum*), rodents, and birds (e.g. *Passer montanus*) are pests of seedlings and seeds (McIntosh et al., 1998; McPartland, 1996).

There are over 100 diseases identified in *Cannabis*, but only a few causes severe yield losses (McIntosh et al., 1998). Gray mold (*Botrytis cinerea*) and hemp canker (*Sclerotinia sclerotiorum*) are the most devastating diseases that cause significant economic losses. *Botrytis cinerea,* a fungus that causes rotted flowering tops and stalks, is more prevalent in humid regions and wet weather. *Sclerotinia sclerotiorum, is* another fungal pathogen that causes watersoaked lesions on stems and branches of plants nearing maturity. This condition can result in the persistent deterioration of affected plants or their eventual wilting and collapse. Varieties that produce dense inflorescences are more vulnerable to bud rot, which results from *Trichothecium roseum* (McPartland et al., 2004). Other diseases include damping off (e.g., *Pythium* species), assorted leaf spots (*Septoria* species), blights (e.g., brown blight caused by two *Alternaria* and two *Stemphylium* species, stem cankers (*Fusarium* species), root rots (e.g., Fusarium root rot caused by *Fusarium solani*), nematode diseases (e.g., root lesion nematodes caused by *Pratylenchus penetrans*). broomrape (*Orobanche ramosa*).

Abiotic disorders such as genetic mutations, nutrient deficiency, drought stress, cold injury, wind injury, and stress due to monoculture cultivation are also sources of diseases in hemp and cause yield losses (McIntosh et al., 1998; McPartland, 1996; Young, 2005).

Hemp has long been renowned as a crop that competes well with weeds and needs little response to prevent yield reductions (Sandler & Gibson, 2019). Hemp can grow and cover the ground quickly after emergence (within four to nine days), enabling the plants to compete with weeds. This could be suitable for fibre cultivars planted in higher planting densities where weed suppression increases with increasing plant population, making chemical weed control unnecessary (Sandler & Gibson, 2019; Young 2005;). However, planting at low density for seed production can be a problem, which could result in significant yield reduction due to weed competition (Hall et al., 2013).

2.4.4 Cultivation Practices

2.4.4.1 Sowing time

The sowing time in hemp cultivation is crucial because of photoperiod and daylength requirements (De Prato et al., 2022), and it varies depending on the desired end-use, either fibre or seed (Visković et al., 2023). In the Northern Hemisphere, sowing from mid-April to the second half of May is recommended. The optimum planting times in New Zealand commence in September. September to October and October to December are optimal sowing times for the North and South Island of New Zealand, respectively (McPartland et al., 2004). Soil temperatures should be 10-12°C at sowing to ensure rapid germination and growth (Merfield, 1999). Earlier plantings go against rains at the end of winter or frost damage, limiting crop growth and yield (McPartland et al., 2004). In general, earlier or later sowing times have an adverse impact on plant growth and yield in reaction to short day lengths, low temperatures, and lack of solar radiation (Hall et al., 2013).

2.4.4.2 Sowing depth and plant densities

Hemp is generally sown at a depth of 1 – 2 cm, but seeds can be placed at a depth of 3cm to minimize bird predation (McPartland et al., 2004). Row spacing can be set from 10 cm (McPartland et al., 2004) to 20 cm (Storier, 2002). A row spacing of 25 cm or more may lead to weed competition (van der Werf, 1991). Optimum plant densities for different environment conditions, varieties, and end-product requirements have been widely researched (Young, 2005). Many experiments have been carried out to find out the optimal plant densities to maximize the fibre yield and quality without a clear consensus (Zatta et al., 2012). A target plant population of 180 -270 plants/m2 appears suitable for a higher fibre yield (Amaducci et al., 2002). Roulac (1997) reported that plant densities up to 500 plants/m^2 are possible if fibre quality is not of interest. However, plant densities for seed production are much lower than fibre production and are needed to maximise the seed yield (Pate, 1999). McPartland et al. (2004) targeted an established population of 90 – 120 plants/m^2 for hemp seed production. Bócsa & Karus (1998) mentioned that seed yields peaking at 125-150 plants/m^2 in Europe. In view of practical and quantitative factors, a plant population of 150 – 225 plants/m^2 has been recommended for oil seed production by Townshend & Boleyn (2008). According to Young (2005), a mid-density sowing rate between the densities recommended for fibre and seed production should be selected when dual-purpose hemp is used to get optimum yield for fibre and seeds.

2.4.4.3 Harvesting and yields

In order to achieve high-quality fibre, hemp plants are harvested right after flowering before the seed set (Cherrett et al., 2005). Bouloc (2019) has stated that early harvesting increases the hurd yield. Hurd weight drops to some extent at maturity; nonetheless, the weight of the fibre remains relatively stable. Thus, the percentage of fibre content is slightly higher for later harvest dates. Harvesting later yields greater amounts for seed production, particularly for late-flowering varieties (Bouloc, 2019). Mediavilla et al. (1998) have reported that hemp plants are harvested when the seeds are nearly 50 % mature. It has also been stated that seed crops can be harvested when 70 - 90 % of the seed heads reach maturity, however, the harvest time depends on the climate and the variety (Wrangham, 2019).

Hemp yields differ based on the purpose of crop production, variety and region. Generally, hemp seed yield ranges between 0.3 and 2.4 t/ha (Tang et al., 2016). A maximum seed yield of 3.5 t/ha has been recorded in Alberta Canada under irrigated conditions (O'Callaghan et al., 2018). The hemp seed yield in Europe is between 900 and 1100 kg/ha. Fibre yield is significantly more variable. France has a production potential ranging between 8 and 13 t/ha in areas having favourable environmental conditions, including deep soil and good water reserves. However, in areas with poor potential, the fibre yield varies between 4 and 7 t/ha (Bouloc, 2019).

2.5 Latitudinal photoperiodic adaptation

The adaptation of hemp growth to different latitudes, influenced by specific photoperiod and temperature conditions, is crucial for successful hemp production (Salentijn et al., 2019; Zhang et al., 2021). *Cannabis* plants exhibit a pattern where their response to daylight hours and overall growth characteristics vary systematically depending on their geographical location. *Cannabis* has developed a global pattern where its response to daylight hours and physical characteristics (stature) gradually change as moving from north to south around the world, and according to Small (2015), this pattern is consistent and genetically determined. During the process of domestication, certain populations of plants may have been intentionally chosen for their ability to grow independently of day length (photoperiodic insensitivity). This selection has been observed in various crops, such as certain strawberry cultivars. However, in the case of *Cannabis*, the selection for photoperiodic insensitivity has not been a significant factor (Small, 2015). Hemp flowering is induced by short-day photoperiods and inhibited by long-day conditions (Blandinières & Amaducci, 2022a; Hall et al., 2014). The optimal photoperiod for flowering induction is approximately 10 - 12 hours of uninterrupted darkness, with a critical photoperiod of around 14-12 hours of daylight (Salentijn et al., 2019). Plants adapted to northern climatic conditions are induced to flower with short days, allowing for a sufficient seed maturity period before late-season frost damage occurs. In contrast, plants adapted to equatorial regions tend to flower slowly as the days shorten. Late maturity is observed when plants adapted to the photoperiod of semitropical areas are grown in north-temperate climatic zones, and these plants fail to withstand low temperatures before seed production (Heslop-Harrison & Heslop-Harrison, 1969). Hemp accessions from northern locations produce seeds within a month, while those from near-equatorial regions remain in vegetative growth even after five months (Small, 2015).

Watson and Clarke (1997) indicated that the majority of hemp cultivars, specifically those intended for fibre production, are specifically bred for northern regions, and exhibit reduced performance as they approach closer to the equator. Sowing an unsuitable cultivar too early can trigger early flowering, particularly in regions with low latitudes. Consequently, this results in a shortened vegetative growth phase and decreased biomass production (Blandinières & Amaducci, 2022b). The success of hemp cultivars selected in a particular geographical region but grown in a significantly different location relies on their adaptation to the local photoperiod (Small, 2015). Therefore, understanding photoperiod adaptation becomes crucial when introducing hemp as a new crop to a country or when introducing new varieties.

2.6 Hemp breeding overview

2.6.1 Reproductive system

Cannabis is predominantly dioecious while breeding efforts have successfully produced monoecious cultivars (Beleggia et al., 2023). X and Y chromosomes govern sex determination. Dioecious nature, X/Y sex determination and wind pollination are rarely observed in plants; however, all three features are present in *Cannabis* (Campbell et al., 2021; Clarke & Merlin, 2016; Hall et al., 2012). Plants that have staminate flowers are taller and slender, while plants with pistillate flowers are shorter and vigorous. Soon after the pollen dehisce, male plants senesce and die, while female plants mature up to a few months after flowering and fertilization (Ranalli, 1999; Small, 2016). After the pollination in the female flowers, paired stigmas will be lost, and ovule and tubular bracts will be enlarged. Harvesting can be done in three to six weeks when the seeds are matured, or the seeds will be scattered to the ground (Ranalli, 1999).

2.6.2 Pollination

Flowering starts in male plants in hemp (Chandra et al., 2017; Pahkala et al., 2008), and the flowering period lasts two to four weeks (Hall et al., 2012). Five sepals of the male flowers open widely during flowering. A single flower can produce 350,000 yellow pollen grains (Aboulaich et al., 2013; DeDecker, 2019) and hundreds of flowers on larger plants (Small & Antle, 2003). Male flowers in monoecious plants are far fewer than in dioecious hemp varieties. Pollen grains of dioecious plants are larger (Migalj, 1969) and more uniform than those of monoecious plants (Small & Antle, 2003; Small, 2015).

The hemp pollen is dispersed by wind in huge amounts and disseminated for long distances (Sarda Estève et al., 2018; Small & Antle, 2003). Cabezudo et al. (1997) reported that wind currents carried pollen from *Cannabis* (marijuana) in North Africa to Southwestern Europe. A study conducted in the Midwestern United States in 2000, before hemp was legalized, found that hemp pollen comprised up to 36 % of total airborne pollen counts (Stokes et al., 2000). These studies evidenced the long-distance dissemination of hemp pollens. As a result, pollen from marijuana fields increases the risk of pollination of hemp, and concerns about cross-pollination among the different hemp varieties are critical (Johnson, 2014). Hybridization of hemp with marijuana can be a critical issue not only for breeding new hemp cultivars but also in the production of pure hemp seeds for planting. If the genes responsible for high levels of THC are transferred to a seed source, the following plant generations could result in a higher THC than the legalized THC limit (Small & Antle, 2003). Small & Antle (2003) also mentioned that “hemp breeders are faced with a perennial problem of ensuring that their plants are adequately protected from contaminating pollen”. Also, cross-pollination during seed multiplication causes male plants to be segregated into monoecious hemp cultivars (Olejar & Park 2022).

The basic strategy for reducing the risk of cross-pollination is maintaining geographical or physical isolation (DeDecker, 2019; Flajšman & Ačko, 2022). Canadian regulations for the production of pedigreed hemp seed recommend an isolation distance of 5 km (Small & Antle, 2003). However, the isolation distance varies among countries. The Vavilov Institute of Plant Industry in Russia maintain an isolation distance of 3 – 4 km for seed multiplication of some hemp germplasm collections (Lemeshev et al., 1994). Strict isolation must be maintained during seed multiplication to maintain a monoecious population. Monoecious pollen is less competitive than contaminating dioecious male individuals, which can reverse their dioecious state (Bócsa & Karus, 1998). Scientific data published on hemp pollen maturity, viability, environmental factors affecting pollination, and correlation between the amount of pollen supply and seed set are limited and remain to be studied precisely. No research has been published related to the pollination of hemp in New Zealand, and work is underway in New Zealand on seed certification requirements for industrial hemp.

2.6.3 Impact of inbreeding and outcrossing

Breeding *Cannabis* poses several challenges due to the predominant dioecious nature of the plants and natural cross-pollination (Hesami et al., 2021; Schluttenhofer & Yuan, 2017). Dioecious hemp plants are open-pollinated and incapable of self-pollination. Breeding programs must incorporate geographic isolation or technical methods to prevent undesired outcrosses since hemp plants are anemophilous, relying on wind for pollination (anemophilous) and freely intercrossing. However, monoecious hemp cultivars can naturally self-pollinate (Schluttenhofer & Yuan, 2017). As mentioned before, monoecious hemp plants do not appear naturally and only exist with the help of human selection. Without ongoing selection efforts, the dioecious nature of hemp plants will be restored in two to three generations (Nelson, 1999).

Naturally intercrossing plants like *Cannabis* exhibit moderate to high levels of heterozygosity, and these plants possess moderate to higher frequency of opposing alleles at a gene locus, particularly for many dominant characters (Clarke & Merlin, 2016; Hesami et al., 2021). Inbreeding is practically challenging in dioecious hemp cultivars. Although developing inbred lines is an effective method for fixing desirable traits (Ranalli, 1999), repeated selfing leads to inbreeding depression in hemp. On the other hand, self-pollination can be performed in monoecious and sub-dioecious hemp cultivars. Monoecious hemp, despite having a lower rate of genetic variation compared to dioecious plants, can serve as parents for unisexual hemp breeding programs, potentially increasing yields in both fibre and grain crops (Hall et al., 2012).

A precise breeding approach is necessary for outcrossing in hemp. This involves searching for variation to create a base population, selecting suitable parents, improving the parents' desirable traits through recurrent selection, and developing and testing experimental varieties (Schluttenhofer & Yuan, 2017). The primary objective and challenge for hemp breeders, as emphasized by Flajšman & Ačko, (2022) and Clarke & Merlin (2016), is to fix selected traits through homozygosity while simultaneously maintaining overall heterozygosity and hybrid vigour in the variety.

2.6.4 Germplasm and natural variability

Germplasm collections are a vital source of genetic and phenotypic variation for selection in crop improvement (Schluttenhofer & Yuan, 2017). *Cannabis* germplasm includes accessions, gene bank collections and populations used in breeding programmes (Ranalli, 2004). Using natural genetic variability is the conventional and most successful breeding approach. This can be achieved through the hybridization of elite breeding material with exotic germplasm (Welling et al., 2016a), which requires access to germplasm conservations. Studies on the germplasm of *Cannabis* showed significant phenotypic diversity in certain traits such as oil content (Grigor'ev et al., 2010), the composition of THC and CBD (Welling et al., 2016a), and xylem composition (de Meijer, 1994).

During the latter half of the 20^{th} century, hemp, as an illegitimate crop, suffered significant losses in germplasm conservation and missed the opportunity to benefit from advanced conservation and breeding technologies (Clarke, 1998; Johnson, 2020; Small & Marcus, 2002). For instance, Dewey (1927), a renowned hemp breeder in the early 1900s, developed numerous valuable hemp varieties for production in the United States. However, due to the prohibition of hemp, the destruction of germplasm and the subsequent loss of these developed varieties occurred (Campbell, 2019; Small & Marcus, 2003). Consequently, modern hemp farmers are faced with the challenge of working with suboptimal genetics. Nonetheless, various organizations are actively involved in addressing and resolving this issue (Johnson, 2020).

Access to and utility of accession collections remain limited due to the lack of a core *Cannabis* germplasm collection (Schluttenhofer & Yuan, 2017), and limited effort has been made for comprehensive characterization of currently available *Cannabis* germplasm resources (Welling et al., 2016b). The renewed interest in hemp presents a promising opportunity for evaluating and breeding with diverse cultivars. However, researchers face challenges in accessing germplasm (Torkamaneh & Jones, 2022). Typically, plant breeders and researchers rely on genebanks worldwide to obtain new traits for developing improved cultivars, including commercial cultivars, landraces, and wild crop relatives. Although there are some public hemp collections available (Torkamaneh & Jones, 2022; Welling et al., 2016a), the growing demand for hemp products emphasizes the urgent need to identify suitable genetics that can be utilized for production and as a source of diversity for future breeding and development efforts. Most of the few seed accessions are held by national gene banks, and the Vavilov Institute of Plant Research (VIR) in Russia maintains the largest collection of hemp germplasm. Table 2.2 shows the details about a few seed banks and the number of *Cannabis* accessions.

Table 2.2. World seed banks for *Cannabis* accessions.

Collection of hemp Germplasm	Number accessions	Special Remarks
Vavilov Institute of Plant Research (VIR) in St. Petersburg, Russia.	563 seed accessions	Twenty-three drug accessions from Afghanistan, Kazakhstan, Syria, Turkey, and Uzbekistan, while the remaining are all hemp and feral accessions from Armenia, Bulgaria, Chile, China, Czechoslovakia, Estonia, France, Germany, Hungary, Italy, Latvia, Moldova, Poland, Portugal, Romania, Russia, Spain, Sweden, Ukraine, the United States, and former Yugoslavia
Wageningen University in the Netherlands	Over 156 accessions	Accessions are from 22 countries and largely sourced from other collections and research institutes. Nearly half of these accessions are from the former USSR and Hungary.
The Institute of Natural Fibres and Medicinal Plants gene bank collections in Poland	139 accessions	Accessions are primarily of European origin, with accessions from France, Hungary, and 54.7% of the Ukrainian collection.
The Yunnan Academy of Social Sciences collection in Yunnan Province, China	350 accessions	Accessions are mainly from East Asian origin.
The Ecofibre Global Germplasm Collection in Australia	The exact number of accessions is not known.	Accessions are mostly Eurasian accessions.
The subterranean Svalbard Global Seed Vault on the Norwegian island of Spitsbergen	43 accessions	Twenty-one accessions from Argentina, Austria, China, Croatia, France, Georgia, Germany, Italy, Poland, Romania, Slovakia, Spain, and Sweden; 16 accessions are of unknown origin.
Millennium Seed Bank in West Sussex, England	Only one *Cannabis* accession	The accession is from Slovakia.

(Source: Clarke & Merlin, 2016)

The limited availability of comprehensive core collections of *Cannabis* seeds, inadequate quantities of viable seeds per accession, the insufficient representation of biodiversity in seed banks, and the absence of coordinated conservation and characterization efforts for *Cannabis* resources pose restrictions on incorporating exotic allelic variants into novel cultivars, thereby limiting their value for breeders. (Ranalli, 2004; Welling et al., 2016b; Clarke & Merlin, 2016; Schluttenhofer & Yuan, 2017). Established commercial hemp breeding programs focus on fibre and seed production in the commercial sector. However, industry-wide knowledge of the available germplasm is lacking (Burton et al., 2022). Specifically, information on elite breeding lines for improved seed size and quality is limited (Burton et al., 2022; Small, 2018). Establishing a core germplasm resource for hemp is essential to address the need for a diverse range of cultivars that can adapt to the varied growing conditions within and across different regions, and various studies have emphasized the importance of this germplasm resource (Burton et al., 2022; Hurgobin et al., 2021; Small, 2018; Welling et al., 2016a). The establishment of well-represented and characterized *Cannabis* genetic resources, including a wide range of genetic and phenotypic diversity, will be a key step towards the development of cultivars and advanced breeding lines capable of coping with industrial requirements and a wider range of growing environments worldwide.

2.6.5 Breeding methods in hemp

Cannabis is a challenging plant to improve by genetic procedures because of its naturally dioecious nature, which leads to outcrossing by nature and the reaction of the plant to day length that affects the phenological development, length of biological cycle, and yield (Ranalli, 2004). Hemp has been recognized as one of the earliest plants in scientific literature where the length of the day was identified as a crucial factor influencing its flowering (Tournois, 1912; as cited in Heslop-Harrison, 1957; Kobayashi & Weigel, 2007). The response of hemp plants to day length leads to extended generation times when cultivated in the field. Consequently, the progress in creating novel hemp varieties can be impeded by the limited potential for field cultivation, with typically only one generation achievable annually (Schilling et al., 2023). The first hemp breeder is known to be Dewey (1927), who was involved in the selection of hemp varieties from Chinese and Italian landraces, and Bredemann (1924) made the first attempt to breed for increased fibre content on hemp (as cited in Ranalli, 2004).

Most of the hemp cultivars available are open-pollinated and synthetic (Salentijn et al., 2015; Vassilevska-Ivanova, 2019). Breeding methods for hemp have changed throughout the years (Meijer, 1995; Koren et al., 2022; Ranalli, 2004; Ranalli & Venturi, 2004). The methods commonly used in hemp breeding today include mass selection, cross-breeding, inbreeding and hybridization (Koren et al., 2022; Shanmugam et al., 2022; Salentijn et al., 2015), and advanced methods have begun to be used in hemp breeding (Koren et al., 2022). At the beginning of the 20th century, mass selection was a predominant approach in producing hemp cultivars, and continuous selections were made for characteristics such as length of vegetative period, plant height, diameter and seed weight from landraces to improve the population by direct selection (Flajšman & Ačko, 2022; Ranalli, 2004). Mass selection refers to selection among individual plants or seeds, which are then bulked together with selected individuals to form a new population (Fehr, 1991). This method resulted in the development of several productive cultivars such as Bolognese, Toscana, and YunMa 1 through the improvement of landrace varieties. However, its effectiveness is constrained by the level of selection intensity and the heritability of the desired traits (Salentijn et al., 2015; Hennink, 1994). Mass selection continues to be utilized to maintain various European dioecious varieties such as Carmagnola, Kompolti, Lovrin, and Novosadska konoplja (Clarke & Merlin, 2016; Flajšman & Ačko, 2022).

Over time, advanced crossing techniques and selective criteria were adopted to improve specific traits in hemp breeding. In cross-breeding method, two parent lines, selected for their uniformity but genetic diversity, are crossed to create offspring with a combination of desirable traits. The progenies are then evaluated and selected based on targeted traits, leading to the development of new plant varieties (Flajšman & Ačko, 2022). Recently, breeders have also utilized varieties as parent lines (Flajšman & Ačko, 2022). Targeted crosses were conducted to improve important traits such as fibre content, stem and seed yield, gender, phenological development, stem quantity, low THC content, resistance to pathogens like root-knot nematodes, lodging resistance, and adaptability to different cultivation regions, considering the influence of genotype and environment on hemp yield and quality (Flajšman & Ačko, 2022; Ranalli, 2004; Salentijn et al., 2015). Half-sib family selection was implemented by evaluating progeny from each open-pollinated mother plant, which is fertilized by multiple pollen male parents. The selection is based on general combining ability[11](GCA), using the entire population as a tester (Salentijn et al., 2015).

[11]General combining ability (GCA) refers to the average performance of a parent within a hybrid combination and mainly reflects the impact of additive gene action (Begna, 2021).

Although early hemp breeders used a relatively primitive approach, they recognized the potential of heterosis[12], or hybrid vigour, and began exploring the possibility of exploiting it alongside selective breeding efforts (Bócsa, 1954; Brian et al., 2019; Koren et al., 2022). *Cannabis* breeders have observed that deliberate crosses between genetically distinct genotypes result in offspring with increased vitality, a phenomenon known as heterosis or hybrid vigour (Flajšman & Ačko, 2022). Dewey (1927) is credited with creating the first intervarietal hybrid, Ferraramington, by crossing Kymington and Ferrara. This hybrid exhibited superior fibre characteristics and was among the successful cultivars created by Dewey. However, all of Dewey's germplasm was lost due to *Cannabis* prohibition in the United States (Brian et al., 2019). Heterosis breeding produced good results of inbred lines crossing, in which F1 are more productive than the initial material (Burczyk et al., 2005; Koren et al., 2022). Heterosis breeding of hemp has been successfully implemented in Hungary and China, leading to the development of various F1 hybrid cultivars. These include Uniko-B, Kompolti hybrid TC, YunMa 2, YunMa 3 and YunMa 4 (Clarke & Merlin, 2016; Salentijn et al., 2015). Among these cultivars, YunMa 2 and YunMa 4 exemplify the successful application of heterosis breeding in China (Salentijn et al., 2015). Uniko-B and YunMa are single cross hybrids, and Kompolti hybrid TC is a three-way-cross hybrid (Salentijn et al., 2015). Although there have been some successes in hybrid breeding, true hemp hybrids through the crossing of pure lines have not been achieved thus far (Flajšman & Ačko, 2022). To achieve advancements comparable to those seen in maize breeding during the 1900s, a significant shift in the paradigm of hemp breeding will be required (Brian et al., 2019; Carlson et al., 2021).

In hemp, molecular-level studies have mostly been employed in forensic applications to differentiate fibre and/or seed type from drug-type *Cannabis*, for the early detection of male plants, trace geographic diffusion routes of *Cannabis* (Salentijn et al., 2015), to study the variability and genetic structure of *Cannabis sativa* (Onofri & Mandolino, 2017). In terms of hemp breeding, molecular technologies mainly aimed at certain traits such as fibre, sex and secondary metabolism (Eržen et al., 2023; Flajšman & Ačko, 2022; Kolenc & Čerenak, 2017; Ranalli, 2004). Finding molecular markers for specific traits in hemp breeding has become crucial to reducing labour-intensive and time-consuming

[12] Hybrid vigour, also known as heterosis or outbreeding enhancement, refers to the superior phenotypes observed in hybrids relative to their inbred parents with respect to the desired traits (Ben-Ari & Lavi, 2012; Chow & Mosher, 2023).

screening methods (Ranalli, 2004; Sebastian et al., 2023). While various types of *Cannabis* molecular markers have been discovered and utilized, research on *Cannabis* lags behind other crops such as rice, wheat, and maize (Pan et al., 2021; Zhao et al., 2022). This is primarily due to the very high genetic variability in *Cannabis* resulting from allogamy and cross-pollination in the past, limiting the practical use of genetic maps and molecular markers (Salentijn et al., 2015; Sebastian et al., 2023; Zhao et al., 2022). Consequently, the density of molecular markers in *Cannabis* remains relatively low, hindering genetic studies like genetic map construction, gene/QTL mapping, and genetic analysis (Pan et al., 2021). At the present stage, less work has been done in hemp employing recent advances in biotechnology (Bouloc, 2019). Given the increasing affordability of high-quality sequence data, genomic selection exhibits significant potential as a breeding strategy to improve complex traits (Brian et al., 2019). Despite the availability of high-quality genome sequences from diverse *Cannabis* strains, including those with balanced CBD and THC levels, as well as CBD/THC-rich strains, the use of genome editing tools to create non-transgenic hemp varieties with enhanced industrially desirable traits remains unexplored (Shiels et al., 2022). According to Feeney and Punja (2003), the gene for isomerase phosphanamose was successfully inserted into hemp using Agrobacterium tumefaciens as a vector. Although this transgenic hemp had no commercial significance, its development showed the potential for genetically modifying hemp when needed (Bouloc, 2019).

2.6.6 Primary trait selection criteria

The diverse applications of hemp drive the need for the development of cultivars that exhibit superior performance in terms of high seed and fibre yield, plant architecture, flowering time, fibre quality, disease and pest resistance, novel cannabinoid profiles, and sensory properties (Shanmugam et al., 2022; Shiels et al., 2022). The main selection criteria for fibre breeding includes high stem productivity in terms of high fibre yield and high fibre quality, low THC, length of the vegetative cycle, degree of monoecy and resistance to pest and diseases (Salentijn et al., 2015). The hemp industry values two crucial traits: high-quality fibre and fibre that can be easily mechanically extracted. High-quality fibre refers to bast fibre that is fine and abundant, with high cellulose content and minimal lignification (Shanmugam et al., 2022). It also requires reduced cross-linking between pectin and other structural cell wall components and efficient decortication properties (Ranalli, 2004). The genetic architecture of fibre quality traits in hemp is complex (Pedró, 2020). The content of cellulose, hemicellulose, lignin, and bast fibre are primarily determined by genetic components, with high heritability and low Genotype × Environment interactions (G × E interactions), hence these traits could constitute of a panel that holds significant potential for maximizing the genetic gain and further studies of the genetics underlying fibre quality traits. However, the environment highly influences the variability in pectin, bast fibre fineness, and decortication efficiency (Shanmugam et al., 2022). Despite the large variability, the genetic means of these traits remain mysterious. This is likely due to the complex interactions between multiple genes and environmental factors (Petit et al., 2020b; Shanmugam et al., 2022). As a result, breeding for these traits is challenging, largely due to the inadequate understanding of their genetic architecture (Shanmugam et al., 2022; Pedró, 2020). In addition, further characteristics such as cleaner fibres with low residual hurd content (Berenji et al., 2013) and late blooming to improve stem yield (Ranalli, 2004) are being considered.

The main selection criteria for seed breeding include high seed-yield (Berenji et al., 2013; Flajšman & Ačko, 2022). The development of monoecious varieties has partially increased seed yield in hemp, but further progress is slow (Flajšman & Ačko, 2022). Seed yield is a quantitative trait, meaning that it is influenced by many genes at multiple loci (Ferfuia et al., 2021). This makes it difficult to improve seed yield through traditional breeding methods (Clarke & Merlin, 2016; Flajšman & Ačko, 2022). Improving the nutritional composition of hempseeds is another crucial aspect. The main goals in this aspect are to increase the protein and oil content in seeds and to improve the fatty acid composition of oil (Bócsa et al., 2005; Flajšman & Ačko, 2022; Shiels et al., 2022). Hemp oil, which contains approximately 80% unsaturated fatty acids, is prone to oxidation and perishability at room temperature, prompting breeding efforts to enhance stability (Bielecka et al., 2014). Another objective is to improve the favourable ratio between linoleic and α-linolenic acid and increase the gamma-linolenic acid content (Berenji et al., 2013; Ranalli, 2004). Compared to increasing yield, the genetic pathways responsible for protein and fatty acid synthesis involve fewer genes, making the selection of suitable lines and breeding of varieties slightly less challenging (Clarke & Merlin, 2016).

In addition to the specific selection criteria for fibre and seed production and the previously mentioned traits, major breeding objectives include developing new varieties with high levels of non-psychoactive cannabinoids and low levels of THC (Hesami et al., 2021). Other goals encompass achieving early canopy closure to maximize intercepted radiation during growth, reducing sensitivity to day length for minimizing the photoperiod's impact on yield, adapting to diverse growing regions while considering G × E interactions, and enhancing genetic and phenotypic uniformity (Berenji et al., 2013; Hesami et al., 2021; Salentijn et al., 2015).

2.7 Selection in plant breeding

Selection is a fundamental process in plant breeding that aims to alter the frequency distribution of genes associated with desirable traits within a population (Allard, 1999; Falconer, 1989; Moose & Mumm, 2008). It involves carefully selecting individuals with desired characteristics, leading to the genetic improvement of cultivated crops (Kersey et al., 2020). The initial step in any plant breeding program is establishing a breeding pool or base population (Fehr, 1991). This involves assembling diverse genetic resources, ranging from wild germplasm to commercially available cultivars. Breeders introduce genetic diversity by crossing various germplasm sources and create a foundation for achieving their breeding objectives (Lusser et al., 2012). Once the breeding pool is established, the focus shifts to identifying and selecting superior individuals (Acquaah, 2012). Selection in plant breeding can be applied to enhance specific plant traits directly or to target characteristics associated with the reliability of production, harvestability, and marketability (Fehr, 1991). Phenotypic selection, based on observable traits, has been a traditional and effective approach in conventional breeding. However, phenotypic selection may yield suboptimal results for traits that are difficult to measure accurately or exhibit low heritability (Moose & Mumm, 2008). The success of selection critically depends on the availability of genetic variation within the breeding population (Fisher, 1919). Genetic variation provides the raw material for breeding programs to select and combine favourable alleles in subsequent generations. A profound understanding of heredity enables the development of valuable genetic diversity and the selection of superior individuals. Improving knowledge of the genetic basis of expression of traits will increase the effectiveness of plant breeding programmes (Fehr, 1991). Therefore, breeders must study the genetic variation in targeted traits (Sharma, 2001) and carefully manage and incorporate genetic diversity into their breeding pools by crossing diverse parental lines and utilizing genetic resources effectively. Understanding the available genetic variation and incorporating and maintaining genetic diversity in the breeding pool is essential, as the effectiveness of selection greatly depends on the presence of genetic variation in the observed trait (Fisher, 1919).

The phenotypic expression of an individual is the product of its genotype (G), its environment (E), and the interaction between the two (G × E interaction) (Falconer, 1989). The phenotypic variation in a population is given as:

$$\sigma_p^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2 \quad (1)$$

where σ_p^2 is the phenotypic variance, σ_G^2 is the genetic variance, σ_E^2 is the environmental variance and σ_{GE}^2 is the variance associated with genotype and environmental interaction (G × E interaction).

In a random mating population, the genetic component of the variance can be further partitioned into three components, as follows (Fisher, 1919; Falconer, 1989):

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2 \quad (2)$$

where σ_A^2 is the additive variance (variance from additive gene effects), σ_D^2 is the dominance variance (the interactions between alleles at the same locus), and σ_I^2 is the interaction effect (the interactions of alleles between loci).

The extent of additive and non-additive genetic variance in traits crucial in plant breeding significantly influences the selection of optimal breeding strategies (Nguyen et al., 2022; White et al., 2007). Additive genetic variance, also known as additive variance, refers to the variation in breeding values among individuals (Fehr, 1991) and is responsible for the similarity observed among relatives. The additive genetic variance is often expressed as the ratio σ_A^2 / σ_G^2 or "narrow-sense heritability" (Silvertown & Charlesworth, 2009). When studying a breeding population, additive genetic variance is the most easily estimable component among others (Acquaah, 2009). It is the main factor influencing the observable genetic characteristics of a population and its response to selection.

2.7.1 Response to selection

As mentioned before, once variability has been created, the breeder must engage in the critical task of selection to advance the population (Swami et al., 2023). Selection is the act of differentiating among a genetically diverse population to identify and choose specific individuals for the next generation. Selection exerts a pressure that alters the frequencies of alleles within a population (Silvertown & Charlesworth, 2009). As a result, the differential reproduction of genotypes within a population leads to gene frequency changes, which subsequently modify the genotypic and phenotypic values of the desired traits (Wricke & Weber, 2010). The breeder aims to select superior individuals with high genetic potential from a diverse population, anticipating that this selection will lead to a positive shift in the population mean of the desired trait in the subsequent generation (Gjedrem, 2005). Response to selection is the difference between the selected individuals' mean and the parental population's mean (Acquaah, 2012; Falconer, 1981; Falconer, 1989; Fehr, 1991). The response to selection is simply the change of the population mean from one generation to the next after the selection process has taken place (Acquaah, 2012; Fehr, 1991). Response to selection is also referred to as "genetic gain". However, response to selection may better explain changes that are not necessarily favourable (Rutkoski, 2019).

Genetic gain from selection, or simply genetic gain, refers to the improvement of the average genetic or phenotypic value within a population resulting from selection applied across successive breeding cycles (Hazel & Lush, 1942). Genetic gain quantifies the average superiority of offspring from selected parents compared to the preceding generation before the selection process (Gjedrem & Thodesen, 2005). Genetic gain serves as a valuable indicator to compare the effectiveness of various plant breeding techniques (Acquaah, 2012; Bertan et al., 2007; Fehr, 1991; Nyquist & Baker, 1991). The extent of genetic gain in a given plant species is influenced by several factors, including the heritability of target traits, species biology, breeding cycle duration, arrangement of spaced-plants in field trials, and resource availability across different breeding methods (Fehr, 1991). Genetic gain is achievable for almost any heritable trait (Rutkoski, 2019).

To predict the response to a single generation of selection, it is necessary to know about the trait's heritability to be improved (Cobb et al., 2019; Walsh, 2001). Heritability is the ratio of genetic variation to total phenotypic variation (Falconer, 1989; Nyquist, 1991) and is a quantitative measure that indicates the proportion of the observed variation in a progeny that can be attributed to the inheritance of traits from parents to their offspring (Nakaya & Isobe, 2012). The primary objective of estimating heritability (also the genetic parameters) involved in assessing and comparing the potential improvements achievable through different selection strategies (Holland et al., 2003). Plant traits with higher heritability can be improved more rapidly compared to traits with lower heritability, as indicated by Nyquist and Baker (1991). Furthermore, heritability measurements play a crucial role in determining the optimal selection strategy and breeding scheme (Nyquist & Baker, 1991). Traits with higher heritability are effectively improved using simpler methods, such as mass selection, while family-based methods are more advantageous for traits with lower heritability (Nyquist & Baker, 1991). Heritability is influenced by population-specific factors and is determined by a combination of additive and non-additive genetic factors and environmental variance (Nyquist & Baker, 1991; Visscher et al., 2008). The factors, such as allele frequencies, mode of gene actions, and environmental variables, can vary between populations, leading to variations in heritability estimates (Visscher et al., 2008).

There are two main types of heritability: broad-sense heritability and narrow-sense heritability (Acquaah, 2012; Falconer, 1960; Nguyen & Sleper, 1983; Nyquist & Baker, 1991). Broad-sense heritability is the proportion of phenotypic variance due to all genetic variance, including additive, dominance, and epistatic effects (Eq. 3) (Bernardo, 2002).

$$h_b^2 = \frac{\sigma_G^2}{\sigma_p^2} \tag{3}$$

where: h_b^2 is the broad-sense heritability, σ_G^2 is the genetic variance, σ_p^2 is the phenotypic variance.

Narrow-sense heritability is the proportion of phenotypic variance due to additive genetic variance (Eq. 4) (Bernardo, 2002).

$$h_n^2 = \frac{\sigma_A^2}{\sigma_p^2} \tag{4}$$

where: h_n^2 is the narrow-sense heritability, σ_n^2 is the additive genetic variance, σ_p^2 is the phenotypic variance.

Broad sense heritability is the proportion of phenotypic variance that is due to all genetic effects (Acquaah, 2009; Nyquist, 1991; Falconer & Mackay, 1996). Narrow sense heritability refers to the proportion of variation in a trait among individuals within a population that can be attributed to genetic factors (Nyquist, 1991). Dr J. L. Lush initially introduced this concept of narrow-sense heritability under the term "heritability" (Bell, 1977; Holland et al., 2003). Narrow-sense heritability is more reliable in predicting genetic gain than broad-sense heritability since the additive component of the genetic variance determines the response to selection (Acquaah, 2009). In the case of clonally propagated species, where both additive and non-additive gene actions are fixed and passed from parents to offspring, broad-sense heritability becomes particularly important (Acquaah, 2012).

Plant breeders select plants based on their observable phenotypic characteristics, which are influenced by both genetics and the environment. If the environment strongly affects a trait, breeders may accidentally select plants with less desirable genotypes (Acquaah, 2015). This will hinder the breeding program's progress, and the expected genetic gain will not be realized (Acquaah, 2015; Falconer, 1981). Heritability measures the reliability of a plant's phenotype in reflecting its breeding value for a particular trait. Heritability does not measure genetic control directly, but rather the extent to which the genetic control can vary (Falconer & Mackay, 1996) and guide to the breeding value of the targeted trait (Acquaah, 2015; Falconer, 1981). Heritability also depends on the trait, population, and environment. Quantitative traits generally have lower heritabilities compared to qualitative traits. Breeding programs aim for high heritability, as it indicates that a trait can be effectively improved through breeding efforts (Lynch & Walsh, 1998).

Heritability and genetic correlation play essential roles as genetic parameters in quantitative traits, enabling the prediction of selection response (You et al., 2016). The heritability of a trait significantly influences breeding program selection methods by determining the effectiveness of traits for selection and their transferability across generations. For an example, highly heritable traits are well-suited for mass selection (Fehr, 1991), making direct selection a suitable approach. On the other hand, when the heritability of the primary trait is lower than that of the secondary trait, and there is a strong genetic correlation between the two, indirect selection is recommended (Acquaah, 2012; Fehr, 1991). This approach is particularly useful for optimizing breeding outcomes.

Heritability estimates can be used to compare the improvement or gain achieved through selection using various experimental designs. This information, along with considerations of the costs associated with additional replications within each environment, more years of evaluations, and additional evaluation locations, can guide the development of optimal breeding strategies (Milligan et al., 1990). When significant changes in ranking occur among families evaluated in different environments due to genotype-by-environment interaction G × E interactions, it is possible to compare heritability estimates reflective of the response to selection based on mean values across all environments with heritability estimates based on mean values within specific local environments. This comparison can help determine the most effective selection strategy (Atlin et al., 2000). Heritabilities of various family structures from the same base population can be compared to identify the most effective structure for maximizing genetic gain over time (Burton & Carver, 1993). The variation of heritability across populations allows us to utilize heritability estimates from different populations to select suitable base populations for more effective selection (Goodman, 1965). Due to the variation of heritabilities among different traits within a population, heritability estimates and genetic correlation estimates among these traits can be utilized to identify not only indirect selection schemes but also direct selection schemes (Diz & Schank, 1995; Bänziger & Lafitte, 1997; Rebetzke et al., 2002).

Resuming the discussion of response to selection, response to selection is determined by multiplying the narrow-sense heritability and the selection differential. The selection differential represents the average phenotypic value of the individuals chosen as parents for the next generation, measured as a deviation from the population mean. The relationship between the response to selection and narrow-sense heritability is defined by the following equation (Falconer & Mackay 1996):

$$R = h_n^2 S \tag{5}$$

where, R is the response to selection, h_n^2 is the narrow-sense heritability, and S is the selection differential.

2.7.2 Half-sib mating in generating half-sib families for quantitative genetics.

Different mating designs are created to estimate genetic variance (Singh et al., 2004), and they are employed to separate variations into distinct genetic components using second-degree statistics (Awata et al., 2018; Singh et al., 2004). Genetic variances in cross-pollinated species like *Cannabis*, can be estimated using mating designs that do not rely on inbred relatives (Moll & Stuber, 1974). Mating design involves the process of generating offspring in plant breeding. Plant breeders and geneticists employ various types of mating designs and arrangements for specific purposes (Nduwumuremyi et al., 2013). The main functions of mating designs are: (1) to gather genetic information about the trait being studied, (2) to predict genetic gain, (3) to create a breeding population for the selection and development of potential varieties, and (4) to evaluate the parents involved in the breeding program (Nyquist & Baker, 1991). Choosing a mating design for estimating genetic variances should consider the study objectives, time, space, cost, and biological constraints. In all mating designs, individuals are randomly chosen and crossed to produce offspring or genetic structures that are either half-sibs or full-sibs (Muthoni & Shimelis, 2020; Nduwumuremyi et al., 2013).

A commonly employed approach involves using half-sib (HS) family variances to estimate additive genetic variances, which in turn allows for the estimation of narrow sense heritabilities and genetic correlations (Riday & Brummer, 2007). Half-sib (HS) matings, such as polycross, topcross, and open-pollination, are valuable for acquiring quantitative genetic data in cross-pollinated plants in plant breeding (Nduwumuremyi et al., 2013; Nguyen & Sleper, 1983). Among these, polycross mating is preferred for generating HS families in quantitative genetic studies (Nguyen & Sleper, 1983). The polycross mating design is particularly suitable for obligate cross-pollinating species (Muthoni & Shimelis, 2020). Polycross mating is advantageous for the following reasons: (1) The crossing plan is simpler compared to the diallel, nested, or factorial mating designs (Comstock & Robinson, 1948; Griffing, 1956), (2) more parents can be utilized in HS matings compared to diallel matings (Nguyen & Sleper, 1983) and (3) by estimating the covariance between parents and HS progenies, as well as the genetic variance among parents and HS families, important genetic information can be obtained (Comstock & Robinson, 1952). The utilization of maternal half-sib families is prevalent in wind-pollinated species due to a high level of confidence that the family members are predominantly composed of half-siblings. In this context, each family member is the outcome of pollination by a distinct male plant (Nyquist & Baker, 1991). Utilizing a polycross half-sib (HS) mating design in hemp (obligate out-crosser, relying on wind pollination) breeding has the potential to develop a genetic structure for estimating quantitative genetic parameters. The estimation of these genetic parameters derived from this mating design would offer valuable guidance for the strategic development of effective hemp breeding programmes.

2.7.3 Estimation of genetic gain and methods of improving genetic gain

The breeder's equation, a key application of quantitative genetics in crop improvement (Cobb et al., 2019), is an invaluable tool for predicting selection-based advancements (Rutkoski, 2019). This equation serves as a benchmark to assess breeding strategies' efficiency and estimating the rate of genetic gain (ΔG) resulting from applied selection pressure on a population (Lush, 1937). Understanding this equation is crucial for comprehending the mechanics of plant breeding, as it serves as a widely used reference to compare the effectiveness of different breeding schemes and resource allocation (Jahufer et al., 2002; Moose & Mumm, 2008). Genetic gain can be evaluated per breeding cycle or annually (Gerald et al., 2013; Heffner et al., 2010). The general plant breeder's equation for predicting ΔG (Eq. 6) is presented below Fehr, (1987).

$$\Delta G = \frac{kc\sigma_A^2}{\sqrt{\sigma_p^2}} \tag{6}$$

where, ΔG is the genetic gain, k is the slection intensity, σ_A^2 is the additive genetic variance, σ_p^2 is the phenotypic variance of the parental population, and c is the parental control. The degree of parental control is quantified in c (Gerald et al., 2013). A half-sibling mating with control over one parent gives a value of 0.5, a full-sibling mating with control over both parents gives a value of 1.0, and a clone or selfed seed gives a value of 2.0.

The additive genetic variation in HS families is distributed both among and within families (Nguyen & Sleper, 1983). One-quarter of the variation is found among HS families, while the remaining three-quarters are within families (Hallauer et al., 2010; Jahufer et al., 2021). By utilizing among-family selection alone, only a quarter of the total additive variation is accessed. The genetic variance among half-sib (HS) families (σ_F^2) can be estimated by calculating the covariance among the HS individuals; $cov(HS)$, which provides an estimation of the additive genetic variance (σ_A^2); $\sigma_F^2 = cov(HS) = 1 / 4\, \sigma_A^2$. This estimation assumes that the population follows a diploid random mating equilibrium, with no epistatic variance, and that the parents of the HS families are non-inbred (Falconer, 1981; Nguyen & Sleper, 1983). On the other hand, among- and within-HS family selection (AWF-HS) takes advantage of the within-family variation, allowing for a larger magnitude of genetic gain. AWF-HS selection involves first choosing the top-performing families and then selecting the best individual plants within those families, effectively utilizing the remaining three-quarters of the additive genetic variation contained within HS families (Falconer, 1981; Hallauer et al., 2010; Jahufer et al., 2021; Nguyen & Sleper, 1983).

In addition to the traditional plant breeder's equation for predicting genetic gain, Casler and Brummer (2008) introduced another form of a prediction equation for genetic gain by considering both among HS family selection (Eq. 7) and among- and within-HS family selection (Eq. 8) shown below:

$$\Delta G_{\mathrm{AF-HS}} = \mathrm{k_f c}\frac{{}^{1}\!/_{4}\ \sigma_{\mathrm{A}}^{2}}{\sigma_{\mathrm{PF}}} \tag{7}$$

where: $\Delta \mathrm{G_{AF-HS}}$ is the genetic gain based on selection and random mating of the top performing HS families; k_f is the among family selection intensity; c is the parental control, taken as 0.5 for HS family selection; σ_{A}^{2} is the additive variance; and σ_{PF} is the among-family phenotypic standard deviation.

$$G_{AWF-HS} = k_f c\frac{{}^{1}\!/_{4}\ \sigma_{\mathrm{A}}^{2}}{\sigma_{\mathrm{PF}}} + k_w c\frac{{}^{3}\!/_{4}\,\sigma_{A}^{2}}{\sigma_{PW}} \tag{8}$$

where: ΔG_{AWF-HS} is the genetic gain based on selection and random mating of the top-performing individuals in top-performing HS families; k_f and k_w among- and within-family selection intensity, respectively; c (c = 0.5) is the parental control; σ_{A}^{2} is the additive variance; and σ_{PF} is the among-family phenotypic standard deviation; σ_{PW} is the within-family standard deviation.

Genetic gain is influenced by factors, including the extent of genetic variation present in breeding materials, the heritability of desired traits, the intensity of selection, and the duration needed to complete a breeding cycle. The predicted genetic gain improves with increasing selection intensity (Jahufer et al., 2021). According to the breeder's equation, when the breeder generates and tests a larger number of candidates for selection, while keeping the number of selected candidates constant, it leads to higher selection intensity. This, in turn, enhances the rate of genetic gain (Cobb et al., 2019). Alternatively, selecting fewer parents can also increase selection intensity, but the decision should be based on the breeding program's long- or short-term genetic gain objectives (Bernardo et al., 2006). If selection intensity is increased without increasing population size, this process can reduce genetic diversity in future populations over time (Ehoche et al., 2022; Frankham, 2008; Woolliams et al., 2015; Xu et al., 2017). Therefore, selection is a balancing act that involves applying the appropriate intensity to improve trait mean values in progeny while also considering the need to maintain genetic diversity.

Improving selection accuracy is essential for enhancing genetic gain, as it increases genetic accuracy (Cobb et al., 2019). Selection accuracy pertains to the precision of choosing individuals based on their genetic merit for the specific desired trait (Simm, 1998). Despite being the costliest component in plant breeding, enhancing phenotyping is crucial for improving selection accuracy. In most breeding programs, the most effective approach to enhance genetic gain is to improve the representation of the desired population of environments by increasing the number of locations where yield trials are conducted. In other words, by expanding the range of environments in which the trials take place, breeders can obtain a more comprehensive understanding of how a particular trait performs under different conditions, thereby increasing the reliability of genetic evaluations and selection for desired traits (Cobb et al., 2019). Additionally, utilizing agronomic practices with improved precision, such as standardizing protocols, implementing error control, utilizing big data for real-time monitoring, and employing cloud-based tools with satellite imagery, can be employed in genetic research and breeding programs to improve the selection accuracy and consequently, selection genetic gain (Xu et al., 2017).

Among the various parameters in the breeder's equation, cycle time stands out as the easiest to comprehend, least expensive to manipulate, and the most influential factor for enhancing genetic gain. Cycle time, also known as generation interval, involves promptly reintroducing breeding material into the crossing block once a breeder identifies a genotype with above-average breeding value for a specific quantitative trait. Despite its apparent simplicity, careful planning and consideration of breeding strategy are necessary when manipulating cycle time, as a breeder can progress quickly in the wrong direction as they can in the right direction (Cobb et al., 2019). By incorporating integrated breeding approaches like marker-assisted selection and doubled haploid development, breeding procedures can be expedited, resulting in a reduction of the breeding cycle time (Xu et al., 2017).

Enhancing genetic gain in plant breeding involves increasing heritability and exploring a wider range of genetic variation (Voss-Fels et al., 2019). Genetic variation forms the fundamental basis for genetic gain. Currently, only a fraction of available genetic variation has been utilized to maximize genetic gain (Xu et al., 2017). The increase of additive genetic variance and associated heritability can be accomplished through various methods. Molecular and genomic techniques, such as mutation, gene mapping, discovery, transgene utilization, and genome editing, offer the means to unlock and generate beneficial genetic variation from germplasm sources like landraces and wild relatives. This process has the potential to increase the additive genetic variation. Factors like the types of germplasm, their origins, and their relationships with the breeding population contribute to genetic variance, influencing genetic gain. Furthermore, refining field experiments by controlling environmental factors or employing envirotyping techniques enables improved estimation of heritability (Xu et al., 2017), eventually enhancing genetic gain.

Despite the foundational role of quantitative genetics in crop improvement, existing literature indicates that these principles are not fully grasped by crop breeders and scientists (Rutkoski, 2019). This lack of understanding potentially leads to inefficient or unsuccessful breeding programs. While some efforts have been made to estimate genetic parameters in hemp (Carlson et al., 2021; Hennink, 1994), comprehensive research focusing on a wide range of genetic parameters related to hemp breeding is limited. Addressing these gaps could significantly advance the understanding and improve the efficiency of hemp breeding programmes.

2.8 Molecular markers in *Cannabis*: Estimation of genetic variability

The breeding and utilization of *Cannabis* (2n=20), a genus of flowering plants, has resulted in two different types of *Cannabis sativa*, one for fibre and seeds (industrial hemp) and another for medicinal use (medicinal or drug-type *Cannabis* or marijuana) (Flajšman & Ačko, 2022; Soorni et al., 2017). Though these two strains or subspecies are morphologically similar, these types can be differentiated by the type and level of cannabinoids produced. Levels of THC, a psychoactive compound found in leaves and inflorescenes and CBD, an isomer of THC in all plant tissues are being used to distinguish hemp and drug-type *Cannabis* (Hurgobin et al., 2021; Soorni et al., 2017). Hemp produces low levels of THC while drug-type varieties possess relatively higher level of THC (Broseus et al, 2010; Hillig, 2005). However, growing conditions can affect THC levels so it is possible that cultivars may exceed the THC levels of hemp, due to conditions during growing, thus changing the status of the crop from legal to illegal. Drug-type varieties also must be constantly selected to maintain its high THC levels and will revert to low levels without such intervention (Merfield, 1999; Dufresnes et al., 2017). Therefore, molecular markers have been extensively used by scientists to differentiate the hemp and drug-type varieties (Forlani & Petrollino, 2021; Marcińska et al., 2022; Yamamuro et al., 2021).

The majority of current *Cannabis* genomics and transcriptomics work is aimed at: the identification of sex determining coding regions (Hesami et al., 2020; Mandolino et al., 1999; Mandolino et al., 2002; Sakamoto et al., 1995; Sakamoto et al., 2005), forensic investigations on the drug-type *Cannabis* (Cascini et al., 2019; Cisana et al., 2022; Gilmore & Peakall, 2003; Gilmore et al., 2003), DNA typing and genetic relatedness analyses (Alghanim & Almirall, 2003; Hesami et al., 2020). Also, this work is used for the development of molecular markers to differentiate hemp and marijuana genotypes along with determining genetic structure and divergence through domestication (Hakki et al., 2007; Lynch et al., 2016; Sawler et al., 2015).

As per Salentijn et al. (2015), the following genetic aspects of hemp make it necessary to investigate the genetic structure and variability within and among hemp cultivars: (1) hemp is an open pollinated and outcrossing crop, which creates high genetic variation and heterozygosity, (2) there is high genetic differentiation within varieties, even in female inbred lines and (3) hemp has limited cultivar boundaries with a widely shared gene pool. Various molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat amplification (ISSR) have been used to assess the genetic variation in *Cannabis* strains (Hesami et al., 2020; Kojoma et al., 2002; Varshney et al., 2005; Sarwat et al., 2012).

Although a variety of markers are employed to evaluate genetic variation, each type has its own advantages and disadvantages. Restriction Fragment Length Polymorphism (RFLP) markers, for instance, have several merits: they are co-dominantly inherited, moderately polymorphic, are well-distributed across the genome, and allow for the simultaneous screening of multiple samples (Agrawal & Shrivastava, 2013; Arti et al., 2018). Nonetheless, RFLP markers also present several challenges. They necessitate high-quality DNA, and the method is time-intensive, relatively costly, and technically complex, requiring considerable labour (Agrawal & Shrivastava, 2013; Fairbanks & Andersen, 1996). Moreover, it is worth noting that only a subset of these markers may exhibit polymorphism (Agrawal & Shrivastava, 2013; Amiteye, 2021).

Random Amplified Polymorphic DNA (RAPD) markers offer several advantages: they are efficient and quick to analyze, requiring only small quantities of DNA, typically between 5 to 50 nanograms per reaction (Babu et al., 2021; Singh et al., 2010). Additionally, RAPD markers are highly abundant and randomly distributed across the genome and eliminate the need for specific sequence data in primer construction (Agrawal & Shrivastava, 2013; Grover & Sharma, 2016). However, RAPD markers have notable limitations. Their reproducibility is low within and between laboratories (Schierwater & Ender, 1993; Singh et al., 2008). They are also not locus-specific cannot be used across populations nor across species (Singh et al., 2008). Furthermore, RAPD markers exhibit dominance, and fragments of similar size may not actually be homologous (Powell et al., 1996; Singh et al., 2008).

Amplified Fragment Length Polymorphism (AFLP) markers are known for their enhanced yield, high reproducibility, resolution, and sensitivity at the whole genome-wide level (Agrawal & Shrivastava, 2013; Mba & Tohme, 2005). They are capable of amplifying between 50 and 100 fragments at one time (Agrawal & Shrivastava, 2013). One of the key advantages of AFLP markers is that they do not require prior sequence information for amplification (Brugmans et al., 2003). Despite these benefits, AFLP markers have certain drawbacks. They are labour-intensive and expensive to use and require high-quality DNA (Holliday et al., 2019; (Muñoz-Colmenero, 2018). While these markers are mainly scored as dominant, it can be challenging to distinguish between different alleles (Al-Samarai & Al-Kazaz, 2015) and have medium reproducibility (Agrawal & Shrivastava, 2013). AFLP requires purified and high molecular weight DNA. The polymorphism observed in AFLP markers is often due to point mutations occurring at restriction sites (Singh et al., 2008).

Inter simple sequence repeat (ISSR) markers, recognized for their high polymorphism and robustness (Sarwat, 2012), are effective even with low DNA quantities (Agrawal & Shrivastava, 2013) and are randomly distributed across the genome (Singh et al., 2008). However, they are not without challenges, particularly concerning the non-homology of fragments (Arti et al., 2018) and issues with reproducibility (Sarwat, 2012). Given that ISSR is a multilocus technique, there is a risk of misinterpreting genetic similarity estimates. This risk stems from fragments of identical size and mobility, which may originate from nonhomologous regions, leading to potential inaccuracies in genetic similarity estimates (Agrawal & Shrivastava, 2013).

Single nucleotide polymorphism (SNP) markers are notable for their abundance and genetic stability, and ideal for high-throughput analysis (Gupta et al., 2008; Thomson, 2014). SNP markers are robust in nature, allows clear identification of polymorphisms, and they are compatible with a variety of detection methods (Singh et al., 2008). These markers are suitable for automation and high throughput detection formats and platforms (Mammadov et al., 2012). Despite these advantages, SNP genotyping still presents significant challenges. It necessitates specialized equipment and is reliant on the availability of advanced, costly technology (Agrawal & Shrivastava, 2013). Development of SNP markers require time and cost investment (Khlestkina & Salina, 2006) and requires detailed sequence information (Singh et al., 2008). Moreover, Employing SNP markers, which are bi-allelic, could lead to less discriminative power compared to, for example, the use of multi-allelic SSRs, particularly when the number of markers remains unchanged (Arca et al., 2021; Hamblin et al., 2007; Laval et al., 2002).

Different types of markers each exhibit distinct strengths and weaknesses, thereby influencing their applicability in various genetic diversity research studies. Common limitations of above mentioned markers include issues such as poor repeatability and dominance. However, within *Cannabis* breeding programs, microsatellite markers stand out for their exceptional value. They have proven to be more effective than other markers typically used in genetic diversity research, highlighting their significance in this specific domain (Borin et al., 2021; Kovalchuk et al., 2020).

2.8.1 Estimation of genetic variability in *Cannabis* using microsatellite markers

Microsatellite markers, also known as Simple Sequence Repeats (SSRs), are the most useful markers with short repeats of DNA sequences that consist of one to six nucleotide repeats (Maria et al., 2015). These markers have greater utility than many other marker types, as they are highly polymorphic (Zhang, 2004). In addition to this, these markers are abundant, distributed throughout the entire genome, including both coding and non-coding regions, and are highly informative (Miah et al., 2013; Vieira et al., 2016). These properties make microsatellite markers one of the best DNA markers for assessing and clarifying genetic diversity within a species (Yulianti et al., 2022).

Only small-scale microsatellite markers were reported in *Cannabis* (Chandra et al., 2011) before the complete genome and transcriptome sequence was published in 2011 (van Bakel et al., 2011). Microsatellite studies were the first to reveal specific features of the

Cannabis sativa genome (Onofri & Mandolino, 2017). First, microsatellite loci comprising a simple sequence repeat motif of six bp (CACCAT) in *Cannabis sativa* were isolated by Hsieh et al. (2003), with variation in the repeat unit length from 3 to 40. Again in 2003, Alghanim & Almirall (2003) identified GA/CT as the most prevalent motif in the *Cannabis* genome representing ~50% out of a total of eight different microsatellite repeats identified. This study also developed eleven polymorphic microsatellite markers, and a total of 52 alleles were detected, averaging 2.4 effective alleles per locus. The observed heterozygosity of the eleven loci ranged between 0.152 and 0.727, with an average of 0.529. Another study conducted by Gilmore et al. (2003) detected 79 alleles by profiling 93 *Cannabis* samples representing nine drug and six fibre accessions, using five microsatellite loci. This study revealed that the diversity among fibre accessions was higher than among drug-type accessions. This study also showed that the contribution of within individuals/accession variation to the total genetic variance was 73%, and the genetic differentiation among the fibre and drug crops accounted for only 6% of the total variance, compared with an average of 21% difference among accessions.

Mendoza et al. (2009) applied a single-reaction sixplex microsatellite tool to 98 *Cannabis* samples (14 hemp and 84 marijuana) and successfully differentiated each sample as a distinct individual. This study also detected 29 alleles, averaging 4.8 alleles per locus. Twenty-two genotypes of hemp were analysed with 16 microsatellite markers by Maria et al. (2015) and the number of alleles ranged from one to seven alleles with an average 4.75 per locus. Six microsatellite markers suitable for identification of industrial hemp (ANUC203, ANUC205, CAN0039, CAN0093, CAN0110 and CAN2913) and one uniform marker (CAN1690B) were detected in this study of genetic variability.

Dufresnes et al. (2017) analysed intra- and inter-population variation at 13 published STR markers in more than 1300 *Cannabis* samples, including 48 hemp accessions (fibre). This study showed that inter-variety diversity was relatively smaller among hemp accessions but featured higher diversity within varieties. The homogenous gene pool of hemp accessions identified in this study also suggested more frequent cross-breeding compared to drug varieties. Zhang et al. (2020) estimated the genetic diversity and population structure of 199 *Cannabis* germplasm resources from 20 regions in China using 59 SSR markers. They identified 310 alleles, with allele frequencies ranging from 0.26 to 0.85. The expected heterozygosity varied from 0.28 to 0.81, averaging at 0.56. Additionally, population structure analysis revealed two subgroups within the germplasm collection:

one consisting primarily of foreign accessions and the other composed exclusively of Chinese accessions. Borin et al. (2021) developed 20 highly informative loci and assessed them in 104 individuals from 11 different hemp varieties. These markers effectively measured homozygosity, genetic uniformity, and genetic diversity within and between varieties. By analyzing the population structure, they identified eight distinct genetic groups that clustered individuals based on their sexual behaviours (dioecious and monoecious) and geographical origins.

Until recently, the genetic diversity of hemp remained under-investigated partly due to complicated regulations of *Cannabis* imposing restrictions even for conducting scientific research. While several studies using microsatellite markers have been conducted in recent years (Borin et al., 2021; Dufresnes et al., 2017; Zhang et al., 2020), more investigations on genetic diversity among and within hemp cultivars bred for fibre and/or for seeds need to be carried out, and there is a crucial need for a reference database providing information on diversity within and among hemp varieties. Knowledge of genetic diversity and relationships among hemp cultivars commonly grown in New Zealand may play a significant role in breeding programs and give beneficial information to plant breeders as a selection tool.

2.9 Genotype × Environment interaction of industrial hemp cultivars

Environmental factors have a greater effect on plant breeding, as the phenotype of an individual is controlled by its genotype, and the relative performances of genotypes differ among the environments (Falconer & Mackay, 1996; Fehr, 1991; Moose & Mumm, 2008). Breeders and geneticists continually strive to broaden the genetic base of crop species to prevent challenges associated with genetic vulnerability (Brown, 1983; Ceccarelli et al., 2010; Cooper et al., 2001; Kang, 1997; Kashyap et al., 2022; Smith et al., 2015; Stander, 1993). According to Falconer and Mackay (1996) and Fehr (1991), the performance of a particular genotype that is superior in one environment might be poorer in another environment due to different climatic factors confronted at different sites and/or years. These differential genotypic expressions across environments are referred to as Genotype × Environment interaction (G × E interaction) (Ceccarelli et al., 1994; de Leon et al., 2016; Falconer & Mackay, 1996; Fehr, 1991; Hayward et al., 2012; Uchendu et al., 2022). Understanding the impact of G × E interaction, the influence of diverse environments on its development, and the associated challenges and opportunities in plant breeding are crucial for designing effective breeding programs (Annicchiarico, 2002; Katsenios et al., 2021; Li et al., 2021). Plant breeders need to assess how genotypes perform across various environments and the extent of G × E interaction. This is important because the full potential of cultivars, as determined by their genetic makeup, can only be realized within specific environmental contexts.

Multi-environments trials that explore the G × E interaction benefit both fitness (yield in the case of a crop) of current cultivars in new target environments and the ability to achieve stability of commercially important traits (Campbell et al., 2019). These trials are important in plant breeding since they enable the breeder to identify cultivars with broad and specific adaptations. Cultivars with broad adaptability can cope with the environment's uncertainty and show stable performance across diverse environments (Hayward et al., 2012). Cultivars with specific adaptation show improved agricultural production in marginal environments and avoid the process of fitting the cultivars to an environment by altering the environment by adding fertilizer, water and pesticides (Ceccarelli, 1994). Plant breeding programmes and cultivar evaluation trials typically involve conducting experiments across multiple locations and years. By conducting trials in different locations over multiple years, breeders can understand the influence of G × E interactions and improve the selection accuracy by reducing the confounding effects (Byth, 1981; Cooper & Byth, 1996). The estimation of G × E allows for the quantification of variances related to Genotype × Location, Genotype × Year, and Genotype × Location × year interactions (Atlin et al., 2011; Van Eeuwijk et al., 2016). G × E interactions occur in two forms. Genotypes differ in performance across environments in the first form, but their rankings remain consistent. This allows for predictable performance across environments. In the second form, genotype rankings change depending on the environment (Bernardo, 2002). Unreliable performance predictions across environments arise when significant G × E interactions result in the re-ranking of entries (Cooper & Byth, 1996). Evaluating genotypes in multiple environments with proper randomization and replication is the most effective approach to address this issue. However, this method is often costly and time-consuming (Moose & Mumm, 2008).

According to Legros et al. (2013), genotype and environment have substantial effects on hemp fibre and seed production. Research conducted with 14 commercial hemp cultivars in four contrasting European environments proved that the variation of stem (fibre) and seed yield among genotypes, mainly due to differences in flowering time, which is controlled by environment, temperature and photoperiod (Tang et al., 2016). According to Petit et al. (2020a), hemp fibre quality traits, including pectin-related monosaccharide content and fitness traits like fineness and decortication efficiency, show variability across different environments due to significant G × E interaction. The authors suggest that this phenotypic variation influenced by G × E interactions could be attributed to "plastic genetic systems," where specific genes are expressed in response to particular environmental conditions. Earlier studies by Struik et al. (2000) and Tang et al. (2016) also examined G × E interactions in hemp, specifically in the context of fibre yield and fibre characteristics.

There has been limited exploration of the G × E interaction effect on grain and total biomass yield in hemp using multivariate statistical methods (Ferfuia et al., 2021). Campbell et al. (2019) revealed that traits such as hemp seed yield, plant height and water use are strongly influenced by the environment and G × E interaction. The study also showed that individual cultivars exhibited very diverse degrees of sensitivity to the environment. In their study, Ferfuia et al. (2021) demonstrated the significance of G × E interaction and its substantial impact on various quantitative traits of agricultural significance in hemp, including seed yield, biomass, seed weight, and seed oil content. The researchers highlighted the potential role of G × E interaction in causing yield instability, particularly in seed yield, and suggested that it could be a contributing factor to the inconsistency observed in seed yield across different environmental conditions. In their three-year field trials of industrial hemp in Greece, Irakli et al. (2019) observed that hemp seeds' nutritional and phytochemical profile, including the total antioxidant activity, is influenced by both genotype and growing year. The study emphasized that genetic factors primarily affect the protein and oil contents and the fatty acid composition, which are important nutritional components of hemp seeds. Conversely, the total phenolics, tocopherols, carotenoid contents, and antioxidant activity were predominantly influenced by the growing year and genotype.

The interaction between genotype and environment also contributed to the variability in cannabinoid production, specifically in terms of THC and CBD percentages (de Prato et al., 2022). While previous studies (de Meijer et al., 2003; Campbell et al., 2019) have suggested that the biosynthesis of cannabinoids is predominantly regulated by genetics, recent research by Yang et al. (2020) and Mostafaei Dehnavi et al. (2022) has indicated that the cannabinoid content in industrial hemp accessions can vary across different environmental conditions.

Since no hemp germplasm was conserved or maintained through the long-spanning prohibition for growing hemp in New Zealand, trials on unacclimatized cultivars from abroad was a logical initiation for reintroducing hemp. Considering that most offshore cultivars (tested in 2001) showed low productivity, Donald James MacIntosh was involved in creating cultivars specific to New Zealand environments. This led him to develop five approved cultivars, Aotearoa 1 and the four A1 series (A1 Monopurp, A1 MACMONO, A1 COMSEED, and A1 HCFX) (McIntosh, 2021). The environmental variations throughout New Zealand and the lack of available information on hemp cultivars impose challenges on farmers in choosing suitable cultivars to achieve the maximum yield outputs. Furthermore, including traits exhibiting significant G × E interaction in breeding programmes may introduce biased selection outcomes due to the uncertainties surrounding their performance in untested environments (Petit et al., 2020a). Hence, it is essential to conduct evaluation trails developed for different environments in view of the fact that cultivars are commonly bred for specific environments and cropping conditions. Since New Zealand lacks studies on G × E interaction in hemp, conducting multi-environment trials with various hemp cultivars could provide valuable insights. These findings would enable breeders to design more efficient breeding programs.

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CHAPTER 3

Evaluation of the performance and potential of industrial hemp (*Cannabis sativa* L.) cultivars across two environments and years in the North Island of New Zealand

Abstract

Industrial hemp (*Cannabis sativa* L.) has recently reemerged as a globally valued multipurpose crop, a trend that includes New Zealand, where it had been prohibited since the early 20th century. Despite recent legislative reforms that have relaxed restrictions and enabled commercial hemp cultivation, New Zealand still faces challenges in growing hemp due to a lack of locally adapted cultivars. The country remains reliant on offshore-bred material, as there are few cultivars tailored to its unique climate. Also, information on the performance of approved cultivars in New Zealand is limited. Despite these challenges, local growers are interested in reinvigorating hemp cultivation. Genotype × Environment (G × E) interactions significantly influence the expression of key agriculturally important traits in hemp. Although hemp is a versatile crop adaptable to diverse climates and soils, its yield can be inconsistent, largely because of its sensitivity to environmental changes. Results from multisite trials that evaluate the performance of different cultivars will provide information that will help identify specific or broad adaptation across environments. Based on the importance of estimating the magnitude and significance of G × E interaction associated with New Zealand's emerging hemp industry, a preliminary study was conducted to, (1) analyse and compare yield components (biomass and seed), seed quality traits and other agronomic traits that are important in terms of industrial use within a set of imported hemp cultivars currently used in New Zealand, and (2) investigate the effects of genotype and environment on hemp seed and biomass production by characterizing the type and magnitude of G × E interaction estimated from multisite industrial hemp trials conducted across two years in the North Island, New Zealand. Two two-year field trials were conducted during the 2019/2020 and 2020/2021 growing seasons to evaluate six of New Zealand's 20 approved hemp cultivars: CFX-2, CRS-1, Férimon 12, Katani, Futura 75, and Finola. Experiments during the 2019/2020 growing season were conducted at two different locations in the North Island: Palmerston North and Wairarapa. During the 2020/2021 season, experiments continued at two sites in Palmerston North, with one being irrigated and the other rainfed, each featuring distinct soil characteristics. The same set of cultivars was evaluated in both growing seasons. The analysis of genotypic differences among the six hemp cultivars for all the traits studied, generated results that generally align with those published from experiments conducted in different overseas environments. Yields varied significantly, with biomass ranging from 1.43 to 28.41 t/ha and seed yield varying between 0.018 to 3.78 t/ha. The monoecious cultivars, Futura 75 and Férimon 12, consistently outperformed other cultivars across diverse environments and appear well-suited for dual-purpose production. The G × E interactions observed in this study, based on the performance of the six evaluated cultivars, present a complex narrative that contrast with the findings from other studies. While most traits showed genotypic variation, only a few, specifically stand establishment in the 2019/2020 season, along with plant height and stem diameter in the 2020/2021 season, and biomass yield across both years, revealed significant G × E interactions. The traits of plant height, stem diameter, biomass yield, seed yield, and thousand seed weight all exhibited high line mean broad sense heritability, suggesting the presence of exploitable genetic variation that could be used to generate breeding pools for cultivar development. Overall, outcomes from this study serve as a valuable foundation for shaping future hemp breeding programs in New Zealand, offering initial insights into the development of superior, stable, and uniform cultivars that are well-adapted to the country's unique climatic conditions.

Keywords: Industrial hemp (*Cannabis sativa* L.), cultivar, Genotype × Environment interactions, genotype, environmental factors, growing year

3.1 Introduction

The interest in industrial hemp (*Cannabis sativa* L.) as an ideal multipurpose crop is increasing worldwide. Hemp is a high-yielding crop that requires little technical input and impacts positively on the environment (Amaducci et al., 2015; Campiglia et al., 2017; Finnan & Styles, 2013; Tang et al., 2016). Hemp holds potential for numerous industries for its fibre, seeds, and medicinal compounds (Crini et al., 2020; Rehman et al., 2021) and as a feedstock for bioenergy (Parvez et al., 2021). Its stem contains high-quality cellulose (de Meijer, 1994), the seed contains high-quality oil and protein (Callaway, 2004a), and the inflorescence contains valuable resins (Bertoli et al., 2010). Hemp cultivars can be classified according to different attributes including (1) plant-use as fibre cultivars (for long fibres or pulp), seed cultivars, dual-purpose (both fibre and seeds) and high-cannabidiol (CBD) hemp cultivars (Schluttenhofer & Yuan, 2017; Stramkale et al., 2023; Toth et al., 2021); (2) flowering time, which includes early ripening, intermediate-ripening and late-ripening cultivars (de Meijer, 1994; Ranalli, 2004; Salentijn et al., 2015); (3) reproductive biology, whether they are dioecious or monoecious cultivars (Punja et al., 2017; Ranalli, 2004; Salentijn et al., 2015) and (4) breeding region/origin (Borin et al., 2021; Forapani et al., 2001). Hemp is primarily grown for its fibre and seeds. However, maximising profits often involves using both for various purposes (Tang et al., 2016; Van der Werf, 1997). Different hemp cultivars exhibit distinct characteristics, making them better suited for specific end products (Tsaliki et al., 2021).

Cultivars of crops are grown under varying conditions, such as diverse soil types, fertility, moisture, temperatures, and management practices, collectively constituting the environment. Consequently, a cultivar's performance can differ depending on the environment (Annicchiarico, 2002; Fehr, 1991); one might yield the best in a particular environment, while another might be better elsewhere. This discrepancy in performance across environments is termed the Genotype × Environment interaction (G × E interaction), the relative change in their performance across environments (Byth, 1981; Fehr, 1991; Ferfuia et al., 2021; Kang, 1998; Knight, 1970; Jahufer et al., 2002). The plant phenotype is influenced by its genotype (G), the surrounding environment (E), and their combined effect (G × E interaction) (Amiruzzaman et al., 2011; Dosho et al., 2022; Ferfuia et al., 2021; MacRobert et al., 2014). The influence of genes on the variation in quantitative traits may differ across different environments (Cooper et al., 1993; Jahufer et al., 2002). While the influence of genotype remains relatively stable because of the cumulative actions of many genes, the environment, fluctuating across locations and time, has a pronounced impact on phenotypic behaviour (Ferfuia et al., 2021). Therefore, the yield of a genotype can differ depending on the environment. Such variations are attributed to both G × E interaction and the direct influence of the environment. Stable genotypes with broad adaptation, which remain relatively consistent across fluctuating conditions, often show comparable performance in diverse environments (Annicchiarico, 2002; Becker & Leon, 1988; Carbonell et al., 2004; Ferfuia et al., 2021). Genotypes that are specifically adapted perform well in a particular set of environments within a target region (Annicchiarico, 2002).

With higher variability of climatic conditions, the primary goal for plant breeders, crop producers and agronomists is to achieve high and consistent yields (Abou-Khater et al., 2022; Brummer et al., 2011; Ceccarelli & Grando, 1996; Ortiz et al., 2008). Every genotype's response to these climatic conditions is vital for understanding and ensuring yield and crop performance stability. Still, the best-performing genotypes are emphasised when recommending specific cultivars for planting (Annicchiarico, 2002). In the long term, a well-planned breeding strategy leads to improved crop adaptation and steady yields. In the short term, the focus is on choosing the best crop cultivars, whether local or imported, and either traditional or released from private or public breeding institutions. The decisions related to the breeding approach and crop cultivar choices should be based on scientific understanding of the plant's interaction with growing environments in the target region (Annicchiarico, 2002). Multi-environment (multisite) trials help determine and reduce the confounding effects of G × E interactions (Annicchiarico, 2002; Campbell et al., 2019; Fehr, 1991; Yan & Kang, 2002). Quantifying the magnitude of G × E interactions helps determine if the observed crop features are due to inheritable genetic factors or environmental conditions. Partitioning of phenotypic trait variation into their specific variance components is an essential preliminary step in cultivar development programs (Campbell et al., 2019).

In recent years, hemp has regained global attention as a prominent crop, including New Zealand, which has faced prohibitions since the early 20th century (McIntosh et al., 1998; McPartland et al., 2004). New Zealand's longstanding legal restrictions resulted in limited availability of local genetic resources (McIntosh et al., 1998). Subsequently, even as legislative action relaxed the legal prohibition and made hemp a commercial crop, there remained challenges to its production due to the low availability of local cultivars (Marsh, 2020; McIntosh et al., 1998; McPartland et al., 2004; Moore et al., 2021; Venture Taranaki, 2022). New Zealand's reliance on offshore hemp cultivars persists due to the limited availability of cultivars specifically developed for its climatic conditions. Upon expanding the area of hemp cultivation, introduced offshore cultivars may perform poorly due to a difference between the new genotype and the environmental conditions (Hammami et al., 2022; Wojczynski & Tiwari, 2008). While New Zealand's climate is suitable for hemp growth, there is limited data on the productivity of recently registered cultivars. Yet, many New Zealand growers are keen on introducing hemp cultivation, and according to the New Zealand Industrial Hemp Association (NZIHA), the New Zealand industrial hemp market is projected to be a 2-billion-dollar industry by 2030 (Marsh, 2020).

In hemp, it is widely recognised that biomass and seed yield are significantly influenced not only by genotype but also by environmental factors and G × E interactions (Ferfuia et al., 2021; Legros et al., 2013; Tang et al., 2016; Tsaliki et al., 2021), the impact of which often varies depending on the origin and breeding environment of the specific cultivar (Ferfuia et al., 2021). G × E interaction plays a significant role in hemp, exerting a relatively strong influence on the expression of key agricultural traits like seed yield, biomass, seed weight, and seed oil and protein content (Aubin et al., 2016; Baldini et al., 2020; Campbell et al., 2019; Irakli et al., 2019; Petit et al., 2020). As mentioned earlier, hemp performance in the field can vary due to interactions between genotype and climatic conditions, as well as interactions between genotype and crop management techniques, both of which are considered environmental factors. Key crop management techniques influencing biomass and seed yield and quality include sowing date (Sengloung et al., 2009; Visković et al., 2023), harvesting date (Amaducci et al., 2008; Hoppner & Mange-Hartmann, 2007), plant density (Amaducci et al., 2008; Tang et al., 2016), fertiliser application (Struik et al., 2000; Tang et al., 2016), and irrigation regime (Campbell et al., 2019). Multisite variety trials in hemp conducted over different years are often used to assess the suitability of cultivating a multipurpose crop in a specific location (Baldini et al., 2020). Longstanding prohibition in many countries, including in New Zealand, has led to a lack of expert knowledge in hemp cultivation. The limited scientific insight into the strong interaction between genotype and environment continues to challenge hemp cultivation, impacting both seed and biomass yield and quality (Blandinières & Amaducci, 2022). In New Zealand, there are no published studies on G × E interactions in hemp to date.

In essence, while hemp is a versatile crop that can thrive in various climates and soil types, its yield can be inconsistent, largely because of its sensitivity to environmental changes. As a consequence, there is significant importance in identifying cultivars that are both stable in a range of environments and widely adaptable. Effective management practices combined with these cultivars can reinforce production and acreage. Multisite trials are essential in this regard. By evaluating different cultivars using multisite trials, breeders and agronomists can understand the complex dynamics of G × E interaction. Also, this helps in identifying genotypes that perform well across various environments. Recognising the significance of G × E interaction studies as the initial step to choosing or breeding suitable hemp cultivars for new environments in New Zealand, this study aimed

(1) to analyse and compare yield components (biomass and seed), seed quality traits and other agronomic traits that are important in terms of industrial use within a set of hemp cultivars from Canada, Finland and France and (2) to explore the effects of genotype and environment on hemp seed and biomass production by characterising the type and magnitude of G × E interaction estimated from multisite industrial hemp trials conducted across two years in the North Island, New Zealand.

3.2 Materials and methods

3.2.1 Plant material

Out of the 20 approved cultivars in New Zealand, six cultivars were chosen for field trials that were conducted over two growing seasons (growing seasons 2019/2020 and 2020/2021). The list of approved cultivars in New Zealand is presented in Table 3.1.

Table 3.1. Approved cultivars of industrial hemp in New Zealand (MoH, 2023).

No.	Approved Cultivars	Date Approved	Breeding region
1	A1 HCFX	22 September 2021	New Zealand
2	Canda	31 July 2020	Canada
3	Joey		Canada
4	A1 COMSEED	30 June 2020	New Zealand
5	A1 MACMONO		New Zealand
6	A1 Monopurp	26 February 2019	New Zealand
7	Sirius		
8	Katani	2 November 2018	Canada
9	CFX-1	27 November 2014	Canada
10	CFX-2		Canada
11	CRS-1		Canada
12	Futura 75		France
13	Fedora 17	25 March 2010	France
14	Férimon 12	15 October 2009	France
15	Aotearoa 1	31 July 2008	New Zealand
16	Anka	7 September 2006	Canada
17	Finola		Finland
18	Fasamo		Germany
19	Kompolti		Hungary
20	USO31		Germany

A diverse group of cultivars was selected for the study based on; origin, cultivar type (monoecious and dioecious), and end-use. The final selection of cultivars was CFX-2, CRS-1, Férimon 12, Katani, Futura 75 and Finola. The details of the selected cultivars are given in Table 3.2.

Table 3.2. Characteristics of selected cultivars for the experiments.

Variety	Breeding region	Reproductive Biology (Cultivar type)	Purpose
CFX-2	Canada	Dioecious	Seed
CRS-1	Canada	Dioecious	Seed
Férimon 12	France	Monoecious	Seed & fibre
Katani	Canada	Dioecious	Seed
Futura 75	France	Monoecious	Fibre
Finola	Finland	Dioecious	Seed

3.2.1.1 Sources of planting material and germination tests prior to planting

Seeds of five of the six cultivars, CFX-2, CRS-1, Férimon 12, Katani and Futura 75 were obtained from Midlands Seed Limited, and Finola seeds were obtained from Hemp Connect, New Zealand. Germination tests were carried out prior to planting in both years according to ISTA (International Seed Testing Association) (2018) protocols to adjust the seed sowing rates. The results are presented in Appendix 3.

3.2.2 Site description

3.2.2.1 Site description for the field trials planted in 2019/2020

The field experiments were conducted at two different sites in the North Island of New Zealand in the growing season 2019/2020 (Figure 3.1A). The trial sites were located at the Plant Growth Unit (PGU) Orchard, Massey University in Palmerston North (40° 22' 40.656" S 175° 36' 48.6936" E) and at the Moiki farm, Bidwells Cutting, South Wairarapa (41° 10' 10.9848" S 175° 28' 1.4226" E). These two sites were selected as they represented typical commercial hemp production environments.

Soil characteristics and soil nutrient status at each experimental site were obtained by soil sample analysis before planting. Four corners of the field are determined, and sampling was done diagonally. These soil samples were taken at a depth of 15 cm. The measured soil characteristics are presented in Table 3.3. Climatological data: Monthly mean maximum temperature (Tmax), minimum temperature (Tmin) and the total rainfall during the growing season were obtained from the National Climate Database via the nearest observing authority, "NIWA/AgResearch", in Palmerston North. The nearest weather stations were accessed based on the location of the Plant Growth Unit at Palmerston North and the Moiki farm.

Table 3.3. Soil characteristics at the two trial sites – 2019/2020.

Soil characteristic	Level found at the site in Palmerston North	Level found at the site in Wairarapa
pH	6.0 pH unit	5.9 pH units
Olsen Phosphorus	50 mg/L	37 mg/L
Potassium	0.65 me/100g	10 MAF units
Calcium	8.5 me/100g	14 MAF units
Magnesium	1.62 me/100g	31MAF units
Sodium	0.07 me/100g	6 MAF units
Cation Exchange Capacity	15 me/100g	19 me/100g
Total base saturation	75%	73 %
Volume weight	0.92 g/mL	0.96 g/mL
Sulphate Sulphur	3 mg/kg	7 mg/kg
Potentially available nitrogen	139 kg/ha	111 kg/ha
Anaerobically mineralisable N	101 µg/g	78 µg/g
Soil type	Manawatū silt loam	Ahikouka silt loam

3.2.2.2 Site description for the field trials planted in 2020/2021

The trials were planted at two sites in Palmerston North, New Zealand (Figure 3.1B). The trial sites were located at the Plant Growth Unit (PGU) Orchard, Massey University (40° 22' 40.656" S 175° 36' 48.6936" E) and the Pasture and Crop Research Unit (PCRU), Massey University (40° 23' 11.5872" S, 175° 36' 27.5904" E). These two sites were 1.03 km apart. The site at the PGU was irrigated (IR site) using a sprinkler irrigation system so that the soil moisture level was maintained above 50% field capacity (FC) throughout the trial period. Moisture sensors (Model EC-5; METER Group, Inc., USA) installed in the trial area were used to determine the FC of the soil. The other site at PCRU was under rainfed conditions (RF site).

Soil characteristics and nutrient levels were determined via pre-planting soil analysis at each site. Baseline data collected on soil characteristics for both sites is presented in Table 3.4. Meteorological data for the IR and RF sites were sourced from The National Climate Database via the nearest observing authority, "NIWA/AgResearch", in Palmerston North. This location is situated equidistantly between both trial sites, at a distance of approximately 511 m from the IR site and 524 m from the RF site. Monthly mean maximum temperature (Tmax), minimum temperature (Tmin) and the total rainfall during the growing season were obtained from the database.

Table 3.4. Soil characteristics at the two trial sites – 2020/2021.

Soil characteristic		Level found at the IR site	Level found at the RF site
pH	pH unit	6.0	5.7
Olsen Phosphorus	(mg/L)	38	24
Potassium	(me/100g)	0.56	0.15
Calcium	(me/100g)	7.2	5.0
Magnesium	(me/100g)	1.54	0.89
Sodium	(me/100g)	0.08	0.12
Cation Exchange Capacity	(me/100g)	13	11
Total base saturation	%	72	55
Volume weight	g/mL	0.97	0.99
Sulphate Sulphur	Mg/kg	3	3
Potentially available nitrogen	kg/ha	92	132
Anaerobically mineralizable N	µg/g	63	89
Soil type		Alluvial soil	Pallic soil
Soil, texture		Manawatū silt loam	Tokomaru silt loam

Figure 3.1. Trial sites in the growing season 2019/2020 (A). Trial sites in the growing season 2020/2021 (B).

3.2.3 Field experiments

3.2.3.1 Field experimental description for the field trials in 2019/2020

The field experiments were laid out in a split-plot design consisting of six varieties and two sowing dates, replicated four times (Figure 3.2). The dimension of each experimental unit was 2.7 m by 6m with 15 cm of constant distance between the rows. Including the border rows, there were 18 rows in an experimental unit. The same experimental design and field layout was established at both sites, with different randomization of the entries. The total trial area at each site was 2178 m^2.

Note: The initial experiment, planted in a split-plot design with six varieties and two sowing dates, aimed to investigate the impact of cultivars, sites and different sowing dates on hemp performance. However, an early harvest of the plants from the second sowing date due to the nationwide lockdown, from March to May 2020, associated with the Covid-19 pandemic meant that only data from the first sowing date could be used. The data collected from the plants from the first sowing date were effectively used to study the performance of the selected six hemp cultivars within and across the two sites.

3.2.3.2 Field experimental description for the trials in 2020/2021

The six cultivars were evaluated under irrigated and rainfed conditions. The trials at both sites were arranged in a randomized complete block design with four replicates as shown in Figures 3.3a and 3.3b. Each plot was 3.0m by 6.0m with 15cm of constant distance between the rows. Plots consisted of 18 rows including border rows. The experimental design and field layout were fundamentally the same for both sites, with different randomization of the entries. The total trial area at each site was 828m^2.

3.2.4 Crop establishment and maintenance

3.2.4.1 Seed rate calculation

Seed rates were calculated for each cultivar for both years using the following equation:

$$\text{Seed rate (kg/ha)} = \frac{\text{Target plant population (plants/m}^2\text{) x 1000 Seed weight (g) x 100}}{\text{\% germination x \% emergence}}$$

(The Foundation for Arable Research, 2018)

Seed weight for packaging was obtained using the following equation.

Packet size = Plot size × target plant population × TSW/1000 × 100/germination × 100/survival

(Seed preparation instructions for trial contributors, 1995)

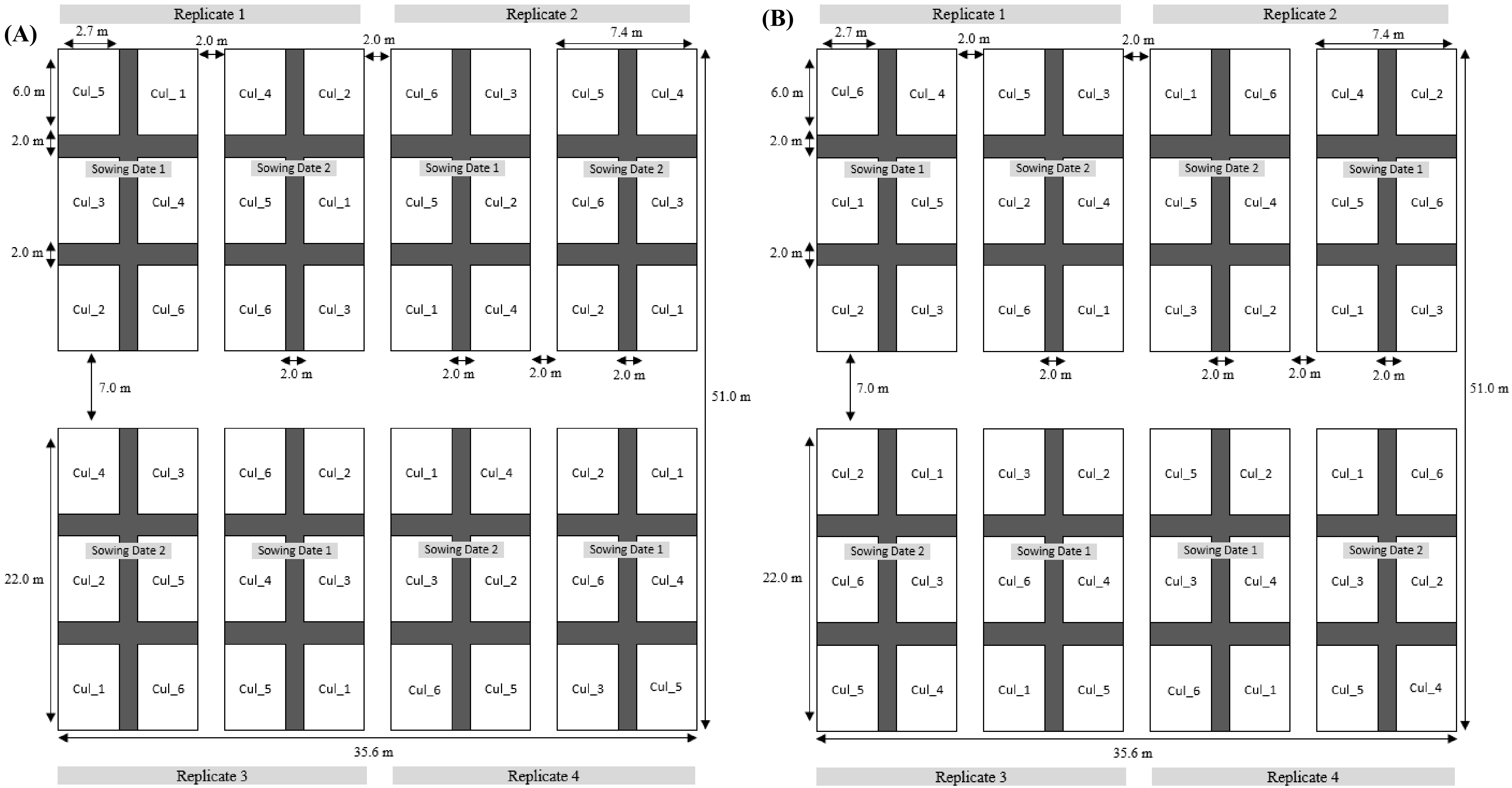


Figure 3.2. Trial design for Plant Growth Unit Massey University, Palmerston North (A), Trial design for Moiki farm, Wairarapa (B).

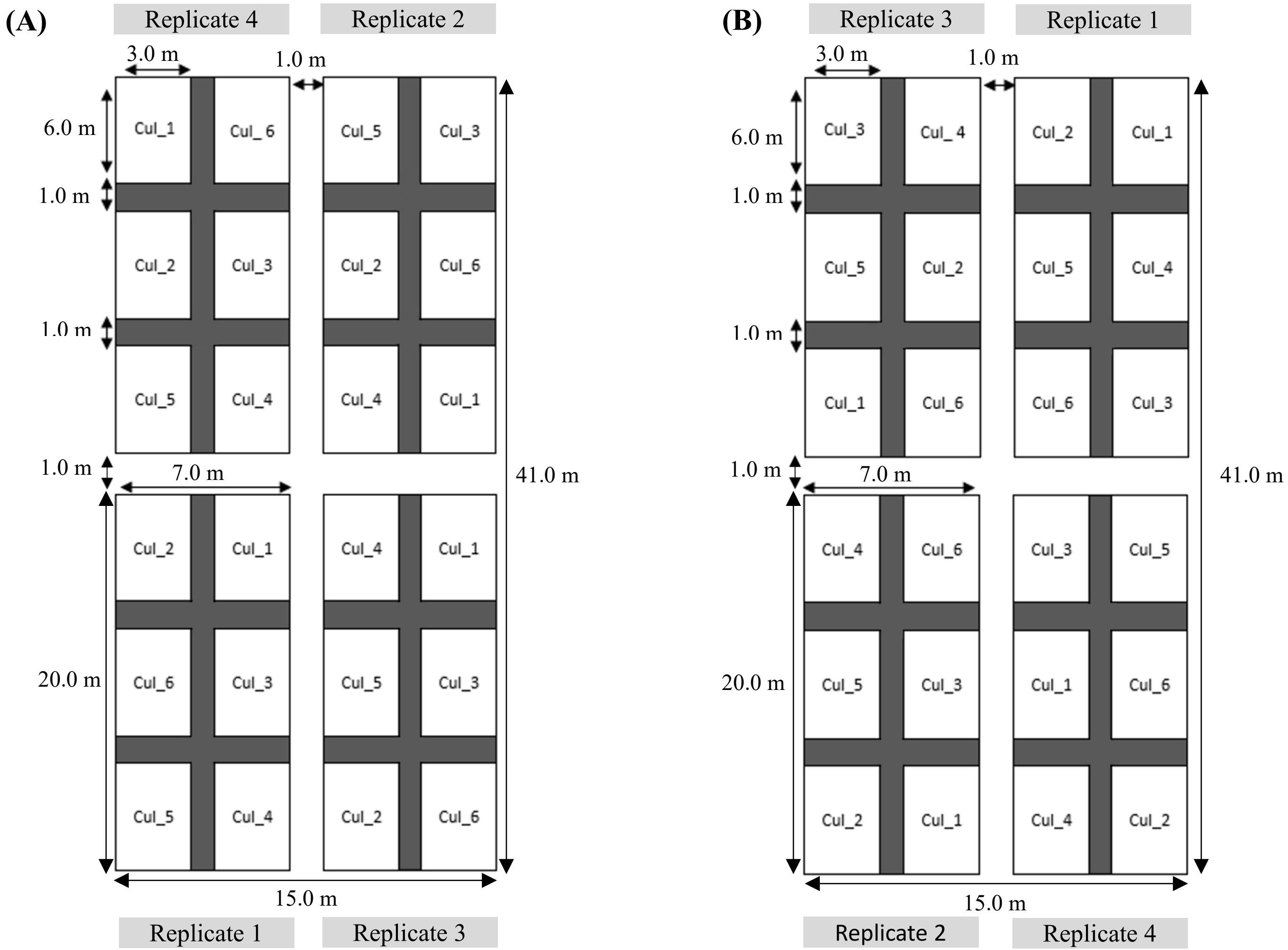


Figure 3.3. Trial design for irrigated site [IR site] (A). Trial design for rainfed site [RF site], (B).

3.2.4.2 Land preparation and sowing

3.2.4.2.1 Land preparation and sowing of the field trials in 2019/2020

The field trial area was ploughed to a depth of 30 cm and the soil was tilled finely with two passes using a disk. The paddock was maintained free of weeds using a disk harrow cultivator at the plant growth unit, Massey University. The paddock in Wairarapa was ploughed to a depth of 30 cm and the last pass was with a power harrow to create an even seedbed.

The first sowing of the trials was on the 27th of November 2019 at Wairarapa and on the 28th of November 2019 at the PGU, Massey University. A TAEGE direct drill with a computerized sponge metering system was used to sow the seeds. The seeds were placed at a depth of 3 cm in the soil to reduce bird predation (McPartland et al., 2004). The target plant population density for seed and dual-purpose cultivars was 125 plants/m^2 and 200 plants/m^2 for the fibre cultivar. Nets were used to cover the paddocks after sowing to avoid birds picking the emerging seedlings.

3.2.4.2.2 Land preparation and sowing for the field trials in 2020/2021

The trial areas at both sites were ploughed and tilled finely with a disk. A disk harrow cultivator was used to break the weeds and clods. At both sites the soil was prepared as a seedbed. The sowing was carried out on 24th November 2020 and sowing methods were the same at both sites. The seeds were directly drilled into the prepared plots using an Oyjord based cone plot seeder at a depth of 3 cm. The target plant population density for the seed and dual-purpose cultivars was 125 plants/m^2 and 200 plants/m^2 for the fibre cultivar. Nets were used to cover the paddocks after sowing. Due to extremely poor germination and seedling establishment at both sites as a result of 106.2mm rainfall from the 25th of November to the 9th of December, the entire trial was re-sowed on 17th December 2020.

3.2.4.3 Fertilizer application and irrigation

3.2.4.3.1 Fertilizer application and irrigation for the field trials in 2019/2020

Fertilizer application rates were calculated based on the soil analysis results of both trial sites presented in Table 3.3. Since base fertility levels were very high at both sites, fertilizer was applied in the third week of the trials. Fertilizer was incorporated to achieve the target fertilizer levels: 150 Kg/ha nitrogen, 80 Kg/ha phosphate and 80 Kg/ha potash

(McIntosh et al., 1998). Irrigation frequency varied depending on the rainfall. The irrigation systems equipped at Wairarapa and Palmerston North were gun irrigation and overhead sprinkler systems, respectively.

3.2.4.3.2 Fertilizer application and irrigation for the field trials in 2020/2021

The application rates for fertilizer were determined using the soil analysis results from both sites, as presented in Table 3.4. Fertilizer was applied in the third week of the trials at each site to achieve the target fertilizer levels: 150 Kg/ha nitrogen, 80 Kg/ha phosphate and 80 Kg/ha potash as McIntosh et al. 1998 recommended. Irrigation was applied as described in section 3.2.2.2.

3.2.4.4 Weed, pest and disease management

3.2.4.4.1 Weed, pest and disease management for the field trials in 2019/2020

Early and thorough ground preparation was done to control weeds to eliminate the use of herbicides. However, at the early growth stages, hemp plants showed an inability to compete with weeds. Many weeds emerged in between the plots (pathways). Even though rapid growth and canopy closure have a competitive advantage over weeds, weed emergence was still noted during the early phases of the cultivation period. Hand weeding at both sites was done to remove weeds between plants and around the plots, and a Jobmate 30cc Petrol Straight Shaft Brushcutter® was used to cut down the weeds between plots.

No diseases were observed throughout the growing period at both sites. During the experimental period, damage caused by pests such as rabbits (*Oryctolagus cuniculus*), brown field slugs (*Deroceras panormitanum*), and birds (predominantly *Passer montanus*) were observed. Preventive measures were taken against the birds and slugs. Bird scarers and bird protective net bags were used to avoid bird damage at the Palmerston North site. Slug bait “Slug out®” was applied immediately after observing slug damage during the seedling stage at the Palmerston North site.

3.2.4.4.2 Weed, pest and disease management for the field trials in 2020/2021

The intensity of weed pressure was quite high at the IR site. Hand weeding was done in between plants. Jobmate 30cc Petrol Straight Shaft Brushcutter® was used to trim the weeds between plots. The plots were kept weed-free by manual weeding at the RF site. No herbicides were used to control the weeds in both sites.

The trials at both sites were completely covered with nets to deter birds from feeding on the mature seeds. Insect pests: budworms and green stink bugs were observed at both sites. Aphids and thrips were observed only in the IR site. Even though these pests were observed, none required pesticides.

Flowering tops and stalks from infection with *Sclerotinia sclerotiorum* were observed in the trial at IR site. However, losses were not regarded as significant. The plants identified with the disease were removed from the field immediately. No diseases were observed throughout the growing period at RF site.

Crop establishment and the growing practices in both years were duplicated at both experimental sites to reduce the variation in terms of agronomic and cultural practices.

3.2.5 Harvesting and sampling scheme

3.2.5.1 Harvesting and sampling scheme for the field trial in 2019/2020

As described earlier, the experimental design at each site was a split-plot design with four replicates, and each experimental unit was 16.2 m^2. An area of 1 m^2 in each plot, discarding four border rows, was utilized at maturity to harvest and measure seed and crop biomass yields. Plants were cut manually at ground level. Harvesting for the first sowing was performed at the seed maturity stage; when bracts began to dehisce, seed colour changed from green to greyish brown, and the first signs of seed scattering were observed (Townshend & Boleyn, 2008). A subsample of cultivar Futura 75 was harvested directly after flowering. Hand harvesting was done at both sites.

3.2.5.2 Harvesting and sampling scheme for the field trial in 2020/2021

The plants from all six cultivars were harvested when the seeds reached the maturity stage. A subsample of cultivar Futura 75 was harvested directly after flowering. The sampling area was 1m^2, leaving four border rows and border plants within the rows. All plants in the sampling area were cut manually above the soil surface at each harvesting time.

3.2.6 Data collection

Each year, the agronomic measurements and seed characteristics were measured. Plant height at harvest, stem diameter, biomass yield, seed yield, and thousand seed weight were measured for ten representative plants from each sampling area.

3.2.6.1 Agronomic measurements

a. Seedling establishment

Seedling emergence is severely affected by environmental factors such as type of soil, soil moisture, light intensity and soil temperature (Gusovius & Lühr, 2017). Also, seedling emergence was observed to be deferred between hemp cultivars (Aubin et al., 2016). Therefore, the seedling establishment was recorded to observe any differences among cultivars and any differences across both sites. The seedling counts were conducted on every plot two weeks after planting, according to Hansen et al. (2017).

b. Stand establishment

Substantial G × E interaction for stand establishment has been observed, with environment being the most influential factor (Campbell et al., 2019; Struik et al., 2010). Therefore, stand establishment was assessed in this study to determine differences among cultivars within and across sites. This measure was obtained by dividing the number of plants standing at harvest by the total seeds planted, then multiplying by 100 (Campbell et al., 2019).

c. Height of the plant at the time of harvest

In hemp, plant height is strongly influenced by genetic and environmental factors (Campbell et al., 2019), such as day length, water availability and soil nutrient availability (Gusovius & Lühr, 2017). Therefore, plant height was measured to characterize differences among cultivars within and across sites. Plant height was measured using a tape measure, from the base of each stem.

d. Stem diameter

Studies showed that there were significant differences among cultivars for stem diameter across contrasting locations in Europe (Tang et al., 2017). Therefore, stem diameter was measured to investigate differences among cultivars within and across sites. Stem diameter was measured in ten representative plants using a vernier calliper at 10cm from the base of each plant, according to Tang et al. (2017).

e. Biomass yield

The biomass of hemp is significantly influenced by environmental factors (Campbell et al., 2019) and genotype variability (Aubin et al., 2016).

Therefore, biomass yield was measured to characterize differences among cultivars within and across sites. Plants were oven-dried at 75℃ for 48 hours and weighed to determine the biomass yield.

3.2.6.2 Seed characteristics

Studies have revealed that there is G × E interaction for seed yield (Aubin et al., 2016). Galasso et al. (2016) reported a significant effect of genotype on thousand seed weight, seed protein and oil content. Moreover, studies have reported variations in seed oil content (Anwar et al., 2006) and seed protein (Galasso et al., 2016) from contrasting locations. Therefore, seed yield, thousand seed weight, seed oil and protein content were chosen to study the effect of different cultivars within and across different environments. In the 2019/2020 season, seeds from Futura 75 were neither collected nor studied for thousand seed weight, seed oil content, and seed protein content because the cultivar is primarily bred and grown for fibre. However, in the 2020/2021 growing season, seeds from Futura 75 were collected, and seed characteristics were assessed across different sites and years to evaluate its potential for dual-purpose use.

a. Seed yield

Inflorescences of representative plants were air-dried, and seeds were separated manually. Sieves were used to remove the large inert matter, and an Oregon seed blower (Hoffman Manufacturing, Inc., Corvallis, OR) was used to remove the smaller leaf and stem particles and empty seeds. The seeds were blown using successive manometer settings of 30 to 60, and the blowing time was five minutes per subsample. The seeds remaining after blowing were weighed to determine the seed yield.

b. Thousand seed weight

Thousand seed weight was determined on five replicates of 100 seeds each. Seeds were manually counted and weighed (Galasso et al., 2016).

c. Seed protein content and oil content

The seed protein content was determined using the ‘Dumas’ procedure in the Association of Official Analytical Chemist method (AOAC 968.06). Seed oil content was determined using a method described by House (1997).

3.2.7 Data analysis

3.2.7.1 Random and fixed effect analysis

Data collected were analysed using the DeltaGen (Jahufer & Luo, 2018) software package. Data collected from the six hemp cultivars for all the traits from Wairarapa and Palmerston North in the growing season 2019/2020 and the irrigated (IR) and rainfed (RF) sites in the growing season 2020/2021, were analyzed within each site and across both sites. Given that the experimental site in Palmerston North during the 2019/2020 growing period and the irrigated site in 2020/2021 served as common sites for both years, the data collected from this site were subsequently analysed together to present insights across these two consecutive years. These analyses were conducted using the variance component analysis based on the Residual Maximum Likelihood (REML) (Harville, 1977; Patterson & Thompson, 1971; Patterson & Thompson, 1975) procedure in DeltaGen (Jahufer & Luo, 2018). In single-site, across-site and across-year analyses, when the cultivars were analyzed as fixed effects, F ratio tests were carried out, and $LSD_{0.05}$ estimates were generated. The statistical significance of the variance components was assessed using deviance of log-likelihood (Galwey, 2006; Holland et al., 2003).

The data analysis from the trial conducted during the 2019/2020 growing season utilized data from bulk samples per cultivar per replicate. The trial during the 2020/2021 growing season was analysed using data obtained from ten individual plant samples for each replicate within each cultivar. To be able to conduct the analysis across years, the data obtained from ten individual plant samples per cultivar per replicate in the growing season 2020/2021 were averaged within each replicate.

The random linear model used in REML analysis for analyses within site (growing season 1; 2019/2020),

$$\boldsymbol{Y_{ij}} = \mu + \boldsymbol{g_i} + \boldsymbol{b_j} + \boldsymbol{\varepsilon_{ij}} \tag{1}$$

$\boldsymbol{Y_{ij}}$ is the value of an attribute measured from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{j}$, and $\boldsymbol{i} = 1,...,$ $\boldsymbol{n_g}$, $\boldsymbol{j} = 1,..., \boldsymbol{n_b}$; where $\boldsymbol{g}$ and $\boldsymbol{b}$ are cultivars and replicates, respectively; μ is the overall mean; $\boldsymbol{g_i}$ is the random effect of cultivar $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_g^2)}$; $\boldsymbol{b_j}$ is the random effect of replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_b^2)}$; $\boldsymbol{\varepsilon_{ij}}$ is the residual effect of cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_\varepsilon^2)}$.

This model was also used as a mixed linear model by considering *cultivars* as fixed effects, where $\boldsymbol{g_i}$ is the fixed effect of cultivar $\boldsymbol{i}$.

The mixed linear model used in REML analysis for analyses across sites (growing season 1; 2019/2020),

$$\boldsymbol{Y_{ijk}} = \mu + \boldsymbol{g_i} + \boldsymbol{l_j} + (\boldsymbol{gl})_{\boldsymbol{ij}} + \boldsymbol{b_{jk}} + \boldsymbol{\varepsilon_{ijk}} \tag{2}$$

$\boldsymbol{Y_{ijk}}$ is the value of an attribute measured from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{k}$ at site $\boldsymbol{j}$, and $\boldsymbol{i} =$ 1,..., $\boldsymbol{n_g}$, $\boldsymbol{j}$ = 1,..., $\boldsymbol{n_l}$, $\boldsymbol{k}$ = 1,..., $\boldsymbol{n_b}$; where $\boldsymbol{g}$, $\boldsymbol{l}$ and $\boldsymbol{b}$ are cultivars, sites and replicates, respectively; μ is the overall mean; $\boldsymbol{g_i}$ is the random effect of cultivar $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_g^2)}$; $\boldsymbol{l_j}$ is the fixed effect of site $\boldsymbol{j}$; $\boldsymbol{b_{jk}}$ is the random effect of replicate $\boldsymbol{k}$ within site $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_b^2)}$; $(\boldsymbol{gl})_{\boldsymbol{ij}}$ is the random effect of the interaction between cultivar $\boldsymbol{i}$ and site $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_{gl}^2)}$; $\boldsymbol{\varepsilon_{ijk}}$ is the residual effect of cultivar $\boldsymbol{i}$ in replicate $\boldsymbol{k}$ in site $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_\varepsilon^2)}$.

This model was also used as a mixed linear model by considering *cultivars*, *sites* and the *interaction between cultivar and site* as a fixed effects, where $\boldsymbol{g_i}$ is the fixed effect of cultivar $\boldsymbol{i}$, $\boldsymbol{l_j}$ is the fixed effect of site $\boldsymbol{j}$ and $(\boldsymbol{gl})_{\boldsymbol{ij}}$ is the fixed effect of the interaction between cultivar $\boldsymbol{i}$ and site $\boldsymbol{j}$.

The random linear model used in REML analysis for analysis within site (growing season 2; 2020/2021),

$$\boldsymbol{Y_{ijk}} = \mu + \boldsymbol{g_i} + \boldsymbol{b_j} + \boldsymbol{c_{ik}} + \boldsymbol{\varepsilon_{ijk}} \tag{3}$$

$\boldsymbol{Y_{ijk}}$ is the value of an attribute measured from sample $\boldsymbol{k}$ in cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{j}$, and $\boldsymbol{i} =$ 1,..., $\boldsymbol{n_g}$, $\boldsymbol{j}$ = 1,..., $\boldsymbol{n_b}$, $\boldsymbol{k}$ = 1,…, $\boldsymbol{n_c}$; where $\boldsymbol{g}$, $\boldsymbol{b}$ and $\boldsymbol{c}$ are cultivars, replicates and samples, respectively; μ is the overall mean; $\boldsymbol{g_i}$ is the random effect of cultivar $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_g^2)}$; $\boldsymbol{b_j}$ is the random effect of replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_b^2)}$; $\boldsymbol{c_{ik}}$ is the random effect of sample $\boldsymbol{k}$ from cultivar $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_c^2)}$; $\boldsymbol{\varepsilon_{ijk}}$ is the residual effect of sample $\boldsymbol{k}$ from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_\varepsilon^2)}$.

This model was also used as a mixed linear model by considering *cultivars* and *samples within cultivars* as fixed effects, where $\boldsymbol{g_i}$ is the fixed effect of cultivar $\boldsymbol{i}$, $\boldsymbol{c_{ik}}$ is the fixed effect of sample $\boldsymbol{k}$ from cultivar $\boldsymbol{i}$.

The mixed linear model used in REML analysis for analyses across sites (growing season 2; 2020/2021),

$$\boldsymbol{Y_{ijkm}} = \mu + \boldsymbol{g_i} + \boldsymbol{l_j} + (\boldsymbol{gl})_{\boldsymbol{ij}} + \boldsymbol{b_{jk}} + \boldsymbol{c_{im}} + \boldsymbol{\varepsilon_{ijkm}} \tag{4}$$

$\boldsymbol{Y_{ijkm}}$ is the value of an attribute measured from sample $\boldsymbol{m}$ from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{k}$ at site $\boldsymbol{j}$, and $\boldsymbol{i} =$ 1,..., $\boldsymbol{n_g}$, $\boldsymbol{j}$ = 1,..., $\boldsymbol{n_l}$, $\boldsymbol{k}$ = 1,..., $\boldsymbol{n_b}$; $\boldsymbol{m}$ = 1,…, $\boldsymbol{n_c}$; where $\boldsymbol{g}$, $\boldsymbol{l}$, $\boldsymbol{b}$ and $\boldsymbol{c}$ are cultivars, sites, replicates and samples, respectively; μ is the overall mean; $\boldsymbol{g_i}$ is the random effect of cultivar $\boldsymbol{i}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_g^2})$; $\boldsymbol{l_j}$ is the fixed effect of site $\boldsymbol{j}$;$(\boldsymbol{gl})_{\boldsymbol{ij}}$ is the random effect of the interaction between cultivar $\boldsymbol{i}$ and site $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_{gl}^2})$; $\boldsymbol{b_{jk}}$ is the random effect of replicate $\boldsymbol{k}$ within site $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_b^2})$;$\boldsymbol{c_{im}}$ is the random effect of sample $\boldsymbol{m}$ from cultivar $\boldsymbol{i}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_c^2})$; $\boldsymbol{\varepsilon_{ijkm}}$ is the residual effect of sample $\boldsymbol{m}$ from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{k}$ at site $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_\varepsilon^2})$.

This model was also used as a mixed linear model by considering *cultivars, sites* and the *interaction between cultivar and site* as a fixed effects, where $\boldsymbol{g_i}$ is the fixed effect of cultivar $\boldsymbol{i}$, $\boldsymbol{l_j}$ is the fixed effect of site $\boldsymbol{j}$ and $(\boldsymbol{gl})_{\boldsymbol{ij}}$ is the fixed effect of the interaction between cultivar $\boldsymbol{i}$ and site $\boldsymbol{j}$.

The mixed linear model used in REML analysis for analyses across years (growing season 1 and 2; 2019/2020 and 2020/2021),

$$\boldsymbol{Y_{ijk}} = \mu + \boldsymbol{g_i} + \boldsymbol{y_j} + (\boldsymbol{gy})_{\boldsymbol{ij}} + \boldsymbol{b_{jk}} + \boldsymbol{\varepsilon_{ijk}} \tag{5}$$

$\boldsymbol{Y_{ijk}}$ is the value of an attribute measured from cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{k}$ in year $\boldsymbol{j}$, and $\boldsymbol{i} =$ 1,..., $\boldsymbol{n_g}$, $\boldsymbol{j}$ = 1,..., $\boldsymbol{n_y}$, $\boldsymbol{k}$ = 1,..., $\boldsymbol{n_b}$; where $\boldsymbol{g}$, $\boldsymbol{y}$ and $\boldsymbol{b}$ are cultivars, years and replicates, respectively; μ is the overall mean; $\boldsymbol{g_i}$ is the random effect of cultivar $\boldsymbol{i}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_g^2})$; $\boldsymbol{y_j}$ is the fixed effect of year $\boldsymbol{j}$; $\boldsymbol{b_{jk}}$ is the random effect of replicate $\boldsymbol{k}$ within years $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_b^2})$; $(\boldsymbol{gy})_{\boldsymbol{ij}}$ is the random effect of the interaction between cultivar $\boldsymbol{i}$ and year $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_{gy}^2})$; $\boldsymbol{\varepsilon_{ijk}}$ is the residual effect of cultivar $\boldsymbol{i}$ within replicate $\boldsymbol{k}$ in year $\boldsymbol{j}$, $\boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_\varepsilon^2})$.

This model was also used as a mixed linear model by considering *cultivars*, *years* and *interaction between cultivar and year* as fixed effects, where $\boldsymbol{g_i}$ is the fixed effect of cultivar $\boldsymbol{i}$, $\boldsymbol{y_j}$ is the fixed effect of year $\boldsymbol{j}$ and $(\boldsymbol{gy})_{\boldsymbol{ij}}$ is the fixed effect of the interaction between cultivar $\boldsymbol{i}$ and year $\boldsymbol{j}$.

3.2.7.2 Heritability

The estimates of broad sense heritability ($\boldsymbol{h_b^2}$) on a line mean basis, and the associated standard errors were generated using the software DeltaGen (Jahufer & Luo, 2018). Line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) for each trait were estimated using the corresponding estimates of genotypic and error components of variance using the following equations from Falconer and Mackay (1996).

Heritability on a line mean basis based on genotypic analysis within sites (growing season 1; 2019/2020),

The estimates of variance components generated from Eq. 1, were used to generate estimates of broad sense heritability ($\boldsymbol{h_b^2}$) on a line (cultivar) mean basis, for all the traits measured. The line mean broad sense heritability ($\boldsymbol{h_b^2}$) estimates were estimated using the following equation:

$$\boldsymbol{h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_\varepsilon^2}{n_r}}} \tag{6}$$

where: $\boldsymbol{h_b^2}$ is the line mean broad sense heritability; $\boldsymbol{\sigma_g^2}$ is the line (cultivar) genotypic variance component; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component and $\boldsymbol{n_r}$ is the number of replicates.

Heritability on a line mean basis based on genotypic analysis within sites (growing season 2; 2020/2021),

The estimates of variance components generated from Eq. 3, were used to generate estimates of broad sense heritability ($\boldsymbol{h_b^2}$) on a line (cultivar) mean basis, for all the traits measured. The line mean broad sense heritability ($\boldsymbol{h_b^2}$) estimates were estimated using the following equation:

$$\boldsymbol{h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_\varepsilon^2}{n_r n_s}}} \tag{7}$$

where: $\boldsymbol{h_b^2}$ is the line mean broad sense heritability; $\boldsymbol{\sigma_g^2}$ is the line genotypic variance component; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component, $\boldsymbol{n_r}$ is the number of replicates and $\boldsymbol{n_s}$ is the number of plants sampled within a cultivar within a replicate.

Heritability on a line mean basis based on genotypic analysis across sites (growing season 1; 2019/2020),

The estimates of variance components generated from Eq. 2, were used to generate the estimates of broad sense heritability ($\boldsymbol{h_b^2}$) across sites for all the traits measured. The line mean broad sense heritability ($\boldsymbol{h_b^2}$) estimates across sites were estimated using the following equation:

$$\boldsymbol{h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gl}^2}{n_l} + \frac{\sigma_\varepsilon^2}{n_l n_r}}} \tag{8}$$

where: $\boldsymbol{h_b^2}$ is the line mean broad sense heritability; $\boldsymbol{\sigma_g^2}$ is the line (cultivar) genotypic variance component; $\boldsymbol{\sigma_{gl}^2}$ is the cultivar × site (G × E) interaction; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component, $\boldsymbol{n_l}$ is the number of sites and $\boldsymbol{n_r}$ is the number of replicates.

Heritability on a line mean basis based on genotypic analysis across sites (growing season 2; 2020/2021),

The estimates of variance components generated from Eq. 4, were used to generate estimates of broad sense heritability ($\boldsymbol{h_b^2}$) on a line (cultivar) mean basis, for all the traits measured. The line mean broad sense heritability ($\boldsymbol{h_b^2}$) estimates across sites were estimated using the following equation:

$$\boldsymbol{h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gl}^2}{n_l} + \frac{\sigma_\varepsilon^2}{n_l n_r n_s}}} \tag{9}$$

where: $\boldsymbol{h_b^2}$ is the line mean broad sense heritability; $\boldsymbol{\sigma_g^2}$ is the line genotypic variance component; $\boldsymbol{\sigma_{gl}^2}$ is the cultivar × site (G × E) interaction; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component, $\boldsymbol{n_l}$ is the number of sites, $\boldsymbol{n_r}$ is the number of replicates and $\boldsymbol{n_s}$ is the number of plants sampled within a cultivar within a replicate.

Heritability on a line mean basis based on genotypic analysis across years (growing season 1 and 2; 2019/2020 and 2020/2021),

The estimates of variance components generated from Eq. 5, were used to generate the estimates of broad sense heritability ($\boldsymbol{h_b^2}$) across years for all the traits measured. The line mean broad sense heritability ($\boldsymbol{h_b^2}$) estimates across years were estimated using the following equation:

$$\boldsymbol{h_b^2} = \frac{\boldsymbol{\sigma_g^2}}{\boldsymbol{\sigma_g^2} + \frac{\boldsymbol{\sigma_{gy}^2}}{\boldsymbol{n_y}} + \frac{\boldsymbol{\sigma_\varepsilon^2}}{\boldsymbol{n_y n_r}}} \tag{10}$$

where: $\boldsymbol{h_b^2}$ is the line mean broad sense heritability; $\boldsymbol{\sigma_g^2}$ is the line (cultivar) genotypic variance component; $\boldsymbol{\sigma_{gy}^2}$ is the cultivar × year (G × E) interaction; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component, $\boldsymbol{n_y}$ is the number of years and $\boldsymbol{n_r}$ is the number of replicates.

As seed oil and protein content were measured from bulk samples of only two replicates, within-site and across-site analyses were conducted using Eq. 1 and Eq. 2, respectively. Heritability estimates on a line mean basis based on genotypic analysis within and across sites for seed oil and protein content were analyzed using Eq. 6 and Eq. 8, respectively.

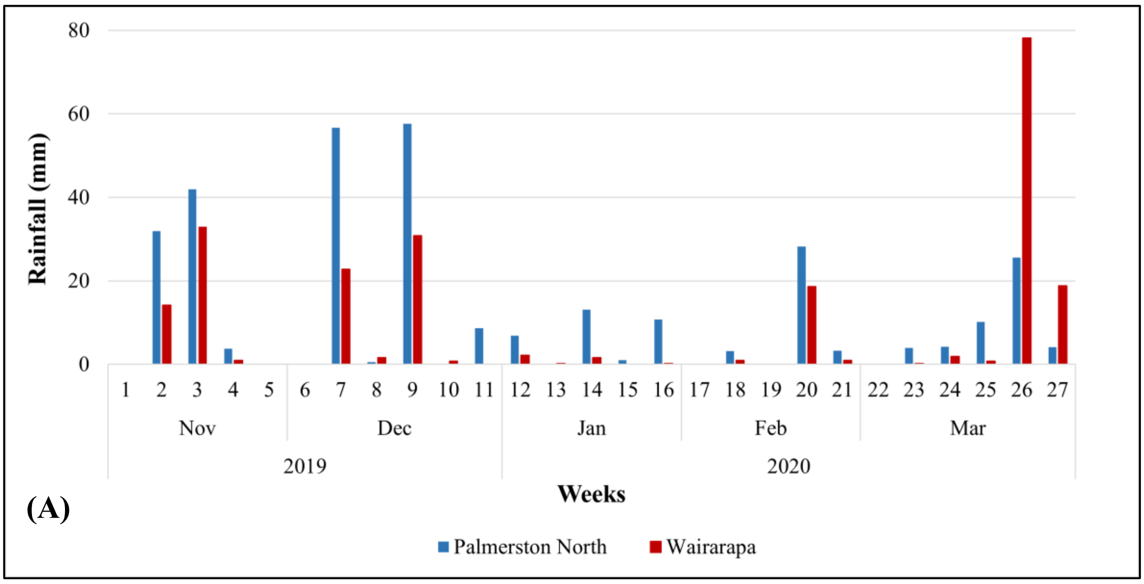
3.3 Results

3.3.1 Meteorological conditions

3.3.1.1 Growing season 1; 2019/2020

Meteorological data from both Palmerston North and Wairarapa were collected, with daily observations aggregated to compile weekly averages and totals for rainfall, as well as maximum and minimum air temperatures, as illustrated in Figures 3.4 (A) and (B). Over the years 2019 and 2020, the annual total rainfall for Palmerston North was 976.8mm and 826.4mm, respectively, while Wairarapa received 567.6mm and 735.1mm. During the trial period, the total rainfall at Palmerston North was 202.9mm, while in Wairarapa it was 87.8mm. Annual mean maximum temperature for Palmerston North in 2020 was 18.3°C and the minimum was 9.5°C. In contrast, Wairarapa's mean maximum temperatures for 2019 and 2020 were close, at 19°C and 18.9°C, while the mean minimum temperatures were 8.3°C and 8.5°C, respectively. The highest mean daily maximum temperature recorded during the trial period was 30.5°C at Palmerston North and 31.8°C at Wairarapa.

Based on the Meteorological data gathered, Palmerston North generally experienced higher rainfall than Wairarapa during the growing season 2019/2020. While overall annual rainfall was higher in Palmerston North in 2019 and 2020, this trend was particularly pronounced during the trial period. The average temperatures were quite similar in both regions, although Wairarapa recorded slightly higher annual mean maximum temperatures. Meanwhile, Palmerston North had marginally warmer minimum temperatures.



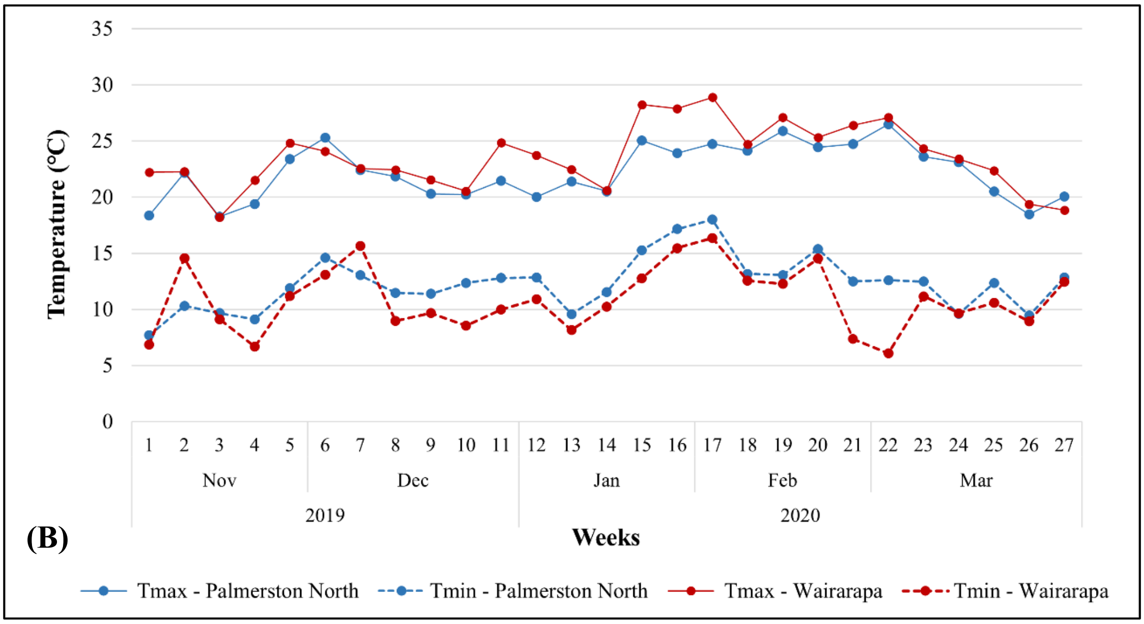


Figure 3.4. Total weekly rainfall at Palmerston North and Wairarapa (A) and Mean maximum and minimum weekly air temperatures (°C) at Palmerston North and Wairarapa during the growing season 1; November 2019 to March 2020 (B).

3.3.1.2 Growing season 2; 2020/2021

Meteorological data from Palmerston North for the growing season 2: 2020/2021 was collected and summarized, with daily observations being aggregated to weekly totals and average values for rainfall, and maximum and minimum air temperatures, which are presented in Figures 3.5 (A) and (B). The annual total rainfall for the region in 2021 was notably high, reaching 1083.8mm compared to the annual rainfall in 2019 and 2020. During a specific trial period within that year, the region experienced 252.1mm of this total rainfall. Temperatures remained moderate, with the annual mean maximum temperature at 18.8℃ and the mean minimum at 9.7℃. The highest mean daily maximum temperature recorded in the year was 27.6℃.

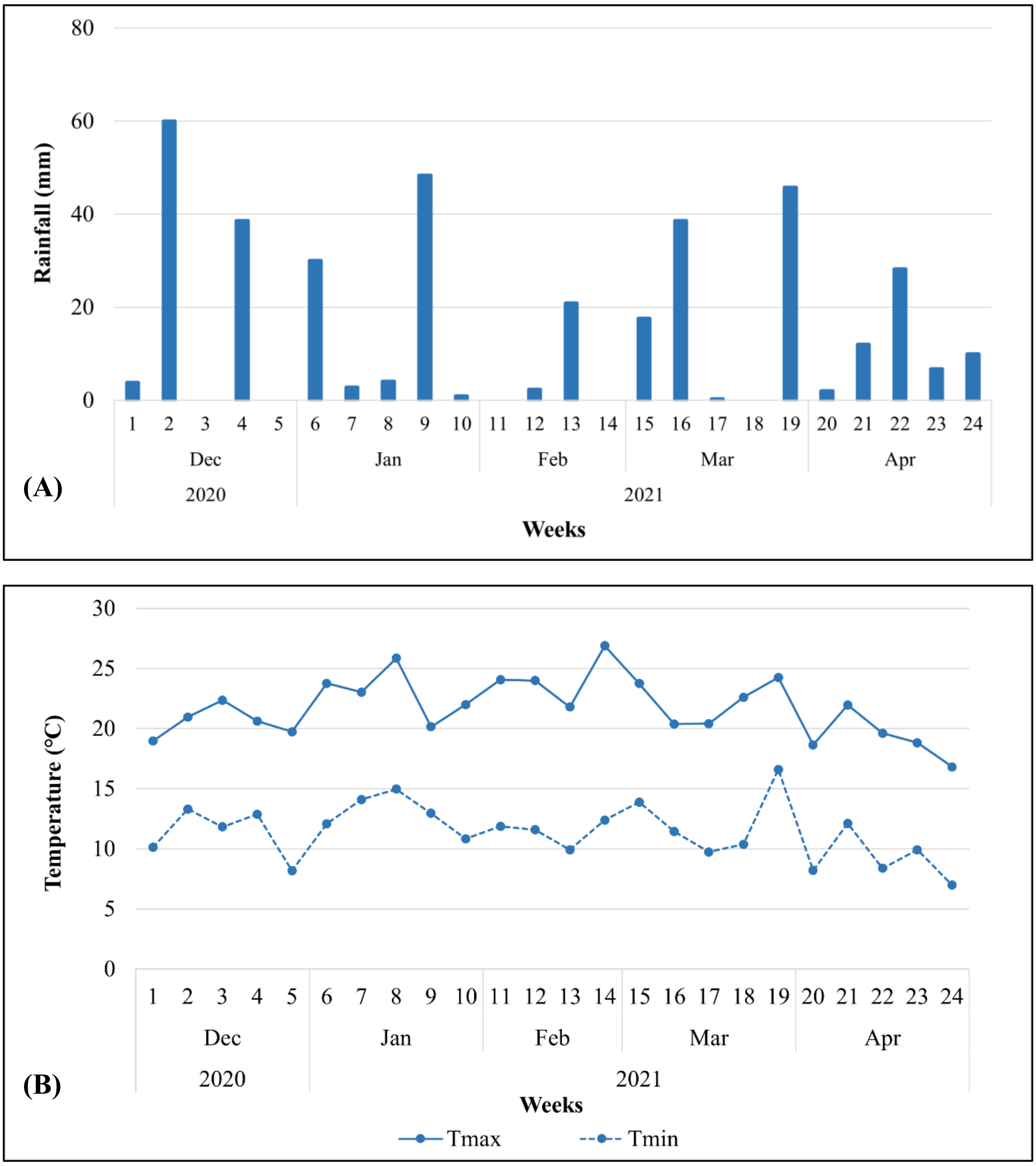


Figure 3.5. Total weekly rainfall (A) and Mean maximum and minimum weekly air temperatures (°C) (B) at Palmerston North during the growing season 2; December 2020 to April 2021.

Figure 3.6 displayed the differences in moisture levels between the irrigated and rainfed sites. At the irrigated site, the soil moisture level maintained above 50% of field capacity. Moisture levels at the rainfed site ranged from 31.66% to 74.13% of field capacity. Moisture level at the irrigated site varied from 50.69% to 85.42% of field capacity.

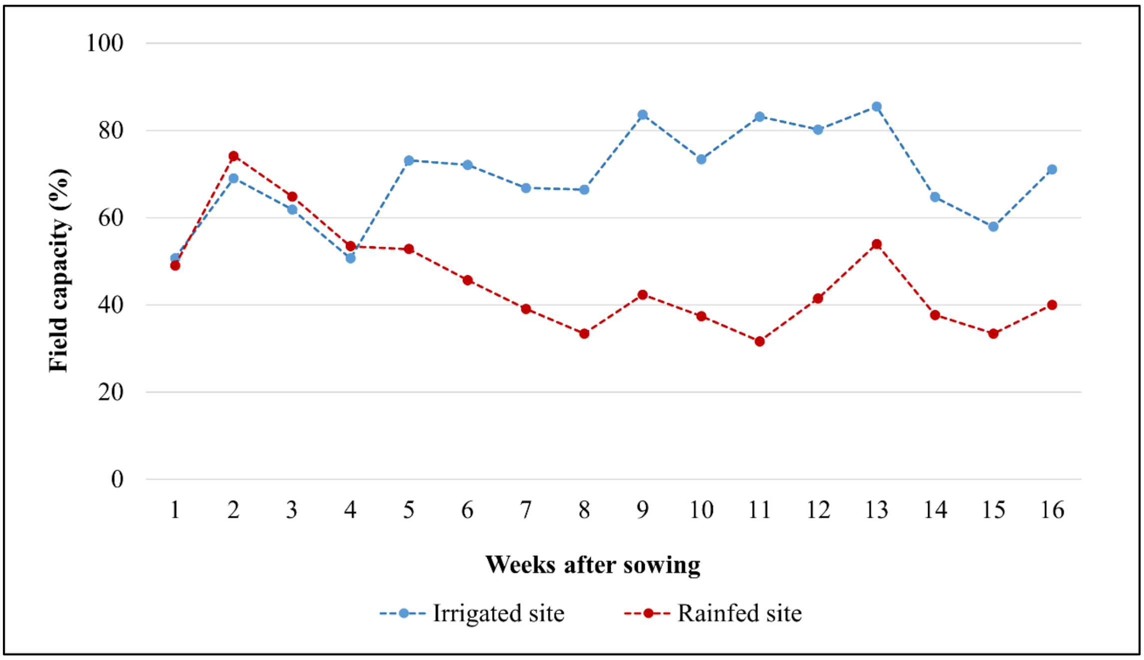


Figure 3.6. Field capacity (%) of the irrigated and rainfed sites during the trial period; from 17th December 2020 to 8th April 2021 in growing season 2 (2020/2021).

3.3.1.3 Growing seasons 1 & 2; 2019/2020 and 2020/2021.

Figure 3.7 provides a comprehensive illustration of the meteorological conditions for the two growing seasons of 2019/2020 and 2020/2021, summarizing data on monthly rainfall and mean monthly temperatures. Throughout the trial period conducted in the growing season 2019/2020, recorded rainfall was 202.9mm, with mean maximum and minimum temperatures ranging from 16.4°C to 30.5°C and 5.8°C to 19.5°C, respectively. In contrast, the subsequent 2020/2021 trial period demonstrated an increase in rainfall to 252.1mm, with the temperature ranging between 16.8°C and 27.6°C for mean maximum temperatures and 3.1°C and 17.5°C for mean minimum temperatures.

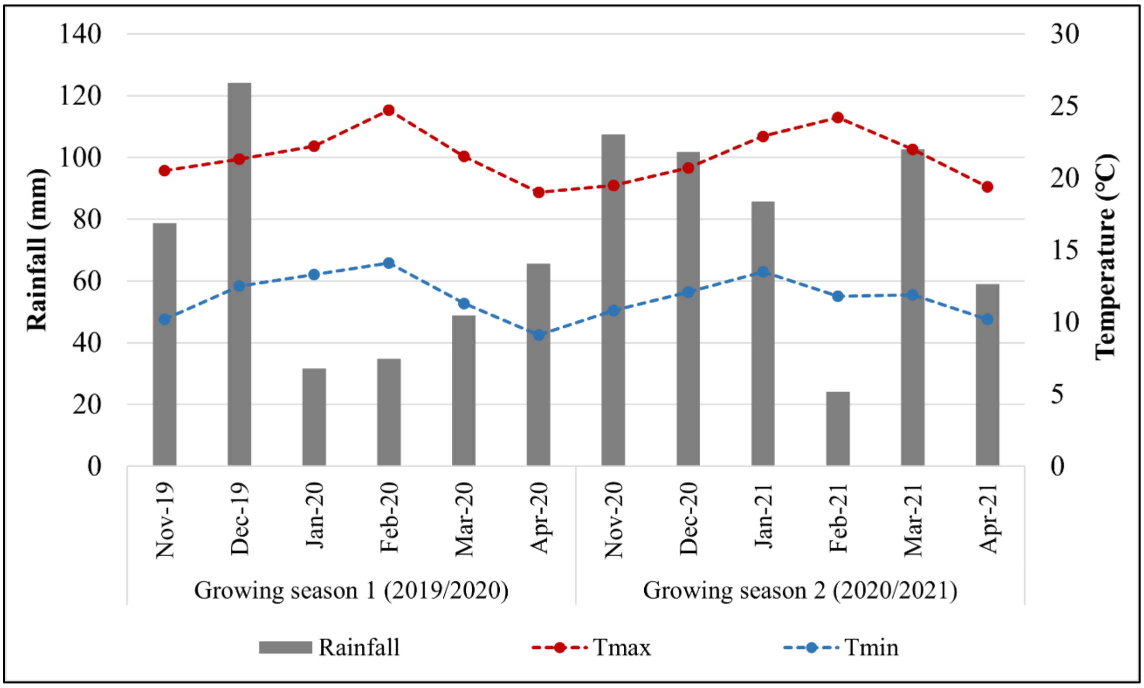


Figure 3.7. Total monthly rainfall (mm) and mean maximum and minimum monthly air temperatures (°C) at Palmerston North across both growing seasons 1 & 2; 2019/2020 and 2020/2021.

3.3.2 Within site and across sites analyses - growing season 2019/2020

3.3.2.1 Within sites

The results demonstrated that there were significant ($P < 0.05$) differences among the six hemp cultivars for the traits; seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm), evaluated at Palmerston North (Table 3.5). The highest seedling establishment was observed in cultivar Finola, and the least was observed in cultivar Futura 75. The results showed that the highest stand establishment was in cultivar Férimon 12, and the lowest was in cultivar CFX-2. Although the seedling establishment of CFX-2 did not significantly ($P > 0.05$) differ from CRS-1, Katani or Finola. The highest biomass yield was observed in Futura 75. The least biomass yield was recorded in CFX-2, which was statistically comparable with the biomass yield of the cultivars Férimon 12, CRS-1, Katani and Finola. The results illustrated that the highest plant height was observed in Futura 75, and the shortest plants were observed in Finola, which was on par with the cultivars; Katani and CFX-2. Among the cultivars evaluated, Futura 75 recorded the highest stem diameter, and Finola recorded the lowest stem diameter.

Table 3.5. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm) measured from the six hemp cultivars evaluated at Palmerston North during the growing season 2019/2020.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	67.36^{bc}	30.57^{c}	4.78^{b}	101.38^{cd}	7.23^{ab}
CRS-1	68.43^{bc}	34.54^{bc}	8.35^{b}	122.94^{bc}	8.11^{ab}
Férimon 12	69.85^{abc}	56.57^{a}	8.96^{b}	139.51^{b}	7.88^{ab}
Katani	71.51^{ab}	35.23^{bc}	6.21^{b}	90.95^{cd}	7.44^{ab}
Futura 75	66.10^{c}	42.73^{b}	28.47^{a}	185.71^{a}	9.47^{a}
Finola	73.57^{a}	37.11^{bc}	5.00^{b}	81.15^{d}	6.56^{b}
± SE	0.98	2.28	2.00	8.50	0.58
Significance	***	***	***	***	*

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

There were significant ($P < 0.05$) differences among five hemp cultivars for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) evaluated at Palmerston North (Table 3.6). The seed yield for the cultivars varied from 1.37 t/ha to 2.64 t/ha. The cultivar Férimon 12 produced the most seed, while Finola produced the lowest. The highest thousand seed weight was recorded in CRS-1 (19.26 g), and the lowest was in Finola (13.54 g). Estimates for seed oil content measured from the five cultivars ranged from 30.66% to 34.02 %. The cultivar Finola had the highest seed oil content, and Férimon 12 had the lowest. Estimates for seed protein content measured from the five cultivars ranged from 22.19% to 26.75%, where Férimon 12 contained the highest and Katani contained the lowest. However, it should be noted that both oil and protein the percentages did not differ significantly amongst most cultivars.

Table 3.6. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the five hemp cultivars evaluated at Palmerston North during the growing season 2019/2020.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	1.86^{b}	16.54^{bc}	31.63^{ab}	25.31^{ab}
CRS-1	1.93^{b}	19.26^{a}	32.32^{ab}	22.60^{ab}
Férimon 12	2.64^{a}	17.91^{ab}	30.66^{b}	26.75^{a}
Katani	2.00^{b}	15.50^{cd}	33.87^{a}	22.19^{b}
Finola	1.37^{c}	13.54^{d}	34.02^{a}	22.63^{ab}
± SE	0.11	0.60	0.42	0.80
Significance	***	***	**	*

Values with the same letter within the same column are not significant (P > 0.05); * Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001; ns; not significant.

The results on seedling establishment (%) and stand establishment (%) of six hemp cultivars evaluated at Wairarapa showed that there was significant (P < 0.05) difference among the cultivars (Table 3.7). The highest established seedling population was in Katani, and lowest was in Futura 75. Although there was no significant (P > 0.05) difference in seedling establishment between any Futura 75 and the other cultivars except Katani. The highest and the lowest stand establishment was recorded in Futura 75 and Finola, respectively. However, Férimon 12 was not significantly (P > 0.05) different from Futura 75, nor were the other cultivars significantly different from Finola.

Results on biomass yield (t/ha), plant height at harvest (cm), and stem diameter (mm) of six hemp cultivars evaluated at Wairarapa showed that, there were significant (P < 0.05) differences among the cultivars evaluated (Table 3.7). The cultivar Futura 75 was found to have the highest biomass yield (17.56 t/ha). Katani depicted the lowest biomass yield (2.31 t/ha), which is not significantly (P > 0.05) different to the biomass yield of CRS-1 (4.54 t/ha), CFX-2 (3.36 t/ha) and Finola (2.46 t/ha). According to the experiment results, Futura 75 was found to have the highest plant height (144.04 cm) and was followed by Férimon 12 (124.21 cm). The lowest plant height was recorded in Katani (55.64 cm), which was on par with the height of Finola (56.42 cm). Among the cultivars evaluated, Futura 75 had the highest stem diameter (6.73 mm), which was not significantly (P >

0.05) different to Férimon 12 (6.49 mm) and CRS-1 (5.74 mm). The lowest stem diameter was recorded in Katani (4.55 mm). Based on this result, Futura 75 showed highest expression for the traits; biomass yield, plant height and stem diameter, and stand establishment, while Katani showed the lowest expression for traits; biomass yield, plant height and stem diameter in Wairarapa.

Table 3.7. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm) measured from the six hemp cultivars evaluated at Wairarapa during the growing season 2019/2020.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	72.15^{ab}	36.27^{b}	3.36^{bc}	74.66^{cd}	5.26^{bc}
CRS-1	69.98^{ab}	37.37^{b}	4.54^{bc}	88.10^{c}	5.74^{ab}
Férimon 12	73.20^{ab}	53.74^{a}	8.73^{b}	124.21^{b}	6.49^{a}
Katani	75.13^{a}	36.27^{b}	2.31^{c}	55.64^{e}	4.55^{c}
Futura 75	67.81^{b}	56.70^{a}	17.56^{a}	144.04^{a}	6.73^{a}
Finola	68.10^{b}	35.42^{b}	2.46^{c}	56.42^{de}	4.72^{bc}
± SE	1.37	1.98	1.32	4.86	0.29
Significance	**	***	***	***	***

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

Significant ($P < 0.05$) differences were observed for seed yield and thousand seed weight among the five hemp cultivars evaluated at Wairarapa, but seed oil and protein content remained statistically the same (Table 3.8). Férimon 12 exhibited the highest seed yield (0.05 t/ha), whereas Finola, was not significantly ($P > 0.05$) different to cultivars Katani (0.03 t/ha), CRS-1 (0.02 t/ha), and CFX-2 (0.02 t/ha), which showed the lowest yield (0.02 t/ha). Katani recorded the highest thousand seed weight (12.68 g), followed by Férimon 12 (9.69 g) and CFX-2 (9.00 g), whose thousand seed weights were not significantly ($P > 0.05$) different to Katani. Conversely, Finola and CRS-1, with comparable measures, exhibited the lowest thousand seed weight (8.10 g and 8.26 g, respectively). The seed oil content across the five hemp cultivars evaluated at Wairarapa varied between 32.27% and 33.93%, while protein content ranged from 24.88% to 25.85%. Despite insignificance ($P > 0.05$), Finola marked both highest seed oil (33.93%) and protein content (25.85%).

Table 3.8. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the five hemp cultivars evaluated at Wairarapa during the growing season 2019/2020.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	0.02^{b}	9.00^{ab}	32.89^{a}	24.88^{a}
CRS-1	0.02^{b}	8.26^{b}	32.35^{a}	25.10^{a}
Férimon 12	0.05^{a}	9.69^{ab}	32.27^{a}	25.82^{a}
Katani	0.03^{b}	12.68^{a}	32.42^{a}	25.28^{a}
Finola	0.02^{b}	8.10^{b}	33.93^{a}	25.85^{a}
± SE	0.01	0.87	0.74	0.41
Significance	*	*	ns	ns

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

3.3.2.2 Across sites

Table 3.9 presents the data on seedling and stand establishment percentages, biomass yield (t/ha), harvest plant height (cm), and stem diameter (mm) for the six hemp cultivars evaluated across two sites: Palmerston North and Wairarapa. Significant ($P < 0.05$) differences among the cultivars were evident for seedling and stand establishments, while no significant ($P > 0.05$) differences were observed for biomass yield, plant height, and stem diameter across both sites.

Although seedling establishment for other cultivars did not differ significantly ($P > 0.05$) from that of Katani or Futura 75, Katani had the highest rate of seedling establishment (73.32%), while Futura 75 had the lowest (66.95%). Férimon 12 reported the highest stand establishment (55.15%), while CFX-2 showed the lowest (33.42%). However, the stand establishment for Férimon 12 and CFX-2 was not significantly ($P > 0.05$) different from that of Futura 75 and the other three cultivars (CRS-1, Katani, and Finola), respectively.

For biomass yield, plant height at harvest, and stem diameter, Futura 75 recorded the highest values (23.01 t/ha, 164.88 cm, and 8.10 mm, respectively), with Finola registering the lowest measures for all three traits (3.73 t/ha in biomass yield, 68.79 cm in height and 5.64 mm in stem diameter). Férimon 12, was not significantly (P > 0.05) different to the other cultivars and followed Futura 75 in terms of biomass yield (8.84 t/ha) and plant height (131.86 cm).

Table 3.9. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm), measured from the six hemp cultivars, based on analysis across the sites Palmerston North and Wairarapa during the growing season 2019/2020.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	69.76^{abc}	33.42^{b}	4.07^{b}	88.02^{cd}	6.25^{bc}
CRS-1	69.20^{bc}	35.95^{b}	6.44^{b}	105.52^{c}	6.92^{ab}
Férimon 12	71.52^{ab}	55.15^{a}	8.84^{b}	131.86^{b}	7.18^{ab}
Katani	73.32^{a}	35.75^{b}	4.26^{b}	73.29^{de}	5.99^{bc}
Futura 75	66.95^{c}	49.71^{a}	23.01^{a}	164.88^{a}	8.10^{a}
Finola	70.84^{ab}	36.26^{b}	3.73^{b}	68.79^{e}	5.64^{c}
± SE	0.84	1.51	1.20	4.90	0.32
Sources of variation					
Cultivars	***	***	***	***	***
Sites	ns	ns	***	**	**
Cultivar × Site	**	**	ns	ns	ns

Values with the same letter within the same column are not significant (P > 0.05); * Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001; ns; not significant.

Table 3.10 displays results on seed yield, thousand seed weight, seed oil, and protein content measured across the Palmerston North and Wairarapa from the six hemp cultivars. Significant (P < 0.05) differences were observed among cultivars for seed yield, thousand seed weight, and protein content, while seed oil content was not significant (P > 0.05).

Férimon 12 and Katani recorded the highest seed yield and thousand seed weight across both sites, respectively. Although for thousand seed weight, Katani did not differ significantly (P > 0.05) from that of three other cultivars: Férimon 12, CRS-1, and CFX-2. Across sites, seed yield varied from 0.70 t/ha to 1.35 t/ha, and thousand seed weight ranged between 10.82 g to 13.80 g. Despite being the highest yielder, Férimon 12 was one of the cultivars with the lowest seed oil content (31.46%), while Finola, with the lowest yield, had the highest seed oil content (33.97%). Seed protein content ranged from 23.74% to 26.28%, with Férimon 12 having the highest and Katani one of the lowest.

Table 3.10. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the five hemp cultivars, based on analysis across the sites Palmerston North and Wairarapa during the growing season 2019/2020.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	0.94^{b}	12.77^{ab}	32.26^{bc}	25.09^{ab}
CRS-1	0.98^{b}	13.76^{a}	32.33^{bc}	23.85^{b}
Férimon 12	1.35^{a}	13.80^{a}	31.46^{c}	26.28^{a}
Katani	1.02^{b}	14.09^{a}	33.14^{ab}	23.74^{b}
Finola	0.70^{c}	10.82^{b}	33.97^{a}	24.24^{b}
± SE	0.06	0.53	0.42	0.45
Sources of variation				
Cultivars	***	***	**	*
Sites	***	***	ns	ns
Cultivar × Site	***	***	ns	*

Values with the same letter within the same column are not significant (P > 0.05); * Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001; ns; not significant.

3.3.2.3 Genotypic variance components, cultivar × site (G × E) interaction variance components and line mean broad sense heritability estimates

There was significant (P < 0.05) genotypic variation ($\boldsymbol{\sigma_g^2}$) among the cultivars for all the traits evaluated, except for SD; stem diameter within Palmerston North (Table 3.11). The traits Se. EST; seedling establishment, St. EST; stand establishment, PH; plant height at harvest, SD; stem diameter, BM; biomass yield and TSW; thousand seed weight had significant (P < 0.05) genotypic variation at Wairarapa. There was significant (P < 0.05) cultivar × site ($\boldsymbol{\sigma_{gl}^2}$) interaction for the traits; Se. EST; seedling establishment, St. EST;

stand establishment, SY; seed yield, TSW; thousand seed weight and PRO; seed protein content. Notably, of these traits, only St. EST; stand establishment showed significant (*P* < 0.05) genotypic variation across both sites.

The line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) for all the traits measured within Palmerston North, except for SD; stem diameter (0.72) were higher than 0.85 (Table 3.11). The line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) estimated within Wairarapa were comparable to the corresponding heritability estimates from Palmerston North for the traits, St. EST; stand establishment (0.97), PH; plant height at harvest (0.99), and BM; biomass yield (0.95). The heritability for the other traits, except SD; stem diameter (0.72 in Palmerston North and 0.93 in Wairarapa), were lower than those obtained in Palmerston North. The heritability estimates across both sites aligned with Wairarapa, except Se. EST; seedling establishment (0.29), SY; seed yield (0.10), and TSW; thousand seed weight.

Table 3.11. Genotypic variance (σ_g^2), cultivar × site (G × E) interaction variance (σ_{gl}^2), residual variance (σ_ε^2), their associated standard errors (±SE) and line mean broad sense heritability (h_b^2) for nine traits measured from six industrial hemp cultivars, estimated within and across sites, Palmerston North and Wairarapa during the growing period 2019/2020. Traits: Se. EST; seedling establishment, St. EST; stand establishment, PH; plant height at harvest, SD; stem diameter, BM; biomass yield, SY; seed yield, TSW; thousand seed weight, OIL; seed oil content, and PRO; seed protein content.

Trait	Palmerston North			Wairarapa			Across sites			
	σ_g^2	σ_ε^2	h_b^2	σ_g^2	σ_ε^2	h_b^2	σ_g^2	σ_{gy}^2	σ_ε^2	h_b^2
Se. EST	6.65 ± 4.08*	3.84 ± 1.28	0.87 ± 0.08	6.77 ± 4.76*	7.19 ± 2.70	0.79 ± 0.14	1.35 ± 3.06	5.34 ± 3.69*	5.59 ± 1.48	0.29 ± 0.53
St. EST	82.43 ± 46.03*	14.36 ± 5.27	0.96 ± 0.03	93.07 ± 51.19*	13.24 ± 4.85	0.97 ± 0.02	72.47 ± 43.70*	15.29 ± 10.41*	13.8 ± 3.6	0.89 ± 0.09
PH	1422.1 ± 788.44 *	237.29 ± 87.04	0.96 ± 0.03	1310.08 ± 702.46 *	68.38 ± 25.01	0.99 ± 0.01	1360 ± 730.27 *	6.01 ± 51.94	152.84 ± 40.61	0.98 ± 0.02
SD	0.70 ± 0.56	1.11 ± 0.42	0.72 ± 0.19	0.76 ± 0.44*	0.22 ± 0.08	0.93 ± 0.04	0.73 ± 0.44*	0.00 ± 0.00	0.66 ± 0.16	0.90 ± 0.06
BM	78.21 ± 43.57*	15.96 ± 5.32	0.95 ± 0.03	33.33 ± 18.57*	6.64 ± 2.44	0.95 ± 0.03	51.54 ± 29.08*	4.19 ± 4.08	11.45 ± 2.7	0.94 ± 0.05
SY†	0.19 ± 0.12*	0.04 ± 0.02	0.95 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.72 ± 0.21	0.01 ± 0.04	0.09 ± 0.06*	0.02 ± 0.01	0.10 ± 0.73
TSW†	4.58 ± 2.79*	1.05 ± 0.43	0.95 ± 0.04	2.72 ± 2.06*	3.01 ± 1.1	0.78 ± 0.15	0.00 ± 0.00	3.60 ± 1.7*	2.24 ± 0.58	-
OIL†	1.91 ± 1.2*	0.36 ± 0.23	0.91 ± 0.07	0.28 ± 0.36	0.39 ± 0.3	0.59 ± 0.43	0.54 ± 0.59	0.54 ± 0.48	0.4 ± 0.21	0.59 ± 0.35
PRO†	3.64 ± 2.44*	0.91 ± 0.67	0.89 ± 0.11	0.02 ± 0.21	0.34 ± 0.15	0.10 ± 0.10	0.12 ± 0.88	1.69 ± 1.22*	0.66 ± 0.35	0.11 ± 0.73

Significance at the 0.05 probability level (based on log-likelihood ratio test).
† Variance components and line mean broad sense heritabilities were estimated among five hemp cultivars.

3.3.3 Within site and across sites analyses - growing season 2020/2021

3.3.3.1 Within site

The results revealed significant ($P < 0.05$) differences among the six hemp cultivars for stand establishment, biomass yield, plant height at harvest, and stem diameter at the irrigated site (Table 3.12). There was no significant ($P > 0.05$) difference in seedling establishment was found across cultivars. The results indicated Férimon 12 having the highest (8.95%) and CRS-1 the lowest stand establishment (2.92%). Futura 75 displayed the highest biomass yield (14.32%), plant height (201.69 cm) and stem diameter (12.94 mm), with Finola recording the lowest in these metrics (1.43 t/ha, 83 cm and 7.73 mm, respectively), yielding statistically ($P > 0.05$) comparable biomass yield with CFX-2, CRS-1, and Katani. The plant height of Finola was not found to be significantly ($P >$ 0.05) different from that of Katani.

Table 3.12. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm) measured from the six hemp cultivars evaluated at the irrigated site during the growing season 2020/2021.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	19.40^{a}	5.11^{bc}	3.83^{c}	98.49^{d}	9.03^{cd}
CRS-1	16.08^{a}	2.92^{c}	3.77^{c}	119.73^{c}	10.27^{bc}
Férimon 12	13.89^{a}	8.95^{a}	10.67^{b}	162.50^{b}	10.93^{b}
Katani	13.37^{a}	4.15^{bc}	2.51^{c}	86.55^{de}	8.44^{d}
Futura 75	9.94^{a}	5.75^{b}	14.32^{a}	201.69^{a}	12.94^{a}
Finola	16.96^{a}	4.08^{bc}	1.43^{c}	83.00^{e}	7.73^{d}
± SE	2.16	0.62	1.07	4.88	0.55
Significance	ns	***	***	***	***

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

Significant ($P < 0.05$) differences were observed for seed yield and thousand seed weight among the six hemp cultivars evaluated at the irrigated site, while seed oil and protein content remained statistically ($P > 0.05$) consistent (Table 3.13). Férimon 12 had the highest seed yield (3.78 t/ha), along with Futura 75 (3.30 t/ha), whereas Finola registered the lowest yield (0.67 t/ha), a figure statistically similar ($P > 0.05$) to the yields of CFX-2 (1.49 t/ha), CRS-1 (1.44 t/ha), and Katani (1.32 t/ha). Futura 75 also had the highest thousand seed weight (17.78 g), which is statistically equivalent to Férimon 12 (17.56 g) and CRS-1 (17.35 g), while Finola reported the lowest (12.96 g). The range of seed oil content across the six hemp cultivars differed from 30.78% (CRS-1) to 33.77% (Finola), while the protein content ranged from 25.56% (Futura 75) to 27.52% (Férimon 12).

Table 3.13. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the six hemp cultivars evaluated at the irrigated (IR) site during the growing season 2020/2021.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	1.49^{b}	15.63^{b}	31.89^{a}	26.28^{a}
CRS-1	1.44^{b}	17.35^{a}	30.78^{a}	25.97^{a}
Férimon 12	3.78^{a}	17.56^{a}	33.23^{a}	27.52^{a}
Katani	1.32^{b}	14.94^{b}	32.95^{a}	26.47^{a}
Futura 75	3.30^{a}	17.78^{a}	31.06^{a}	25.56^{a}
Finola	0.67^{b}	12.96^{c}	33.77^{a}	26.41^{a}
± SE	0.23	0.34	1.31	0.73
Significance	***	***	ns	ns

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

The results on seedling and stand establishment of the six hemp cultivars at the rainfed site revealed no significant ($P > 0.05$) differences among the cultivars (Table 3.14). Significant ($P < 0.05$) differences were observed for biomass yield, plant height at harvest, and stem diameter (Table 3.14). Futura 75 showed the highest biomass yield (7.01 t/ha), plant height (84.46 cm), and stem diameter (4.76 mm), followed by Férimon 12 for each trait. Katani had the lowest biomass yield (1.52 t/ha), not significantly ($P > 0.05$) different to yields from CRS-1 (2.21 t/ha), CFX-2 (1.86 t/ha) and Finola (1.53 t/ha). CFX-2 showed the least plant height (23.78 cm) and stem diameter (2.55 mm), figures that were similar to those of Katani (29.83 cm and 2.75 mm, respectively) and Finola (26.15 cm and 2.56 mm, respectively).

Table 3.14. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm) measured from the six hemp cultivars evaluated at the rain fed site (RF) during the growing season 2020/2021.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	47.67[a]	27.78[a]	1.86[c]	23.78[d]	2.55[d]
CRS-1	30.45[a]	19.79[a]	2.21[c]	35.84[c]	3.23[c]
Férimon 12	35.36[a]	32.24[a]	4.48[b]	55.80[b]	4.06[b]
Katani	33.80[a]	20.12[a]	1.52[c]	29.83[cd]	2.75[cd]
Futura 75	23.58[a]	22.21[a]	7.01[a]	84.46[a]	4.76[a]
Finola	43.12[a]	20.34[a]	1.53[c]	26.15[d]	2.56[d]
SE	7.17	5.97	0.72	5.11	0.23
Significance	ns	ns	***	***	***

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

There were significant ($P < 0.05$) differences for seed yield and thousand seed weight, but no significant ($P > 0.05$) differences for seed oil and protein, content among the six rainfed hemp cultivars (Table 3.15). Futura 75 led in seed yield (3.25 t/ha) and thousand seed weight (16.34 g), the latter comparable to CRS-1 (16.22 g). Finola had the lowest expression for both these traits (0.56 t/ha and 10.88 g, respectively). Seed oil content ranged between 31.38% (Futura 75) and 33.26% (Férimon 12), and protein content from 24.79% (Finola) to 26.24% (CRS-1).

Table 3.15. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the six hemp cultivars evaluated at the rainfed site (RF) site during the growing season 2020/2021.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	1.11[bc]	14.08[b]	32.64[a]	26.00[a]
CRS-1	1.26[bc]	16.22[a]	31.53[a]	26.24[a]
Férimon 12	1.53[b]	14.22[b]	33.26[a]	25.57[a]
Katani	0.99[bc]	13.11[b]	32.11[a]	25.94[a]
Futura 75	3.25[a]	16.34[a]	31.38[a]	25.60[a]
Finola	0.56[d]	10.88[c]	32.72[a]	24.79[a]
± SE	0.28	0.36	1.14	0.76
F value	***	***	ns	ns

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

3.3.3.2 Across sites

Table 3.16 presents results on the traits; seedling and stand establishment percentages, biomass yield, plant height at harvest, and stem diameter from the six hemp cultivars evaluated across two sites: irrigated and rainfed. Significant ($P < 0.05$) differences among the cultivars were evident for biomass yield, plant height, and stem diameter, while no significant ($P > 0.05$) differences were observed for the traits seedling and stand establishments across both sites.

Cultivar CFX-2 had the highest seedling establishment (33.53%), although statistically, this differed only from that of Futura 75, which had the lowest establishment (16.76%). Across both sites, Futura 75 recorded the highest values for biomass yield, plant height at harvest, and stem diameter (10.66 t/ha, 143.08 cm, and 8.85 mm, respectively), while Finola had the lowest values for all three traits (1.48 t/ha in biomass yield, 54.58 cm in height, and 5.15 mm in stem diameter). Férimon 12 followed Futura 75 for biomass yield (7.58 t/ha), plant height (109.15 cm), and stem diameter (7.49 mm).

Table 3.16. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm), measured from the six hemp cultivars, based on analysis across the irrigated (IR) and rainfed (RF) sites during the growing season 2020/2021.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	33.53^{a}	16.45^{a}	2.84^{c}	61.13^{d}	5.79^{d}
CRS-1	23.26^{ab}	11.35^{a}	2.99^{c}	77.78^{c}	6.75^{c}
Férimon 12	24.62^{ab}	20.59^{a}	7.58^{b}	109.15^{b}	7.49^{b}
Katani	23.58^{ab}	12.14^{a}	2.02^{cd}	58.19^{d}	5.60^{d}
Futura 75	16.76^{b}	13.98^{a}	10.66^{a}	143.08^{a}	8.85^{a}
Finola	30.04^{a}	12.21^{a}	1.48^{d}	54.58^{d}	5.15^{d}
± SE	3.74	3.00	0.63	3.49	0.29
Sources of variation					
Cultivar	**	ns	***	***	***
Site	**	**	*	***	***
Cultivar × Site	ns	ns	***	***	***

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

Table 3.17 shows results on seed yield, thousand seed weight, seed oil, and protein content from the six hemp cultivars evaluated across the irrigated and rainfed sites. Cultivar comparison across the sites revealed significant ($P < 0.05$) differences for seed yield and thousand seed weight, while seed oil and protein content remained statistically non-significant ($P > 0.05$).

Futura 75 achieved the highest seed yield, and thousand seed weight across both sites and these traits ranged from 0.61 t/ha to 3.28 t/ha and 11.92 g to 17.06 g, respectively. Although Futura 75 did not differ significantly (P > 0.05) from CRS-1 for thousand seed weight. Finola had the lowest values for these traits. There were no significant differences among other cultivars across the sites for seed oil or protein contents.

Table 3.17. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the six hemp cultivars, based on analysis across the irrigated (IR) and rainfed (RF) sites during the growing season 2020/2021.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	1.30^{c}	14.86^{c}	32.27^{a}	26.14^{a}
CRS-1	1.35^{c}	16.79^{ab}	31.15^{a}	26.10^{a}
Férimon 12	2.66^{b}	15.89^{b}	33.25^{a}	26.54^{a}
Katani	1.15^{c}	14.03^{c}	32.53^{a}	26.21^{a}
Futura 75	3.28^{a}	17.06^{a}	31.22^{a}	25.58^{a}
Finola	0.61^{d}	11.92^{d}	33.24^{a}	25.60^{a}
SE	0.18	0.25	0.87	0.53
Sources of variation				
Cultivar	***	***	ns	ns
site	ns	***	ns	ns
Cultivar × Site	***	*	ns	ns

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

3.3.3.3 Genotypic variance components, cultivar × site (G × E) interaction variance components and line mean broad sense heritability estimates

There was significant ($P < 0.05$) genotypic variation ($\boldsymbol{\sigma_g^2}$) among the six cultivars based on performance within the irrigated site for the traits, St. EST; stand establishment, PH; plant height at harvest, SD; stem diameter, BM; biomass yield, SY; seed yield, and TSW; thousand seed weight (Table 3.18). Of these traits, all the traits except St. EST; stand establishment had significant ($P < 0.05$) genotypic variation within the rainfed site. Across both sites, only PH; plant height at harvest, SD; stem diameter, and TSW; thousand seed weight had significant genotypic variation. There was significant ($P < 0.05$) cultivar × site ($\boldsymbol{\sigma_{gl}^2}$) interaction for the traits; PH; plant height at harvest, SD; stem diameter, BM; biomass yield, and SY; seed yield. However, among the traits that had significant cultivar × site ($\boldsymbol{\sigma_{gl}^2}$) interaction across both sites, only PH; plant height at harvest and SD; stem diameter showed significant across-site genotypic variation.

Line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) for all the traits measured within the irrigated site, except for St. EST; stand establishment, OIL; seed oil content and PRO; seed protein content, were higher than 0.90 (Table 3.18). The line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) estimated within the rainfed site were comparable to the corresponding heritability estimates from the irrigated site for the traits, Se. EST; seedling establishment (0.58), PH; plant height at harvest (0.99), SD; stem diameter (0.98), BM; biomass yield (0.99), SY; seed yield (0.98), and TSW; thousand seed weight (0.97). The line mean broad sense heritabilities ($\boldsymbol{h_b^2}$) for most of the traits evaluated across sites were lower than the corresponding heritability estimates obtained within sites.

Table 3.18. Genotypic variance (σ_g^2), cultivar × site (G × E) interaction variance (σ_{gl}^2), residual variance (σ_ε^2), their associated standard errors (±SE) and line mean broad sense heritability (h_b^2) for nine traits measured from six industrial hemp cultivars, estimated within and across the sites; irrigated (IR) and rainfed (RF) during the growing period 2020/2021. Traits: Se. EST; seedling establishment, St. EST; stand establishment, PH; plant height at harvest, SD; stem diameter, BM; biomass yield, SY; seed yield, TSW; thousand seed weight, OIL; seed oil content, and PRO; seed protein content.

Trait	Irrigated site			Rain fed site			Across sites			
	σ_g^2	σ_ε^2	h_b^2	σ_g^2	σ_ε^2	h_b^2	σ_g^2	σ_{gy}^2	σ_ε^2	h_b^2
Se. EST	6.67 ± 6.52	16.4 ± 6.26	0.62 ± 0.27	43.57 ± 46.59	126.84 ± 47.37	0.58 ± 0.30	25.2 ± 19.54	0.00 ± 0.00	71.54 ± 17.23	0.74 ± 0.16
St. EST	4.06 ± 2.35*	1.29 ± 0.47	0.93 ± 0.05	5.59 ± 20.43	82.48 ± 30.93	0.21 ± 0.65	7.47 ± 7.42	0.00 ± 0.00	39.24 ± 9.46	0.60 ± 0.25
PH	2243.09 ± 1191.9 *	356.24 ± 33.15	1.00 ± 0.00	547.5 ± 297.23 *	185.41 ± 17.25	0.99 ± 0.00	1084.27 ± 665.5 *	310.83 ± 172.59 *	270.83 ± 17.82	0.87 ± 0.10
SD	3.5 ± 1.96*	4.23 ± 0.39	0.97 ± 0.02	0.81 ± 0.45*	0.58 ± 0.05	0.98 ± 0.01	1.66 ± 1.06*	0.5 ± 0.32*	2.4 ± 0.16	0.85 ± 0.11
BM	26.12 ± 14.27*	24.37 ± 2.27	0.98 ± 0.01	4.83 ± 2.69*	2.84 ± 0.26	0.99 ± 0.01	11.27 ± 7.35	4.21 ± 2.52*	13.6 ± 0.9	0.83 ± 0.13
SY	1.5 ± 0.82*	1.57 ± 0.15	0.97 ± 0.01	0.86 ± 0.48*	0.73 ± 0.07	0.98 ± 0.01	0.86 ± 0.56	0.33 ± 0.20*	1.15 ± 0.08	0.83 ± 0.13
TSW	3.42 ± 1.9*	4.41 ± 0.47	0.97 ± 0.02	4.06 ± 2.21*	4.43 ± 0.41	0.97 ± 0.01	3.58 ± 1.97*	0.19 ± 0.17	4.68 ± 0.33	0.96 ± 0.03
OIL	0.59 ± 1.25	1.81 ± 1.26	0.39 ± 0.63	0.00 ± 0.00	1.32 ± 0.59	-	0.53 ± 0.53	0.00 ± 0.00	1.32 ± 0.49	0.62 ± 0.27
PRO	0.00 ± 0.00	0.97 ± 0.38	-	0.00 ± 0.00	0.56 ± 0.25	-	0.00 ± 0.00	0.00 ± 0.00	0.77 ± 0.24	-

*Significance at the 0.05 probability level (based on log-likelihood ratio test).

3.3.4 Across year analyses

Results generated from analysis across two years (year 1; 2019/2020, year 2; 2020/2021) in Palmerston North revealed significant (P < 0.05) differences for the traits; seedling establishment, stand establishment and biomass yield among six evaluated cultivars (Table 3.19). No significant (P > 0.05) differences were observed across two years for plant height and stem diameter.

Across both years, Finola had one of the highest seedling establishments (45.26%), with Futura 75 recording the lowest (38.02%). Finola's seedling establishment was statistically comparable (P < 0.05) to all cultivars except Futura 75. Férimon 12 reported the highest stand establishment (32.76%), and CFX-2 showed one of the lowest (17.84%). Stand establishment of CFX-2 did not differ significantly (P > 0.05) from CRS-1, Katani, and Finola. In terms of biomass yield, Futura 75 ranked highest across both years, with biomass yield of 21.39 t/ha, and Finola recorded the lowest (3.22 t/ha). However, Finola's biomass yield was not significantly (P > 0.05) different from that of CFX-2, Katani, and CRS-1.

Table 3.19. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seedling establishment (%), stand establishment (%), biomass yield (t/ha), plant height at harvest (cm) and stem diameter (mm), measured from the six hemp cultivars, generated from analysis across two years (year 1; 2019/2020, year 2; 2020/2021) at Palmerston North.

Entry	Seedling establishment (%)	Stand establishment (%)	Biomass yield (t/ha)	Plant height at harvest (cm)	Stem diameter (mm)
CFX-2	43.38[a]	17.84[c]	4.30[bc]	99.93[d]	8.13[bc]
CRS-1	42.25[ab]	18.73[c]	6.06[bc]	121.33[c]	9.19[b]
Férimon 12	41.87[ab]	32.76[a]	9.81[b]	151.01[b]	9.40[b]
Katani	42.44[ab]	19.69[c]	4.36[bc]	88.75[d]	7.94[bc]
Futura 75	38.02[b]	24.24[b]	21.39[a]	193.70[a]	11.21[a]
Finola	45.26[a]	20.50[bc]	3.22[c]	82.08[d]	7.14[c]
± SE	1.18	1.18	1.41	5.37	0.47
Sources of variation					
Cultivar	*	***	***	***	***
Year	***	***	***	ns	**
Cultivar × year	*	***	**	ns	ns

Values with the same letter within the same column are not significant (*P* > 0.05); * Significant at *P* < 0.05; ** Significant at *P* < 0.01; *** Significant at *P* < 0.001; ns; not significant.

Table 3.20 shows data on seed yield, thousand seed weight, seed oil, and protein content for the five hemp cultivars evaluated over the two years. There were significant (*P* < 0.05) differences among the cultivars for mean seed yield across the years. There were no significant (*P* > 0.05) differences for the traits of thousand seed weight, seed oil, and protein content across the years. Férimon 12 had the highest seed yield (3.21 t/ha), while Finola had the lowest yield, statistically similar to the other three cultivars. Across the years, the thousand seed weight ranged from 13.25 g to 18.31 g, whereas the oil and protein content ranged from 31.55% to 33.89% and 24.28% to 27.13%, respectively.

Table 3.20. Best linear unbiased estimates (BLUE) and associated standard errors (±SE) generated for the traits seed yield (t/ha), thousand seed weight (g), seed oil content (%) and seed protein content (%) measured from the five hemp cultivars, generated from analysis across two years (year 1; 2019/2020, year 2; 2020/2021) at Palmerston North.

Entry	Seed yield (t/ha)	Thousand seed weight (g)	Seed oil content (%)	Seed protein content (%)
CFX-2	1.68^{b}	16.09^{b}	31.76^{a}	25.80^{ab}
CRS-1	1.68^{b}	18.31^{a}	31.55^{a}	24.28^{b}
Férimon 12	3.21^{a}	17.73^{a}	31.94^{a}	27.13^{a}
Katani	1.66^{b}	15.22^{b}	33.41^{a}	24.33^{b}
Finola	1.02^{b}	13.25^{c}	33.89^{a}	24.52^{b}
± SE	0.17	0.37	0.72	0.56
Sources of variation				
Cultivar	***	***	ns	*
Year	ns	ns	ns	*
Cultivar × year	**	ns	ns	ns

Values with the same letter within the same column are not significant ($P > 0.05$); * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns; not significant.

3.3.4.1 Genotypic variance components, cultivar × year (G × E) interaction variance components and line mean broad sense heritability estimates

Significant genotypic variation ($\boldsymbol{\sigma_g^2}$) was observed among the cultivars based on performance across both years for the traits; PH; plant height at harvest, SD; stem diameter, BM; biomass yield, and TSW; thousand seed weight (Table 3.21). Of these traits, only BM; biomass yield exhibited significant ($P < 0.05$) cultivar × year ($\boldsymbol{\sigma_{gy}^2}$) interaction (Table 3.21). Besides biomass yield, the traits St. EST; stand establishment and SY; seed yield also showed significant ($P < 0.05$) cultivar × year ($\boldsymbol{\sigma_{gy}^2}$) interaction across years at Palmerston North. Line mean broad sense heritability ($\boldsymbol{h_b^2}$) for most traits across years at Palmerston North, excluding Se. EST; stand establishment and OIL; seed oil content, exceeded 0.55 (Table 3.21).

Table 3.21. Genotypic variance (σ_g^2), cultivar × year (G × E) interaction variance (σ_{gy}^2), residual variance (σ_ε^2), their associated standard errors (±SE) and line mean broad sense heritability (h_b^2) for nine traits measured from six industrial hemp cultivars, estimated across two years; (year 1; 2019/2020, year 2; 2020/2021) at Palmerston North. Traits: Se. EST; seedling establishment, St. EST; stand establishment, PH; plant height at harvest, SD; stem diameter, BM; biomass yield, SY; seed yield, TSW; thousand seed weight, OIL; seed oil content, and PRO; seed protein content.

Trait	σ_g^2	σ_{gy}^2	σ_ε^2	h_b^2
Se. EST	2.19 ± 3.58	4.45 ± 4.08	10.19 ± 2.73	0.39 ± 0.47
St. EST	17 ± 18.28	26.25 ± 15.21*	7.82 ± 2.03	0.55 ± 0.34
PH	1799.7 ± 968.57*	21.34 ± 46.57	183.43 ± 48.53	0.98 ± 0.02
SD	1.8 ± 1.11 *	0.16 ± 0.34	1.36 ± 0.36	0.88 ± 0.10
BM	39.94 ± 25.17*	10.57 ± 8.03*	15.83 ± 3.73	0.85 ± 0.12
SY†	0.51 ± 0.38	0.24 ± 0.18*	0.24 ± 0.06	0.77 ± 0.19
TSW†	3.99 ± 2.34*	0.00 ± 0.00	0.95 ± 0.25	0.97 ± 0.02
OIL†	0.51 ± 0.78	0.58 ± 1.05	1.33 ± 0.77	0.45 ± 0.49
PRO†	0.86 ± 0.95	0.78 ± 0.98	1.15 ± 0.54	0.56 ± 0.37

*Significance at the 0.05 probability level (based on log-likelihood ratio test).
† Variance components and line mean broad sense heritabilities were estimated among five hemp cultivars.

3.4 Discussion

3.4.1 Differences in performances of cultivars within and across environments

3.4.1.1 Biomass yield

Hemp cultivation is greatly influenced by the interaction between its cultivars and the surrounding environment, affecting both the yield and quality of the produced biomass (Blandinières & Amaducci, 2022). Factors such as temperature, light quality, stress, and location, as well as crop management techniques like sowing date, plant density, fertilization, and irrigation, all play crucial roles in determining biomass yield (Amaducci et al., 2008; Aubin et al., 2015; Blandinières & Amaducci, 2022; Burczyk et al., 2009; Finnan & Burke, 2013; Struik et al., 2000; Tang et al., 2017; Vera et al., 2010). Biomass yield depends significantly on the type of hemp cultivar grown; the purpose (seed, biomass/fibre, or dual-purpose) for its cultivation, and whether it is monoecious or dioecious. Usually, fibre, and dual-purpose cultivars, particularly monoecious types, yield more biomass than seed-type cultivars (Aubin et al., 2015; Laate, 2012; Slaski, 2015).

The present study indicated considerable differences in biomass yield across different sites and years, with results varying from 1.43 t/ha to 28.41 t/ha. Factors such as environmental conditions, fertilization techniques, plant density, and the specific cultivar chosen are the primary influencers of such differences. Across different hemp growing regions of the world, the biomass yield of hemp has been found to vary substantially. For instance, in Central Italy, the biomass yield ranged from 3.4 to 8.0 t/ha, as reported by Campiglia et al. in 2017. Cappelletto et al. (2001) observed a higher range of 28.6 to 31.2 t/ha in Northern Italy. Similarly, other regions showed varying results, such as 5.2 to 12.8 t/ha in Southwest Germany (Scheliga et al., 2018), 7.8 to 14.5 t/ha in Southern Sweden (Svennerstedt & Sevenson, 2006), 9.9 to 14.4 t/ha in another study from Southern Sweden (Prade et al., 2011), 13.5 to 15.3 t/ha in the Netherlands (Struik et al., 2000), 13.5 to 21.3 t/ha in Latvia (Adamovics et al., 2016), and 13.1 to 26.3 t/ha again in Central Italy (Ascrizzi et al., 2016). This diversity in biomass yield highlights the important roles that both genotype and environmental factors play in hemp cultivation. In a study by Wulff et al. (2021), the average biomass yield in New Zealand was found to range between 8.86 and 13.1 tons per hectare.

The monoecious fibre cultivar Futura 75 consistently yielded the highest biomass, regardless of the environmental conditions (within sites, across sites and years). Numerous studies conducted in varying environments have revealed that Futura 75 typically outperforms other tested cultivars regarding biomass yield. This superior yield of Futura 75 is attributed to a higher stem production with an elevated fibre content (Amaducci et al., 2008). Between 2010 and 2013, a Lithuanian trial assessed hemp cultivars for biomass yield potential and showed Futura 75 as a top performer. In 2010, Futura 75 produced the maximum biomass at 11.8 t/ha and was reported as one of the most productive cultivars in 2011, with a biomass yield of 12.3 t/ha (Jankauskienė & Gruzdevienė, 2015). A separate study carried out in Italy by Amaducci et al. (2008) examined the impact of agronomic factors on the yield and quality of two hemp cultivars. In this study, regardless of the combination of experimental factors (cultivation year, genotype, plant population, and harvesting time), Futura 75 consistently outperformed the other cultivar, Tiborszallasi. The seed cultivar Finola, in the irrigated site during the

2020/2021 growing season, recorded the lowest biomass yield of 1.43 t/ha (Table 3.12). This yield was notably lower than the biomass yields reported in the earlier studies. The observed decrease in biomass was primarily attributed to the poor plant establishment and stand establishment observed at this site during the 2020/2021 growing period, as indicated in Table 3.12.

Irrespective of the site or the growing year, monoecious dual-purpose cultivar Férimon 12 was second only to cultivar Futura 75, consistently producing a higher biomass yield than other seed cultivars. This outcome aligns with the results of a study conducted by Wulff et al. (2021) in Canterbury, New Zealand. This study examined the growth, dry matter accumulation, and composition of hemp crops with a focus on fibre production. Among the cultivars tested, which included Férimon, Kompolti, and Futura 75, the latter consistently yielded the highest average biomass, irrespective of the sowing date (22/10/2020, 11/11/2020, and 9/12/2020 for sowing dates 1, 2, and 3 respectively). On average, across all sowing dates, Futura 75 produced 12.8 t/ha, with Férimon following closely, delivering a yield of 10.5 t/ha of biomass.

In the current study, dioecious seed cultivars CRS-1, CFX-2, Katani and Finola consistently generated lower biomass yields compared with monoecious fibre and dual-purpose cultivars, Futura 75 and Férimon 12, respectively, across all sites and years. This observation is similar to the results from a trial conducted at the Manjimup site in Western Australia during the 2021/2022 growing season. In the Manjimup trial, a notable difference was observed among the eight seed and dual-purpose hemp varieties tested, which included Ruby, CFX-2, CRS-1, Katani, Han NE, Férimon 12, and Henola. Of these, the monoecious dual-purpose cultivar Férimon 12 outperformed the rest, with the highest total dry matter production of 8196 kg/ha, while CFX-2 yielded the least (Miyan, 2022).

3.4.1.2 Other Agronomic traits

3.4.1.2.1 Seedling establishment and stand establishment

During the 2019/2020 growing season, seedling establishment at Palmerston North was between 66.10% and 73.57%, while at the Wairarapa site it ranged from 67.81% to 75.13%. Similarly, stand establishment at the Palmerston North site ranged from 30.57% to 56.57%; in the Wairarapa, it was between 35.42% and 56.70%. These rates were high and consistent among the cultivars at both sites. However, these percentages dropped in the 2020/2021 growing season. Comparing both sites, the rainfed site showed higher seedling establishment percentages (ranging from 23.58% to 47.67%) and stand establishment percentages (ranging from 19.49% to 32.24%) than the irrigated site (seedling: 9.94% to 19.40%; stand: 2.92% to 8.95%). However, the performance differences between the cultivars, based on these two traits, were not significant. This observation was consistent both within sites (whether irrigated or rainfed) and across sites. The only exception was that stand establishment proved to be significant at the irrigated site. Over the years, there were significant differences in seedling and stand establishment. Across all cultivars, seedling establishment and stand establishment percentages were lower in the 2020/2021 growing season, ranging from 9.94% to 19.40% and 2.92% to 8.95%, respectively. In contrast, During the 2019/2020 season, seedling establishment rates ranged from 66.10% to 73.57%, while stand establishment rates varied between 30.57% and 56.57%.

Low seedling and stand establishments consistently pose significant challenges in hemp production, often resulting in crop failures (Ely et al., 2022; Hall et al., 2013; Jeff & Williams, 2019; Kostuik & Williams, 2019; Mi et al., 2020; Miyan, 2022). A primary factor contributing to this is the high soil temperature during sowing. In the week following sowing, the soil temperature at the irrigated site ranged between 20.19°C and 24.08°C, while the rainfed site had temperatures ranging from 20.11°C to 22.95°C. Research suggests optimal 8–10°C soil temperatures ensure fast and uniform germination and emergence (Blandinières & Amaducci, 2022; Desanlis et al., 2013). When planted in warmer soils (> 10°C) with sufficient moisture, most hemp varieties will germinate in 3 to 5 days (Jeff & Williams, 2019). In contrast to the previously mentioned optimum soil temperature, Ely et al. (2022) indicated that hemp is usually sown at the same time as corn (*Zea mays*), ideally when soil temperatures are around 15°C. In the 2020/2021 season, the sowing was delayed, resulting in planting at a time when soil temperatures were higher than the optimal germination temperatures specified in cited studies. This could have negatively impacted seedling germination and establishment.

Seed quality, specifically germination percentage and vigour, play a crucial role in successful field establishment and crop production (Ely et al., 2022). Despite testing seed germination percentages (between 95% and 84% - results presented in Appendix 3) and adjusting seeding rates accordingly, the seedling establishment remained low for all cultivars in the second growing season 2020/2021. Ely et al. (2022) suggested that while increasing seed sowing rates can compensate for low germination to some extent, it does not necessarily address the issue of poor vigour. Thus, seeds might exhibit good germination percentages but still suffer from low emergence due to poor vigour. This lack of vigour is especially concerning with imported seeds (Ely et al., 2022; Jeff & Williams, 2019). The first hemp trial in New Zealand was conducted at 19 sites over two seasons (2001/2002 and 2002/2003) following the New Zealand Ministry of Health's licensing of low-THC hemp seed imports in 2001. This trial evaluated 11 cultivars and consistently noted poor emergence rates of imported cultivars, with rates as low as 1% at one site. Researchers attributed this to the negative effects of phytosanitation treatments, mandated by the Biosecurity Act of 1993, on seed viability (McPartland et al., 2004).

Improper seed placement is another factor that can hinder seedling establishment. While the general recommendation is a planting depth of 0.5–1.0 cm (Ely et al., 2022; McPartland et al., 2004), a depth of 2–3 cm can be more suitable in especially dry conditions to prevent seed desiccation prior to germination (Burczyk, 2003; Desanlis et al., 2013; Visković et al., 2023). In this study, due to notably dry conditions and to deter consumption by birds, seeds were planted at a depth of 3 cm (Desanlis et al., 2013; McPartland et al., 2004). It is worth noting that the optimal sowing depth varies based on factors like seed quality, soil characteristics, ambient conditions, and precipitation patterns during planting (Ely et al., 2022). The reduced seedling establishment observed in the 2020/2021 season might be due to the combination of high soil temperature at the planting time, planting depth, and low seed vigour.

While seedling and stand establishment at the irrigated site were inferior to the rainfed site, plants under irrigation had superior height, stem diameter, biomass yield, seed yield, and thousand seed weight (Tables 3.12–3.15). Soil variation and the moisture levels between the irrigated and rainfed sites may have influenced this result. These results contrasted with previous studies that showed increased yield responses to increased plant densities but aligned with Meijer et al. (1995) and Van der Werf (1994), who reported high biomass yields in their research due to the limited survival of plants until harvest. Similarly, Kerckhoffs et al. (2017) noted that higher sowing rates adversely impacted stem dry weights. Additionally, an experiment by Townshend & Boleyn (2008) with the monoecious cultivar Fasamo, across target populations ranging from 125 to 250 plants/m^2, indicated a slight yet insignificant decline in seed yield against established plant population, suggesting a yield reduction of 0.069 kg/ha for each additional plant/m^2. Tang et al. (2017) found that planting density between 30 and 240 plants/m^2 did not affect seed yield significantly. Studies by Amaducci et al. (2002) and Tsaliki et al. (2021) also observed negligible morphological variances across different plant densities. Moreover, Amaducci et al. (2002) and Hall et al. (2014) indicated that plants exhibit pronounced growth and heavy individual plant weight at low densities. These could explain the observed superior traits at the irrigated site, including greater plant height, stem diameter, biomass yield, seed yield, and thousand seed weight.

Among all cultivars, the monoecious cultivar Futura 75 consistently showed the lowest seedling establishment across all environments. Of the two monoecious cultivars, Férimon 12 exhibited superior stand establishment across all environments except for Wairarapa. This can be attributed to Férimon 12's higher seedling establishment rates than Futura 75. Although dioecious cultivars exhibited higher seedling establishment rates than both monoecious cultivars, monoecious cultivars demonstrated higher stand establishment percentages. This is expected, as dioecious cultivars typically have a higher ratio of male plants (Mediavilla et al., 2001), which die after shedding pollen (Mediavilla et al., 2001; Strzelczyk et al., 2022). Consequently, monoecious Férimon 12 and Futura 75 yielded more biomass and seeds than dioecious types, agreeing with Amaducci et al.'s (2008) findings of monoecious cultivars outperforming in stem yields. Severe wind events are one of the factors that cause plant damage, contributing to crop loss (Gibson, 2007; Merfield,1999). Severe wind events resulting in some degree of plant damage and subsequent crop loss were noted at all sites except the rainfed site. The two factors mentioned above, attributed to the reduction difference between the seedling and stand establishment were common in all environments in both growing seasons. However, as mentioned, no wind-related damages were recorded at the rainfed site. Apart from these factors, *Sclerotinia sclerotiorum* infected some plants, but only in the irrigated site during the growing season 2020/2021, consistent with the disease's prevalence in humid, wet conditions (Bains et al., 2000; McPartland & Rhode, 2005; Meijer et al., 1995), explaining its observation solely at the irrigated site.

3.4.1.2.2 Plant height and stem diameter

The height and stem diameter of plants are influenced by several factors: cultivar (Aubin et al., 2016; Tang et al., 2016), growing year (Tsaliki et al., 2021), climatic conditions, and cultural practices like plant density (Burczyk et al., 2009; Cosentino et al., 2013) and nitrogen fertilization (Campiglia et al., 2017; Vera et al., 2004). Among these, cultivar stands out as the most influential factor (Tang et al., 2016). Typically, seed-type cultivars are bred to be shorter to optimize the seed harvest index (Aubin et al., 2016; Small, 2018), while fibre cultivars are developed for tall, unbranched stems, ideal for hemp fibre extraction from the stems (Clarke, 1999; Clarke & Merlin, 2016; Small, 2018).

In the 2019/2020 growing season, cultivars exhibited significant plant height and stem diameter differences within sites, with no significant differences across sites. Conversely, these parameters differed significantly within and across sites in the 2020/2021 season. This difference was pronounced between irrigated and rainfed sites, reflecting the prevailing environmental conditions at each site. For example, the plant heights at the irrigated site ranging from 83.00 cm to 201.69 cm, compared with 23.78 cm to 84.46 cm at the rainfed site. As Campbell et al. (2019) indicated, it seems that plant height in the present study is primarily influenced by environmental factors, especially irrigation and soil conditions. While hemp can grow between 200–400 cm based on the cultivar (Bouloc et al., 2013), most studies report heights ranging from 70.5 cm to 309 cm (Baldini et al., 2020; Cappelletto et al., 2001; Ferfuia et al., 2021; Sankari, 2000; Tang et al., 2016; Tsaliki et al., 2021; Vera et al., 2004). However, Clarke (1999) highlighted that hemp plants exhibit limited foliage growth in arid areas and can mature, producing seeds at a mere height of 20 cm, corresponding to the shorter heights observed at the rainfed site in 2020/2021. Excluding the shortened height in the rainfed site, plant heights at other sites during both seasons are consistent with general observations reported in other studies. Across both sites and years, Futura 75 had the highest growth, while Finola was the shortest. These observations align with Ferfuia et al. (2021) and Tsaliki et al. (2021), who found Futura 75 to be taller and Vera et al. (2004), who noted Finola to be among the shorter cultivars.

3.4.1.3 Seed yield

Cultivar and the environment have large effects on hemp seed production (Ferfuia et al., 2021; Lan et al., 2019; Legros et al., 2013; Tang et al., 2016). Several factors, including the choice of cultivar, environmental conditions like temperature, soil characteristics, and water availability, as well as cultivation practices such as fertilization, irrigation, sowing and harvesting dates, and plant density, all have a significant impact on seed yield (Baldini et al., 2020; Legros et al., 2013; Maļceva et al., 2011; Stafecka et al., 2016; Tsaliki et al., 2021; Żuk-Gołaszewska & Gołaszewski, 2020). In this study, there were considerable differences in seed yield for the assessed cultivars, both within and across sites, as well as across years, ranging from 0.018 to 3.78 t/ha (Tables 3.8 and 3.13). Diverse seed yields have been recorded across multiple geographic locations. In North Dakota, USA, seed yields ranged between 1890 to 2300 kg/ha (Lan et al., 2019), while in Northern Greece, the range ranged from 2.1 to 2.9 t/ha (Tsaliki et al., 2021). Similarly, in Northern Iran, seed yields varied from 288.70 to 3182.60 kg/ha (Abdollahi et al., 2020), in Poland, the range was 1.5 to 2.1 t/ha (Teleszko et al., 2022), and in North-East Italy, it fluctuated between 0.73 to 1.11 t/ha (Baldini et al., 2020). Additionally, seed yields in Latvia ranged from 0.83 to 2.39 t/ha (Stafecka et al., 2016), in Alberta, Canada, it varied from 0.6 to 3.5 t/ha (Slaski, 2017 as cited in O'Callaghan et al., 2018), and in Italy, France, and the Czech Republic, the range was 0.3 to 2.4 Mg/ha (Tang et al., 2016). The average seed yield in New Zealand ranged from 809 to 1823 kg/ha (McPartland et al., 2004; Townshend & Boleyn, 2008).

In the present study, the highest average seed yield was recorded in the 2020/2021 growing season at the irrigated site, while the Wairarapa site registered the lowest in the 2019/2021 growing season. These observed seed yields were, respectively, higher and lower than the ranges typically noted in other studies. However, the maximum average seed yield recorded in this study (3.78 t/ha) aligns closely with findings from an experiment conducted across irrigated and dryland sites in Alberta, Canada, where a similar maximum seed yield of 3.5 t/ha at the irrigated site was reported (Slaski, 2017 as cited in O'Callaghan et al., 2018).

During the 2019/2020 growing season, the trial at the Wairarapa site resulted in lower seed yield (0.018 t/ha ~ 0.02 t/ha). Despite Wairarapa having a good establishment, resulting in a high stand establishment compared with Palmerston North (Table 3.7 and 3.5, respectively), the seed yield measured among the cultivars was low. This was mainly attributed to bird predation, as the plant at the trial site lacked protection from bird damage, which significantly diminished the seed yield. The issue of bird predation causing excessive losses is a common problem across New Zealand, necessitating the implementation of control measures such as repellents and bird netting (Komahan et al., 2019; McPartland et al., 2004; Swanepoel et al., 2018; Townshend & Boleyn 2008).

Across both Palmerston North and Wairarapa in the 2019/2020 growing season and across two years, Cultivar Férimon 12 consistently exhibited superior seed yield. However, it is important to note that this analysis considered only five cultivars and did not include cultivar Futura 75 (section 3.2.6.2). In the subsequent growing season (2020/2021), Futura 75 outperformed the rest in terms of seed yield at both the irrigated and rainfed sites, with Férimon 12 consistently ranking just behind. Both cultivars are of the monoecious type. Previous studies have frequently noted a trend where monoecious hemp cultivars outperform the dioecious cultivars in terms of seed yield (Aubin et al., 2016; Baxter & Scheifele, 2000; Bócsa & Karus, 1998; Salentijn et al., 2015). Dioecious cultivars typically contain a higher ratio of male plants (Mediavilla et al., 2001), a factor that might contribute to their lower seed yields, thus underlining the advantages of monoecious cultivars. The difference is also evident in comparing seedling and stand establishments between the two types (as discussed earlier). Although dioecious cultivars demonstrated equal or higher seedling establishment, they did not translate into higher seed yield compared with the monoecious cultivars. This could likely be attributed to a higher number of male plants in dioecious cultivars, which are also associated with lower stand establishment rates than monoecious cultivars. These findings highlight the benefit of monoecious cultivars in optimizing seed yield.

Few studies have recognized the considerable seed yield potential of Futura 75, despite it primarily being a fibre cultivar. One such study examined the effect of G × E interaction on hemp stem and seed production (Tang et al., 2016). This research included fourteen commercial cultivars (Beniko, Bialobrzeskie, Epsilon 68, Fedora 17, Felina 32, Férimon, Futura 75, Markant, Monoica, Tygra, CS, KC Dora, Tiborszallasi, Tisza). Tang et al. (2016) evaluated the performances of the cultivars within four distinct European regions: Latvia, the Czech Republic, France, and Italy. The study found that Futura 75 consistently yielded the highest bast fibre among the monoecious cultivars tested in Italy, the Czech Republic, and France, while its seed yield was roughly equivalent to the average yield in the experiment. Thus, the researchers recommended Futura 75 for dual-purpose production, especially when a high fibre yield is a primary objective. However, a contrasting observation was noted in a separate three-year field experiment conducted in northern Greece under Mediterranean conditions. The study, which aimed to evaluate the productivity (in terms of fibre and seeds) of six monoecious hemp varieties (Santhica 27, Fedora 17, Felina 32 and Futura 75, Tygra and Bialobrzeskie), revealed that while Futura 75 delivered the highest fibre productivity, it ranked lowest in seed yield productivity (Tsaliki et al., 2021). All this information underlines the importance of regional environmental considerations when selecting hemp cultivars. It also underscores the need for further research on G × E interactions to optimize the use of cultivars like Futura 75.

A general observation on seed yield among the cultivars, examined within and across sites and two years, mostly agrees with findings from a 2021/2022 growing season trial in Manjimup, Western Australia. This trial evaluated eight seed and dual-purpose hemp varieties, namely Ruby, CFX-2, CRS-1, Katani, Han NE, Férimon 12, and Henola, revealing significant differences in their seed yield. Férimon 12 emerged as the top performer, producing the highest seed yield (1249 kg/ha), whereas Katani lagged behind with the lowest yield (981 kg/ha). The top three performers from the present study, Férimon 12, CFX-2, and CRS-1 (excluding Futura 75 and Finola, which were not included in the trial in Manjimup), reflected the seed yield performance levels in the Manjimup trial, with Katani performing lowest in both scenarios (Miyan, 2022). However, the seed yields in the present study generally exceeded those of the Manjimup trial. Additionally, the substantial seed yield of Férimon 12 in the present study agrees with the results from an Austrian field trial conducted on an organic farm. This trial evaluated the seed yield of 20 hemp varieties over three harvest dates, demonstrating that Férimon 12 consistently yielded high seed quantities on all three occasions (Vogl et al., 2004).

Despite showing comparable or even superior plant establishment rates to other cultivars, the seed yield of Finola was observed to be the lowest in all sites and across the years studied. This observation agrees with the findings of Aubin et al. (2016), who evaluated eleven cultivars: Anka, Alyssa, CanMa, CFX-1, CFX-2, CRS-1, Delores, Finola, Jutta, Yvonne, and Férimon, for biomass, seed yields, and composition across seven contrasting environments in Québec, Canada. Their study found that Finola produced the least seed yield in all locations, varying between 237 kg/ha and 1844 kg/ha, a conclusion that aligns with the present study. Another study in Tasmania investigated the adaptability of five dual-purpose grain cultivars (CFX, Finola, Crag, and Zolo 11) and eleven landraces from different parts of the world across three sowing dates. It further reinforced that Finola's seed yield was consistently the lowest across all sowing dates, ranging between 0.1 and 0.2 t/ha (Boersma et al., 2018).

In contrast to these findings, Callaway (2004a), the breeder of Finola, reported that agricultural trials in Eastern Finland predicted an average seed yield close to 1.7 t/ha.

Callaway also mentioned that this estimated yield surpassed the results of any other industrial hemp variety evaluated until that point. Further, Callaway (2004b) indicated that, under favourable agronomic conditions, independent trials recorded yields that exceeded 2000 kg/ha. It is, however, important to note that Finola is a seed-producing variety specifically bred for Nordic hemp production conditions (Baraniecki et al., 2022; Callaway, 2004b). The findings from the present study and the previous studies underscore the importance of multisite trials with diverse cultivars over multiple years to thoroughly assess cultivar stability and adaptability across different environments.

3.4.1.4 Thousand seed yield

Factors affecting thousand seed weight include cultivar, geographic location, growing year, and their interaction (Abdollahi et al., 2020; Baldini et al., 2020; Tsaliki et al., 2021). For many plant species, environmental variables like temperature, rainfall quantity and pattern, and relative humidity, among others, notably affect seed phenotypic variation (Abdollahi et al., 2020; Cook & Scoot, 1993; Galloway, 2001). In the current study, a significant difference was observed in the thousand seed weight among evaluated cultivars, within and across sites and years, with values ranging from 8.10 g to 19.26 g (Tables 3.6 and 3.8). These findings align with the ranges documented in studies from various regions and on diverse hemp cultivars: 5.20–15.28 g in Northern Iran (Abdollahi et al., 2020), 13.01–18.54 g in Kansas, USA (Xu et al., 2021), 14.1–16.7 g in North Dakota (Hanson et al., 2018 as cited in Xu et al., 2021), 17–23 g in Romania (Mihoc et al., 2012), 12.9–50.6 g in China (Chen et al., 2010), 5.13–18.10 g in North-East Italy (Baldini et al., 2020), 4.1–18.9 g in North-Eastern Italy (Ferfuia et al., 2021), 6.1–10.9 g in Northern Greece (Tsaliki et al., 2021), and 9.4–22.8g in Italy (Galasso et al., 2016).

In the present study, within and across all environments (within sites, across sites and years), the Finola cultivar consistently exhibited the lowest thousand seed weight. Despite the notable advancements in hemp seed production with the introduction of FIN-314 (Finola), a high-yielding auto-flowering grain cultivar (Callaway, 2004b), its seeds are approximately half the size of several commercial cultivars (Schluttenhofer & Yuan, 2017). This observation is aligned with the findings of the present study.

3.4.1.5 Seed oil and protein content

Hemp seeds usually contain 25-35% oil (Abdollahi et al., 2020; Callaway, 2004a; Deferne & Pate, 1996; Farinon et al., 2020; Latif & Anwar, 2009; Pate, 1999) and 20-25% of protein (Callaway, 2004a; Deferne & Pate, 1996; Farinon et al., 2020; Latif & Anwar, 2009; Pate, 1999; Tang et al., 2006). The oil content in the present study was in the range of 30.66–34.02%. This range is similar to the ranges reported by Abdollahi et al. (2020), Calzolari et al. (2021), Galasso et al. (2016), Farinon et al. (2020), Kriese et al. (2004), Lan et al. (2019) and Taaifi et al. (2021). The oil content in the present study is higher than the range reported by Xu et al. (2021) and Baldini et al. (2020). The protein content in the present study ranged between 22.19 and 27.52%. This range is similar to the range reported by Lančaričová et al. (2021), Callaway (2004a), and House et al. (2010). The protein content in the present study is higher than the range reported by Anwar et al. (2006), Baldini et al. (2020), Farinon et al. (2020), Lan et al. (2019), Taaifi et al. (2021), Vonapartis et al. (2015) and Xu et al. (2021).

In recent research, significant differences in seed oil and protein content have been observed, with these differences largely attributed to factors such as cultivar, seed provenience, growing season, and climatic conditions (Abdollahi et al., 2020; Anwar et al., 2006; Calzolari et al., 2021; Ferfuia et al., 2021; Galasso et al., 2016; Kriese et al., 2004; Lančaričová et al., 2021; Miyan, 2022; Ross et al., 1996). Calzolari et al. (2021) asserted that variations in oil content are predominantly due to cultivars, emphasizing seed provenience as a key determinant. Supporting this claim, Miyan (2022) found significant differences in oil content by varieties. However, no significant effect was noted based on different sowing times or their interactions in Miyan’s (2022) study. The influence of cultivar and environment on hemp seed nutritional properties, especially on protein and oil content, has been supported by Irakli et al. (2019). They indicated that protein and oil content are mainly affected by cultivar. While in a study across two years by Baldini et al. (2020), all cultivars evaluated showed consistent seed oil content across years; exceptions were noted for two cultivars (CS and Ermes). Abdollahi et al. (2020) emphasized the significance of the cultivar and climatic conditions in seed oil yield for hemp. The interaction between these factors did not influence the seed oil content in their study. The present study found significant differences among the cultivars evaluated within Palmerston North and across Palmerston North and Wairarapa during the growing season 2019/2020.

The protein content in hemp is influenced by various factors such as environment, cultivar, year of cultivation, and cultural practices (Aubin et al., 2015; Galasso et al., 2016; Irakli et al., 2019). Research by Lančaričová et al. (2021) found that growing location and harvesting time (seed maturity) significantly impact protein content, yet there was consistency in protein levels across different years. Another study conducted by Pavlovic et al. (2019) compared the protein content of Futura 75 and Finola cultivars and found no significant difference. Similarly, Miyan (2022) found no significant difference in seed protein content based on the sowing time or among different cultivars. This aligns with the present study's findings, where no significant difference in seed protein content was observed across Wairarapa, irrigated, and rainfed sites.

However, there is a noticeable inconsistency in findings related to the impact of cultivars and environments on seed oil and protein content. This inconsistency is evident in the findings of the current study, where significant differences in seed oil content were only observed within the Palmerston North site, while significant differences in protein content were seen both within Palmerston North and across sites; Palmerston North and Wairarapa, during the 2019/2020 growing season. This highlights the complex and varied impact of cultivar and environmental factors on the nutritional properties of hemp seeds.

3.4.1.6 Summary of differences in performances of cultivars within and across environments

During the 2019/2020 growing period: (1) at Palmerston North, all traits measured from six cultivars (five for seed traits) showed significant differences, (2) in Wairarapa, all traits from six cultivars differed significantly, except for seed oil and protein contents and (3) across both sites, all traits measured from six cultivars varied significantly, except for plant height, stem diameter, biomass yield and seed oil content. During the trial period, Palmerston North had slightly lower temperatures and more rainfall than Wairarapa, as detailed in section 3.3.1.1. Palmerston North's soil was Manawatu silt loam, whereas Wairarapa's was Ahikouka silt loam. The former has more favourable attributes, such as low clay%, higher aeration in the root zone, and lower water logging and drought vulnerability (Appendix 2). These conditions likely influenced the reduced biomass yield, plant height, and stem diameter in the six cultivars evaluated at Wairarapa. Notably, these traits and seed oil content showed no significant differences across the sites. It should be noted that the influence of environmental conditions on seed yield and thousand seed weight was overshadowed by significant bird predation in Wairarapa.

During the 2020/2021 growing period: (1) At the irrigated site, all traits measured from six cultivars, except for seedling establishment, seed oil, and protein contents, showed significant differences, (2) at the rainfed site, all traits measured from six cultivars, excluding seedling and stand establishment, seed oil, and protein contents, differed significantly, and (3) across both irrigated and rainfed sites, all traits measured from the six cultivars differed significantly, except for seedling establishment, stand establishment, seed oil, and protein contents. These two trial sites (irrigated and rainfed), situated just 1.06 km apart, mainly contrasted in irrigation methods and soil types. As detailed previously, one site used consistent irrigation to maintain soil moisture above 50% of field capacity, while the other relied on rainfed conditions. The irrigated site had Manawatu silt loam (same as Palmerston North's 2019/2020 trial), and the rainfed site had Tokomaru silt loam. Compared to Manawatu, Tokomaru silt loam has less favorable attributes, such as limited root penetration, poor drainage, and susceptibility to both waterlogging and drought when not irrigated (Appendix 2). These distinct environmental factors explain the observed differences in traits, biomass yield, seed yield, thousand seed weight, plant height and stem diameter. Notably, despite these conditions, soil properties and moisture did not affect seed protein and oil contents or seedling and stand establishments, with the latter two even standing out at the rainfed site.

Across two growing seasons (2019/2020 and 2020/2021) in Palmerston North, only seedling and stand establishment, biomass yield, and seed yield showed significant differences among the evaluated cultivars. Section 3.3.1.3 details the weather during the 2019/2020 and 2020/2021 growing seasons: rainfall was 202.9mm, and temperatures ranged from 5.8°C to 30.5°C in the growing season 2019/2020, and in the 2020/2021 season, rainfall increased to 252.1mm with temperatures between 3.1°C and 27.6°C. Thus, the 2019/2020 season had more comprehensive temperature ranges but less rain than 2020/2021. Another key difference between the seasons was the sowing date. The late sowing in 2020/2021, resulted in higher soil temperatures during sowing, likely hindered germination leading to weaker seedling establishment. This, in turn, affected biomass and seed yields, making them higher than those at the rainfed site.

3.4.2 Genotype × Environment interaction and heritability

During the 2019/2020 growing period at Palmerston North, all evaluated traits, with exception of stem diameter, displayed significant genotypic variation. This suggests that the performance varied significantly across different cultivars for all traits, excluding stem diameter. In Wairarapa, all traits except seed yield, seed oil, and seed protein content varied significantly among the five cultivars. Across both sites, there was significant genotype × site interaction for seedling establishment, stand establishment, seed yield, thousand seed weight, and seed protein content. This indicated that there was a change in the relative performance among the cultivars across both sites for these traits. All these traits except stand establishment showed significant genotypic variation among cultivars.

During the 2020/2021 growing season at the irrigated site, all traits exhibited significant genotypic variation except for seedling establishment, seed oil content, and protein content. At the rainfed site, traits biomass yield, seed yield, thousand seed weight, plant height, and stem diameter showed significant genotypic variation among the six cultivars evaluated. Across both sites, only plant height, stem diameter, and thousand seed weight showed significant genotypic variation. There was a significant genotype × site interaction for plant height, stem diameter, biomass yield, and seed yield, indicating a change in relative performance among the cultivars for these traits across the sites. As expected, the cultivars responded to irrigation, resulting in increased biomass and seed yield. This result is consistent with the results from Campbell et al. (2019). It should be noted that among the traits with significant genotype × site interaction, only plant height and stem diameter showed significant genotypic variation.

Across the two years (growing seasons; 2019/2020 and 2020/2021), plant height, stem diameter, biomass yield, and thousand seed weight had significant genotypic variation among the cultivars. Among these traits, only biomass yield exhibited significant genotype × year interaction. Among the traits that did not show significant genotypic variation, both stand establishment and seed yield displayed genotype × year interactions. The insignificant genotype × year interactions for the other traits: seedling establishment, plant height, stem diameter, thousand seed weight, seed oil content and seed protein content, suggest consistent genotype performance over the years.

Research has demonstrated significant genotype × site interactions for various hemp plant traits such as; seedling establishment (Aubin et al., 2016), stand establishment (Campbell et al., 2019), biomass yield (Aubin et al., 2016; Campbell et al., 2019; Ferfuia et al., 2021; Petit et al., 2020), plant height (Aubin et al., 2016; Baldini et al., 2020; Campbell et al., 2019; Petit et al., 2020), stem diameter (Campbell et al., 2019; Petit et al., 2020), seed yield (Aubin et al., 2016; Baldini et al., 2020; Campbell et al., 2019; Ferfuia et al., 2021; Tang et al., 2016), thousand seed weight (Baldini et al., 2020; Ferfuia et al., 2021), seed protein content (Aubin et al., 2016; Baldini et al., 2020; Irakli et al., 2019), and seed oil content (Aubin et al., 2016; Baldini et al., 2020; Ferfuia et al., 2021; Irakli et al., 2019), among others. In the present study, only stand establishment (2019/2020 season), plant height and stem diameter (2020/2021 season), and biomass yield (over two seasons) exhibited significant G × E interactions. There could be various reasons for this discrepancy.

1. With only two sites, the environmental diversity may not have been large enough to show significant genotype × site interactions. For example, despite variation in soil type and moisture during the 2020/2021 season, only two traits, plant height and stem diameter, showed significant genotype × site interaction. The significant genotype × site interaction estimated for the traits plant height and stem diameter may have been due to the differences in soil types and the application of irrigation. However, there was no significant genotypic variation across the two environments for the traits seedling establishment, stand establishment, biomass seed yield, seed oil content and seed protein content.

2. Another potential reason for insignificant G × E interaction for most of the traits may be a small sample size. For example, seed oil and protein content were assessed from bulk samples of only two replicates. With only two environments and six genotypes, results from only two replicates may lack the statistical power needed to detect G × E interactions.

3. While the first-year trial during the growing season 2019/2020 was conducted across Palmerston North and Wairarapa, the second-year trial during the growing season 2020/2021 was carried out across irrigated and rainfed environments. The site at Palmerston North was consistent between the two years, providing an opportunity for estimating genotype × year interactions. However, it is worth noting that neither the first nor the second year's trials were carried out continuously over two years; for example, the trials across Palmerston North and Wairarapa were not repeated in the second year. Conducting these trials across multiple consecutive years would enable a more robust accounting for year-to-year variability, offering insights that a single-year study may not reveal.

The high line mean broad sense heritability estimates for the traits plant height, stem diameter, biomass yield, seed yield and thousand seed weight at all the sites (Palmerton

North, Wairarapa, irrigated site and rainfed site) and consistently high line mean broad sense heritability across all sites indicates the potential underlying genetic variation for these traits among the cultivars evaluated. Their potential use for developing breeding pools is worthwhile being considered.

3.5 Conclusions

Results from this study indicated that:

- The analysis of genotypic differences among the six hemp cultivars for all the traits studied, generated results that generally align with those published from experiments conducted in different overseas environments.
- The findings provide insights into the cultivars that perform well locally. Of the cultivars examined, the monoecious varieties Futura 75 and Férimon 12 consistently outperformed others across various environments and appear suitable for dual-purpose production. While hemp growers should have a primary production goal in mind, it is evident that monoecious hemp cultivars offer versatile crop utilization.
- The observed G × E interactions based on performance of the six cultivars evaluated in this study, present a complex narrative and differ from studies conducted in other countries. While a majority of the traits exhibited genotypic variation, only a few displayed significant G × E interactions. This suggests consistent responses across sites. It is worth noting that operational setbacks such as seed sourcing and limitations in setting up multiple testing sites constrained a more comprehensive analysis of G × E interactions.
- The traits plant height, stem diameter, biomass yield, seed yield, and thousand seed weight have shown high line mean broad sense heritability estimates. This indicates potential genetic variation that can be exploited for the improvement of hemp. Specifically, this can enhance development of new cultivars adapted to New Zealand's climate conditions.

It is important to note that this study was conducted across a small sample of environments. While the study could not fully characterize the G × E interaction, these results will be valuable for planning future hemp breeding programs in New Zealand. They offer foundational insights for future research on developing superior cultivars with better stability, uniformity and highly adapted to New Zealand climatic conditions. Further research should be conducted across the target population of hemp growing environments in New Zealand - expand trial locations, across multiple years, and incorporate more cultivars, including those bred in New Zealand. Multisite trials will provide a better understanding of environmental factors that influence key traits such as seed yield and biomass yield resulting in identifying material with specific and broad adaptation.

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CHAPTER 4

Estimation of quantitative genetic parameters of a population of industrial hemp using half-sib families

Abstract

Industrial hemp (*Cannabis sativa* L.) possesses significant agricultural potential as a versatile crop for food, fibre and medicine. Despite the growing demand, the breeding efforts to improve the adaptation and production of hemp for environments in New Zealand are limited. Presently, 20 hemp cultivars have been approved for growing in New Zealand, with fifteen of them being introduced cultivars. In this context, research aimed at the genetic improvement of hemp to develop cultivars specifically adapted to New Zealand's climatic conditions that would offer both economic and agronomic advantages is paramount. Considering the significance of estimating quantitative genetic parameters in plant breeding, a key objective of this study was to quantify the magnitude of additive genetic variation for a range of traits within a breeding population. This objective was achieved by conducting quantitative genetic analysis focused on six agronomic traits: stem diameter, plant height, number of internodes, seed yield, thousand seed weight, and biomass yield, measured on 50 half-sib families (HS families). The HS families evaluated in this study were generated from isolated polycrossing of a random sample of plants taken from a hemp breeding population, Férimon 12 (dual purpose cultivar), during the growing season 2020/2021. These 50 hemp HS families were established in a field trial at Palmerston North (plant growth unit orchard, Massey University, Palmerston North, Manawatū) in the growing season 2021/2022. The HS families were planted according to a Row and Column experimental design with two replicates. Besides the 50 HS families, each replicate had ten repeated checks comprising of two commercial cultivars: Fasamo and Férimon 12. The estimated additive genetic variation among the 50 HS families was found to be significant ($P < 0.05$) for all traits, except for thousand seed weight, indicating that all traits, except for thousand seed weight, have the potential to achieve genetic gain through selective breeding. Family mean narrow-sense heritability for all traits ranged from low (0.12; thousand seed weight) to high (0.83; the number of internodes). Only the five traits that showed statistically significant ($P < 0.05$) additive genetic variance among the 50 HS families, as determined through variance component analysis, were subjected to further analyses. Moderate to strong positive genetic correlations were estimated

among all five traits and were generally higher than the phenotypic estimates. The genetic correlation coefficients estimated indicate that genotypes with higher seed and biomass yields can be developed from the reference population, represented by the random sample of 50 HS families. The study also demonstrated that both among-HS family and among- and within-HS family breeding strategies resulted in achieving genetic gain for the traits measured. However, the among- and within-HS family strategy showed the highest genetic gains for the traits in comparison to the among-HS family breeding strategy. Nonetheless, it is important to consider a trade-off between genetic gain and population diversity for the long-term success of a breeding programme. The deterministic simulation revealed that increasing the number of replicates in the field trial increased the predicted genetic gain for each trait. However, increasing replication may result in higher trial costs, which would decrease the cost-efficiency of the breeding strategies and increase the cost of achieving a 1% genetic gain. Significant differences were found among the 50 HS families and two commercial checks, Fasamo and Férimon 12, for all traits except thousand seed weight. For each trait, HS families significantly outperformed the commercial checks were identified. The results of this study highlight the potential of utilizing these HS families as elite parents in modern breeding programmes to develop breeding material. While it is important to note that quantitative genetic estimates are population specific, the findings from this study offer valuable initial insights for hemp breeders in New Zealand.

Keywords: Industrial hemp, *Cannabis sativa* L., half-sib families, additive genetic variation and genetic gain

4.1 Introduction

Cannabis sativa L. is an obligate outcrossing species (Welling et al., 2016) of the Cannabaceae family (Small & Cronquist, 1976) and possesses complex genetic makeup and heredity (Razumova et al., 2016). This genetic complexity is potentially explained by the species' phenotype variability, reproduction biology, polymorphism, and extensive adaptability to diverse environmental conditions (Forapani et al., 2001; Welling et al., 2016; Punja & Holmes, 2020; Razumova et al., 2016; Salentijn et al., 2015). Industrial hemp, a botanical class of *Cannabis sativa* L., is an annual herbaceous plant and one of the oldest domestic crops (Wang et al., 2023). The hemp plant represents a valuable source of fibre, food and medicine (Schluttenhofer & Yuan, 2017). Many Western countries banned hemp cultivation in the early 20th century due to its relationship with marijuana (Cherney & Small, 2016). However, since 1990, hemp varieties with low Δ^9-tetrahydrocannabinol (THC) levels have been licensed for cultivation in several countries, considering their significant role in economic development and environmental protection (Fortenbery & Mick, 2014). Therefore, after previously being banned for decades in many countries, hemp cultivation and breeding has gained renewed global interest, with a consistently increasing need for cultivars better adapted to the local climate and/or for a specific end-use.

Hemp is predominantly dioecious (Petit et al., 2020), and its sexual phenotype is highly variable (Faux et al., 2014). In dioecious hemp, morphological differences in male and female plants demonstrate sexual dimorphism (Berenji et al., 2013) in relation to plant morphology (plant size) and precocity (Faux et al., 2014). The sexual dimorphism in dioecious hemp leads to unsynchronized maturity and lack of uniformity, and this phenomenon poses a significant challenge to mechanized harvesting techniques (Amaducci et al., 1998; Faux et al., 2014; Razumova et al., 2016). Sex expression in hemp exhibits phenotypic variability, which can result in abnormal flower development, such as the formation of hermaphrodite flowers or mixed inflorescences like those found in monoecious phenotypes (Ghosh et al., 2023). Monoecious hemp varieties have developed from spontaneous mutations (Strzelczyk et al., 2022), but strict selection is necessary during seed multiplication due to the recessive nature of the trait (Moliterni et al., 2004). Unlike dioecious hemp, monoecious cultivars display higher crop uniformity and easier mechanical harvesting because of synchronized maturity (Mandolino & Carboni, 2004). Monoecious varieties produce higher seed yields and demonstrate greater stability when exposed to varying environmental conditions compared to the dioecious varieties (Baldini et al., 2020). A number of studies indicated that monoecious hemp cultivars enable versatile utilization of the crop for multiple purposes (Baldini et al., 2018; Baldini et al., 2020; Papastylianou et al., 2023; Sorrentino, 2021). Given the several agronomical advantages and multiple end-uses, in which the priority is seed production, monoecious varieties exhibit superior efficiency compared to dioecious cultivars (Baldini et al., 2020).

Cannabis has a long history of human interaction, and its ongoing domestication will likely continue. Early humans may have first used *Cannabis* for fibre, food, or its psychoactive properties. Early hemp growers recognized useful genetic diversity that could be exploited from the wild and gradually developed into improved populations with higher yield and quality fibre, seed yield, and useful secondary metabolites, which led to organized breeding programmes in the twentieth century (Clarke & Merlin, 2016). From hemp, thousands of products can be manufactured (Crini et al., 2020). Given the diverse range of applications, the direction of hemp breeding can be varied depending on the specific breeding objective. Like any other crop breeding programme, it is important to have well-defined goals consistent with the desired end products (Campbell et al., 2019b). Primary goals of hemp breeding can be classified into three main areas: fibre production, seed, and, more recently, secondary metabolites such as non-THC cannabinoids and terpenes (Salentijn et al., 2015). Even though industry-driven breeding programmes for specific fibre uses, such as textile use, are still of interest (Salentijn et al., 2015), breeding for fibre quality ceased to be a top priority due to the introduction of new fibre processing techniques (Bócsa, 1999). With the shift of modern hemp cultivation towards lower fibre quality and seed production, hemp breeders prioritized developing monoecious cultivars deemed appropriate for producing both fibre and seed concurrently (Tang et al., 2016). Furthermore, the utilization of dual-purpose (seed and fibre) cultivars has received significant attention among hemp growers due to its potential to maximize the utilization of the entire plant (Baldini et al., 2018; García-Tejero et al., 2023; Tang et al., 2016) since it appears to be more economically viable (Ceyhan et al., 2022; Sikora et al., 2022; Tedeschi et al., 2022).

Developing crop varieties is a continuous process that involves selecting the right parents to create new genetic variant and choosing superior recombinants with desired features. Economic traits are considered when selecting parents for hybridization and choosing favourable progenies that require improvement of traits or the incorporation of new traits that respond well to extreme climate changes and meet the increasing demand for food and industrial use (Asfaw et al., 2021). Understanding and quantifying the magnitude of heritable variation and genetic association among traits under improvement is crucial when deciding on appropriate breeding methods and also for predicting genetic gain (Asfaw et al., 2017; Mistro et al., 2004). Genetic parameters have been estimated in crop breeding programs to optimize the breeding schemes and increase genetic improvement (Falconer & Mackay, 1996). The quantification of the variability and determining genetic parameters are crucial steps in comprehending the genetic structure of a breeding population (Mistro et al., 2004), and this information can be useful in identifying superior varieties and parental lines, leading to maximizing genetic gains over time (Asfaw et al., 2021). In addition, the other quantitative genetic assessments, such as pattern analysis and performance of breeding lines compared to commercial checks, offer valuable information about the agronomic potential of the breeding population and assist in determining the appropriate breeding strategy (Ehoche et al., 2022). A better understanding of how quantitative genetic parameters can be used to predict the genetic gain from one generation to the next is crucial in plant breeding, and its significance cannot be overstated.

Hemp breeding methods have evolved over time (Salentijn et al., 2015) and commonly include mass selection, crossbreeding, inbreeding, hybrid breeding, and marker-assisted breeding (Sebastian et al., 2023). Most hemp cultivars available are population cultivars, either open-pollinated cultivars resulting from recurrent selection or synthetic cultivars, which are advanced generations of a population generated from crosses among a limited number of selected elite parents (Salentijn et al., 2015). The number of new hemp cultivars has increased in recent years. The number of registered hemp cultivars in Europe increased from 12 up to 46 between 1995 and the present (Sebastian et al., 2023). However, only a few experimental reports have examined the efficacy of breeding programmes based on predicted genetic gains for traits of interest, as determined by quantitative genetic studies. In contemporary plant breeding practices, the fundamentals of quantitative genetics, which form the basis for the concept of genetic gain, are not sufficiently well understood by crop breeders and scientists, leading to inefficient or unsuccessful crop improvement efforts (Rutkoski, 2019b). According to Hennink (1994), progress in a hemp breeding programme aimed at improving bast fibre production encountered challenges due to a lack of understanding of agronomic trait inheritance. No studies have reported any estimates of realized or narrow sense heritabilities, and evidently, the maximization of the response to selection has not been considered. To address this, Hennink estimated genetic parameters such as narrow-sense heritability and response to selection for the traits of interest using parent-offspring (HS families) relationships (Hennink, 1994). Even to this day, there are only very few experimental reports that have focused on the genetic parameters (Carlson et al., 2021), and to the best of available knowledge, only a single study reported the response to selection, specifically limited to a subset of characteristics linked to bast fibre yield (Hennink, 1994).

According to projections, the worldwide hemp market is anticipated to expand to $65 billion by the year 2030 and with an appropriate strategy and regulatory framework in place, New Zealand has the potential to secure a significant share of this market (Scott, 2022). Although many countries have shown an interest in establishing hemp industries, most of them, including New Zealand, are still in the preliminary phases of legalizing hemp and building the necessary supply chain and support systems to promote the industry's growth (Moore et al., 2021). Due to the extensive duration of prohibition, a significant amount of effort is required to overcome the various challenges associated with the industry's revival in New Zealand (Marsh, 2020). New Zealand's government targets of confronting climate change (climate-change work programme), productive and sustainable farming and "Clean and Green" market positioning, align well with the desirable attributes of hemp farming and products (Moore et al., 2021; MPI, 2023). This implies that New Zealand has the potential to participate in and benefit from the hemp industry. However, the industry in the country is still in its infancy. One of the critical factors identified by the Ministry for Primary Industries that influences the hemp industry's development in New Zealand is the generation of novel and productive hemp strains adapted to local climatic conditions to optimize both yield and sustainability (Moore et al., 2021). According to Mathew Johnson (2021), the managing director of Hemp Connect – New Zealand's premier vertically integrated hemp food company and a leading supplier of hemp – there exists a gap between the demand for hemp products, particularly those made from hemp seeds, and the domestic production of these seeds, which currently relies on imported supplies. He proposed investing in genetics as one of two major potential solutions to address this issue. He further emphasized the significance of utilizing the entire plant, as it offers farmers the possibility of generating multiple revenue streams, thus highlighting the necessity of dual-purpose cultivars.

Despite the growing demand, the breeding efforts on hemp remain limited in New Zealand. New Zealand has approved 20 hemp cultivars for growers to plant, of which five are local cultivars developed by Donald J. McIntosh under Hempseed Holdings Ltd. Donald J. McIntosh developed the cultivars based on the Aotearoa 1 breeder's selection (Marsh, 2020). Details of Aotearoa 1 and the 5 A1 series approved cultivars are given in the following Table 4.1.

Table 4.1. Details of Aotearoa 1 and the 5 A1 series of approved cultivars landrace generic New Zealand created sub cultivars (McIntosh, 2021).

Cultivar & the date approved	Reproductive biology	Purpose	Breeder's remarks
Aotearoa 1 31st July 2008	Dioecious	Seed/fibre	▪ Base stock for all subsequent A1 series cultivars. ▪ Better than the average cultivar in the breeder's view. ▪ However, it shows more variation than all other A1 cultivars.
A1 Monopurp 26th February 2019	Monoecious	Seed	▪ Very hardy. ▪ Good seeds but not voluminous ▪ Seeds have high stearidonic acid. ▪ Good for specific use e.g., cosmetic industry.
A1 MACMONO 30th June 2020	Monoecious	Seed/fibre	▪ Commercial seed stock for broad acre farmers and market garden regime of cultivation. ▪ High seed yielding and good seed oil production. ▪ Useful due to plant uniformity. ▪ Multi-branched.
A1 COMSEED 30th June 2020	Dioecious	Seed/fibre	▪ Suitable for traditional broad-acre farmers. ▪ Breeder has not grown in a broad acre context, but it should be very useful. ▪ One stalk only -not branched.
A1 HCFX 22nd September 2021	Dioecious	Seed	▪ Large, multi-branched. ▪ Vigorous growth continuously creating new branches up to 30 naturally. ▪ Frost, disease, and pest resistant. ▪ Large, heavy well-aerated seeds. ▪ It is probably suited to the market garden regime of individual plant husbandry.

Even though five cultivars have been bred in New Zealand, according to the breeder, these cultivars are not well known locally and do not even make up 25% of the cultivars grown in the country. Furthermore, among these five cultivars, only one cultivar, A1 COMSEED, matches current cultivation and harvesting methods (suited for higher planting densities), whereas the others perform well in low planting densities, i.e., 20 - 40 plants/m^2. This is because of the breeder's wisdom in developing high seed-yielding multi-branched cultivars. During a presentation introducing his cultivars, McIntosh conveyed his opinion on the global cultivation of hemp by stating, *"Everywhere in the world, they grow hemp like wheat and barley, and they harvest it like wheat and barley. That isn't going to work long term if you want high yield"*. McIntosh further emphasized that this was his personal opinion rather than a definite statement. However, according to Joanne Townshend, research and development Manager at Midlands Seed Ltd. (Personal communication, 6th November 2019), it is not advisable to grow hemp seed and dual-purpose cultivars in New Zealand at excessively low densities. Instead, a density of 125 plants/m^2 is recommended, for several reasons. Firstly, there are low chances of achieving the desired population with introduced cultivars. Secondly, the high population helps to control weeds. Finally, maintaining an appropriate stem thickness for machine harvesting is necessary. Reducing plant populations may result in inadequate shading to suppress weed growth, making harvesting more difficult as the grain would be situated lower on the plant and during harvesting, incorporation of additional plant material potentially compromises the seed yield (Townshend & Boleyn, 2008; The hemp production guide, 2022). Increasing the planting densities (e.g., according to Townshend & Boleyn (2008), a target established plant population of 150-225 plants/m^2 is suitable for Fasamo oilseed production in Canterbury, New Zealand) not only reduces the weed densities (Vera et al., 2006; Hall et al., 2014) but also reduces harvesting difficulties (Hall et al., 2014; Townshend & Boleyn, 2008). Low planting densities produce tall, heavily branched hemp plants with thick stems (Ehrensing, 1998; Fike, 2016) and complicate the process of harvesting (Legros et al., 2013; Townshend & Boleyn, 2008). The hemp plant is generally tougher on combine harvesters in comparison to other plants, resulting in increased wear to the machinery (Merfield,1999). Forage harvesters are commonly used with large square or round baling machines to harvest Hemp in New Zealand (NZHIA, 2021). According to the New Zealand Hemp Industries Association (2021), although there is a need for more modern equipment designed specifically for hemp harvesting, currently available conventional equipment can still be utilized with some difficulty. The point is that local farmers still need cultivars specifically adapted to New Zealand's climatic conditions that could offer both economic and agronomic advantages (e.g., a plant architecture with easy harvesting). The proposal of a growers' group to the New Zealand government regarding establishing a joint venture with the Ministry of Primary Industries worth $8 million to develop a new acclimatized cultivar further supports the above statement (McIntosh, 2021).

Férimon 12 is a monoecious, dual-purpose (seed and fibre) cultivar (Wulff et al., 2021). Férimon 12 is one of the best-performing monoecious cultivars globally that consistently exhibit high yields of both seed and stem yield (Darby et al., 2018; Hocking, 2023; Hoppner & Mange-Hartmann, 2007; Kerckhoffs et al., 2017; Miyan, 2022; Riffkin, 2022; Vogl et al., 2004). This cultivar was bred by the *National Federation of Hemp Producers* in France and maintained by "Hemp it;" an agricultural cooperative specialized in producing and marketing hemp seeds for industrial and bio-based markets (Global Registry of Cannabis varieties, 2022). All French cultivars are developed from "Fibrimon," a monoecious cross-bred cultivar with high fibre content bred at the German Max-Planck-Institut Hamburg-Volksdorf by von Sengbusch between 1951 and 1955 (Bredemann et al., 1961). The breeding process of Fibrimon involved the use of breeding populations, which included: (1) Inbred material obtained from monoecious plants that naturally occurred in "Schurigs[13]" or "Havelländische" hemp, which was again a selection from Central-Russian origin, (2) dioecious selections with exceptionally high fibre content from Germany, which were also derived from Central-Russian populations, and (3) dioecious late-flowering landraces sourced from Italy and Turkey (de Meijer, 1995). Subsequently, 'Fibrimon' was introduced to various countries, including France, in the late 1950s. The crossbreeding of selected exotic populations with 'Fibrimon' occurred during the 1960s. The present-day cultivars 'Fibrimon 21', 'Fibrimon 24', and 'Fibrimon 56' were selected directly from 'Fibrimon'. The selections were based on different dates of maturity. The more recent cultivar 'Férimon 12' is an early maturing selection from 'Fibrimon 21', primarily intended for seed production (Clarke & Diemenstraat, 1995).

[13] Schurig's hemp is a famous German heirloom variety. It may have originated from a Central-Russian variety or a hybrid of Northern and Southern ecotypes. It has some subdioecious individuals and led to the creation of the first monoecious hemp variety 'Fibrimon' (The hemp seed hub, 2013).

Considering the aforementioned and recognizing the pressing need to advance hemp breeding efforts in the New Zealand context, an experiment was undertaken with the aim of understanding the underlying genetic basis necessary for the development of a cultivar using a New Zealand-approved monoecious dual-purpose cultivar: “Férimon 12”. This investigation assessed the genetic parameters and response to selection of six key traits deemed significant in terms of yield, namely seed yield, thousand seed weight, and biomass yield, as well as those related to plant architecture, specifically stem diameter, plant height, and the number of internodes, the latter being one of the key determinants of final stem length (Carvalho et al., 2002). The current study was designed as an initial undertaking to facilitate the sustainable development of locally bred hemp cultivars in New Zealand. Its primary objective was to investigate the magnitude of additive genetic variation within a breeding population based on quantitative genetic analysis of data for key traits measured from genetically structured families, half-sib families (HS families). Additionally, the study aimed to predict the rate of genetic gain for the measured traits, thereby contributing to the strategic improvement of hemp breeding efforts in New Zealand.

4.2 Materials and methods

4.2.1 Production of half-sib families

4.2.1.1 Plant material

The reference population used in the quantitative genetic experiment was the hemp cultivar Férimon 12. This is a monoecious and dual-purpose (seed/fibre) cultivar bred in France (Fédération Nationale des Producteurs de Chanvre, 2015). The crop cycle duration of Férimon 12 is from 129 to 134 days (Varietal Catalog, 2019).

4.2.1.2 Sampling and maintenance of female and male parents

The HS families evaluated in this study were generated from isolated polycrossing of a random sample of 450 plants taken from the reference hemp population, cultivar Férimon 12. Of the 450 plants, 50 were emasculated, “females” and 400 were the pollen donor “male” plants. Polycrossing was carried out in an isolation glasshouse at the Plant Growth Unit, Massey University, Palmerston North, New Zealand, during the season 2020/2021.

4.2.1.2.1 Females (HS family seed parents)

In December 2020, 250 seeds were randomly sampled from Férimon 12 and planted (five seeds per pot) at a depth of 2.5 cm into pots (15 L) containing Doltons potting mix, which contains the following basics with a five to six months slow release Oscomote fertilizer.

C. A. N. Fines A grade[14]	50%
Coco fibre	30%
Pacific pumice (7 mm)	20%
Serpentine Super	1 Kg/m^3

These pots were maintained in a glasshouse (glasshouse I). After seedling establishment, the mature seedlings were thinned down to one plant per pot. Fifty plants were grown and maintained in the glasshouse until flowering, to be used as female parents.

The crossing of monoecious hemp lines and cultivars is difficult due to:

i. monoecious hemp undergoes 20-25% self-pollination (Berenji et al., 2013)
ii. the flowering period covers 15 to 30 days (Bialousova et al., 1958), and it is practically impossible to mechanically remove all the male flowers (Grabowska et al., 2005).

Considering the above-mentioned reasons, Ethephon: a chemical hybridizing agent (CHA), was used for emasculation to facilitate the crossing process. Four weeks after establishment, when the 50 plants in glasshouse I reached the 10-leaf stage (before budding), the plants were treated with 0.6% Ethephon (Ethrel® 720, 2-chloroethylphosphonic acid – 2,880 mg/L) at the rate of 30 ml/m^2 to achieve 100% chemical castration of male flowers (Mishchenko & Layko, 2017). A week after the treatment, the male sterilized plants were transferred to glasshouse II and arranged among the 400 plants (pollen parents) in a randomized design generated using DeltaGen statistical software package (Jahufer & Luo, 2018).

[14] The C.A.N stands for Calcium Ammonium Nitrate an additive to raw bark as a nitrogen source for the microbial activity during composting. 'A' grade C.A.N. fines is *Pinus radiata* bark size 0 – 12 mm with added C.A.N. and moisture. The pH is adjusted and composted usually for 15 – 18 weeks.

4.2.1.2.2 Males (pollen parents)

Simultaneously, 2000 seeds were randomly sampled from Férimon 12 and planted (five seeds per pot) at a depth of 2.5 cm into the pots (15 L) containing Doltons potting mix with a five to six months slow release Oscomote fertilizer. These pots were kept in glasshouse II. After seedling establishment, the seedlings were randomly thinned, and four-hundred plants were maintained to be used as male parents.

Both glasshouses had a minimum temperature of 15°C and a maximum temperature of 25°C. A drip irrigation system at both glasshouses ensured a continuous water supply for the potted plants.

4.2.1.3 Mating design - Polycross

In early January 2021, male sterilized plants (female plants) were transferred into glasshouse II to encourage prolific and synchronized flowering. From the onset of flowering, at the time of stigma receptivity between 0900-1000 h (Rana & Choudhary, 2010) throughout the flowering period, the male plants were gently shaken to try and maximize pollen dispersion. The pots containing female plants were frequently re-randomized until the end of flowering to ensure a uniform pollen distribution across the plants. In addition, horizontal airflow fans were installed in the glasshouse to spread the pollen.

4.2.1.4 Seed harvesting

Seed heads were harvested from each of the 50 female parents. Harvested mature seed heads were manually threshed and cleaned using an Oregon seed blower (Hoffman Manufacturing, Inc., Corvallis, OR). The cleaned seeds were stored in controlled temperature rooms at 15°C and controlled Relative Humidity (RH) of 15% until the following season. Seed harvested from each female plant, a HS family, was bagged and stored separately.

The steps involved in the production of HS families are shown in a flow chart in Figure 4.1

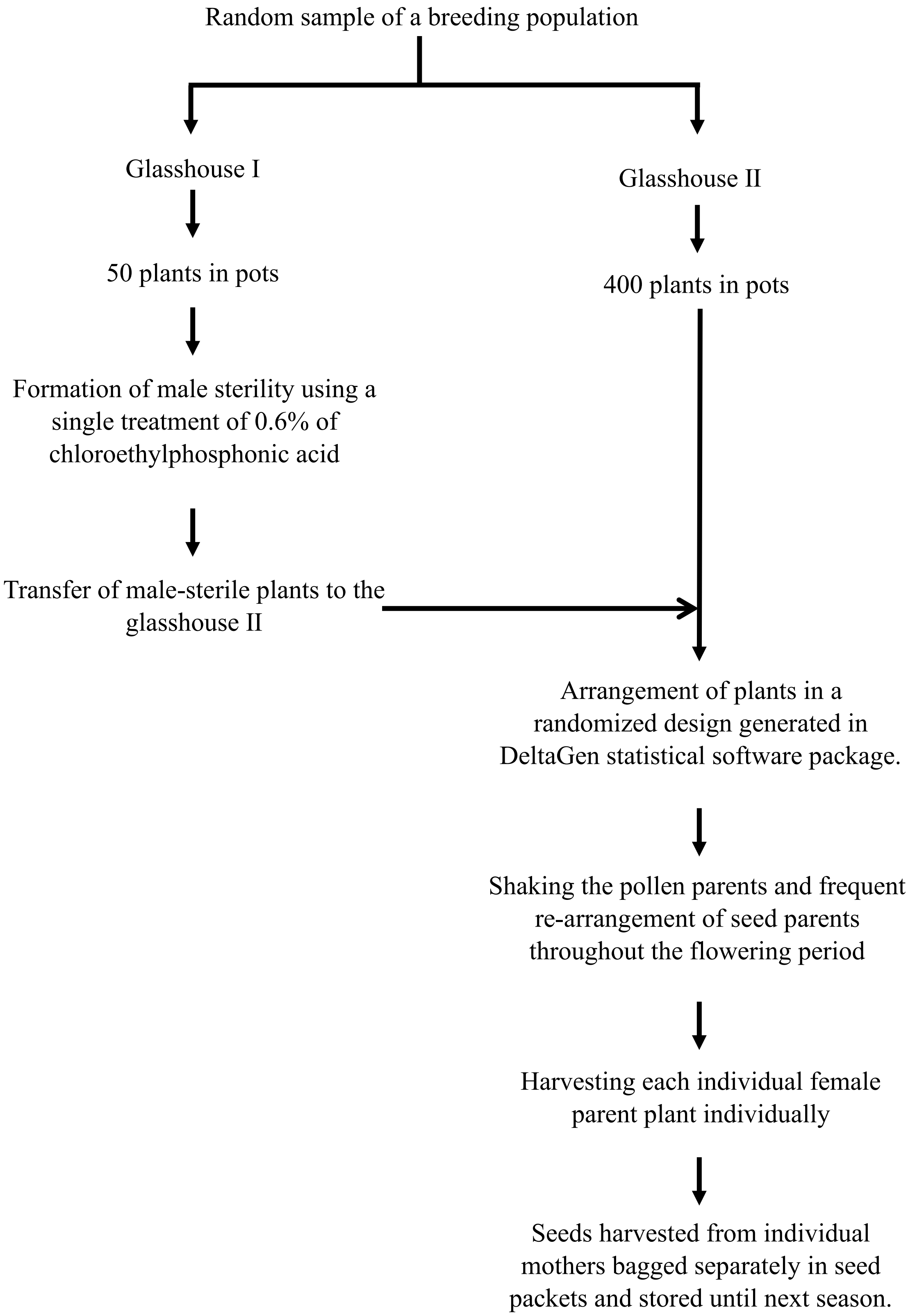

Figure 4.1. Production of HS families.

4.2.2 Establishment of the HS family field trial

4.2.2.1 Trial location

The hemp HS families were established at Palmerston North (plant growth unit orchard, Massey University, Palmerston North, Manawatū), latitude -40.378139 and longitude 175.613500. The annual mean maximum and minimum temperature of this location were 18.8℃ and 9.7℃, respectively, and the average annual rainfall was 1083.8 mm. The soil type at the site is Manawatū silt loam (Oppong, 1998).

4.2.2.2 Experimental design

The field trial was planted according to a Row and Column experimental design with two replicates, as shown in Figure 4.2. The field contained 120 plots with 20 rows and six columns spaced 50 cm apart. Besides the 50 HS families, each replicate had ten repeated checks comprising of the commercial cultivars: Fasamo (RC 01) and Férimon 12 (RC 02). Both these cultivars are monoecious dual-purpose cultivars (Townshend & Boleyn, 2008; Smart et al., 2018) approved to grow in New Zealand (MoH, 2010). The two repeated checks were included: (1) to help reduce spatial variation across the trial replicates and (2) to compare HS family performance to commercially grown cultivars.

Seeds from each HS family and check cultivars were hand-seeded into 0.8 m by 0.4 m plots. The target population was 125 plants/m^2. The experimental design with HS families and check cultivars is given in Figure 4.2.

Row / Col	1	2	3	4	5	6
1	HS 25	HS 17	RC 01	HS 52	HS 18	HS 16
2	HS 41	HS 14	HS 07	HS 43	RC 02	HS 11
3	HS 45	RC 01	HS 37	HS 09	HS 35	HS 51
4	HS 24	HS 28	HS 36	HS 10	RC 01	HS 32
5	HS 29	HS 06	HS 08	RC 02	HS 20	HS 33
6	HS 44	HS 38	HS 50	HS 34	HS 49	RC 01
7	RC 02	HS 21	HS 31	HS 47	HS 40	HS 46
8	HS 12	HS 48	RC 02	HS 26	HS 04	HS 22
9	HS 23	HS 39	HS 13	HS 03	HS 27	RC 02
10	HS 15	HS 05	HS 19	RC 01	HS 42	HS 30
11	HS 51	HS 31	RC 02	HS 36	HS 17	HS 42
12	HS 52	HS 33	HS 09	RC 02	HS 38	HS 41
13	HS 04	HS 01	HS 06	HS 50	HS 11	HS 03
14	HS 19	HS 35	HS 28	HS 25	RC 02	HS 34
15	HS 13	HS 43	HS 15	RC 01	HS 47	HS 08
16	HS 32	RC 02	HS 49	HS 05	HS 45	HS 39
17	HS 21	HS 12	HS 16	HS 20	RC 01	HS 37
18	HS 14	HS 27	HS 26	HS 46	HS 29	RC 01
19	RC 01	HS 24	HS 22	HS 23	HS 07	HS 40
20	RC 02	HS 30	HS 18	HS 48	HS 10	HS 44

Check 1
Check 2
Replicate 1
Replicate 2

0.4 m
0.5 m
17.5 m
0.8 m
0.5 m
7.3 m

Figure 4.2. HS family field trial design.

4.2.2.3 Trial management

4.2.2.3.1 Land preparation and sowing

The seedbed was prepared by disking to a depth of 30 cm. Soil tests were conducted to determine soil characteristics. The measured soil characteristics are presented in Table 4.2.

Table 4.2. Soil characteristics.

Soil characteristic		Level found
pH	pH units	5.9
Olsen Phosphorus	mg/L	56
Potassium	me/100g	0.58
Calcium	me/100g	8.1
Magnesium	me/100g	1.56
Sodium	me/100g	0.07
Cation Exchange Capacity	me/100g	15
Total base saturation	%	70
Volume weight	g/mL	1.04
Sulphate Sulphur	mg/kg	5
Potentially available nitrogen	kg/ha	104
Anaerobically mineralizable N	µg/g	67
Soil type		Manawatū silt loam

The seeds were hand-sown and placed at a depth of 3 cm. After sowing, the trial area was covered with netting to avoid birds picking the emerging seedlings.

4.2.2.3.2 Fertilizer application and irrigation

Based on the soil test results, fertilizer was incorporated to achieve the target fertilizer levels: 150 Kg/ha nitrogen, 80 Kg/ha phosphate and 80 Kg/ha potash (McIntosh et al., 1998).

Irrigation of the trial was applied using an overhead sprinkler system, and the irrigation frequency was varied depending on the rainfall.

4.2.2.3.3 Weed management

The land was well prepared to control weeds at the early stage, especially to reduce the need to weed the crop during the first two or three weeks after planting. Hand weeding was done within the plots to remove the emerging weeds. Weed mats were used in between the rows and columns to suppress the weeds.

4.2.2.3.4 Pest and disease management

The entire trial was under netting to prevent bird predation. No diseases were observed during the trial. No pesticides were applied during the growing season.

4.2.2.4 Harvesting

Random samples of ten plants were harvested from each HS family plot at the seed maturity stage. The harvesting time was determined by visual assessment. The plots were harvested manually when 80% of the seed colour had changed from green to greyish brown, and the first signs of seed scattering were observed (Townshend & Boleyn, 2008).

4.2.3 Data collection

The following traits were measured.

4.2.3.1 Plant height at the time of harvest

The height of ten representative plants from each plot was determined before harvest. The plant height from the stem base was measured using a tape measure.

4.2.3.2 Stem diameter

The stem diameter was measured from the ten representative plants using a vernier calliper at 10 cm from the base, according to the method described by Tang et al. (2016). Two measurements were made perpendicular to each other, and the average was recorded.

4.2.3.3 Number of internodes

The number of internodes was counted from the ten representative plants. Only fully developed internodes were calculated according to Kalousek et al. (2020) when determining the number of internodes.

4.2.3.4 Seed yield

After harvesting, the seed heads of representative plants were air-dried, and the seeds were separated manually. Sieves and an Oregon seed blower (Hoffman Manufacturing, Inc., Corvallis, OR) were used to clean the harvested seeds. The cleaned seeds were weighed to determine the seed yield.

4.2.3.5 Thousand seed weight

The thousand seed weight was determined using 200 seeds in two replicates of 100 seeds each (Schultz et al., 2020). Seeds were counted using a CONTADOR Seed counter (CE Pfeuffer, Baumann Saatzuchtbedarf, Waldenburg, Germany) and weighed.

4.2.3.6 Biomass

The biomass was measured from ten repetitive plants. In order to evaluate the total aboveground biomass, the plant material was oven-dried at 75°C for 48 hours.

4.2.4 Data analysis

4.2.4.1 Assumptions for quantitative genetic analysis

In order to correctly interpret the quantitative genetic parameters estimated in the present study based on a HS family structure, a common set of assumptions proposed by Comstock and Robinson (1952), Cockerham (1963), and Kearsey (1965) were followed:

(i) Regular diploid inheritance.
(ii) Absence of linkage or presence of linkage equilibrium.
(iii) Absence of epistasis.
(iv) Random choice of parents from a random mating population.
(v) No difference between reciprocal crosses.
(vi) Absence of environmental correlation between relatives.

4.2.4.2 Univariate analysis

4.2.4.2.1 Variance components and heritability analysis

All data collected were analysed using the Residual Maximum Likelihood (REML) (Harville, 1977; Patterson & Thompson, 1971; Patterson & Thompson, 1975) procedure based on a linear mix model, using DeltaGen software (Jahufer & Luo, 2018). This mixed model analysis enabled the estimation of variance components for genetic effects and the generation of best linear unbiased predictors (BLUPs) for the traits stem diameter, plant height, number of internodes, seed yield, thousand seed weight, and biomass yield (White & Hodge, 1989), which were then used to construct a BLUP matrix.

The linear model used in the analysis was,

$$\boldsymbol{Y_{ijklm}} = \mu + \boldsymbol{f_i} + \boldsymbol{b_j} + \boldsymbol{r_{jk}} + \boldsymbol{c_{jl}} + \boldsymbol{s_{im}} + \boldsymbol{\varepsilon_{ijklm}} \tag{1}$$

$\boldsymbol{Y_{ijklm}}$ is the value of trait measured from sample $\boldsymbol{m}$ in HS family $\boldsymbol{i}$ in column $\boldsymbol{l}$ and row $\boldsymbol{k}$ of replicate $\boldsymbol{j}$, and $\boldsymbol{i} = 1,\ldots, \boldsymbol{n_f}$, $\boldsymbol{m} = 1,\ldots, \boldsymbol{n_s}$, $\boldsymbol{k} = 1,\ldots, \boldsymbol{n_r}$, $\boldsymbol{l} = 1,\ldots, \boldsymbol{n_c}$, $\boldsymbol{j} = 1,\ldots, \boldsymbol{n_b}$; where $\boldsymbol{f, s, r, c}$ and $\boldsymbol{b}$ are HS families, samples, rows, columns, and replicates, respectively; μ is the overall mean; $\boldsymbol{f_i}$ is the random effect of HS family $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_f^2)}$; $\boldsymbol{b_j}$ is the random effect of replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_b^2)}$; $\boldsymbol{r_{jk}}$ is the random effect of row $\boldsymbol{k}$ in replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_r^2)}$; $\boldsymbol{c_{jl}}$ is the random effect of column $\boldsymbol{l}$ in replicate $\boldsymbol{j}$, $\boldsymbol{N(0, \sigma_c^2)}$; $\boldsymbol{s_{im}}$ is the random effect of sample $\boldsymbol{m}$ in HS family $\boldsymbol{i}$, $\boldsymbol{N(0, \sigma_s^2)}$; $\boldsymbol{\varepsilon_{ijklm}}$ is the residual effect for HS family $\boldsymbol{i}$, from sample $\boldsymbol{m}$ in row $\boldsymbol{k}$ and column $\boldsymbol{l}$ in replicate $\boldsymbol{j}$.

The linear mixed model considered repeated check cultivars as fixed effects, while the random effects consisted of the HS families, replicates, rows, and columns of the experimental design.

The estimates of variance components generated from Eq. 1, were used to generate estimates of narrow-sense heritability ($\boldsymbol{h_n^2}$) on a family mean basis, for the traits stem diameter, plant height, number of internodes, seed yield, thousand seed weight, and biomass yield. Narrow-sense heritabilities ($\boldsymbol{h_n^2}$), for each trait were estimated using the estimates of genetic and error components of variance using the following equation from Falconer (1989):

$$\boldsymbol{h_n^2} = \frac{\boldsymbol{\sigma_f^2}}{\boldsymbol{\sigma_f^2} + \frac{\boldsymbol{\sigma_\varepsilon^2}}{\boldsymbol{n_r n_s}}} \tag{2}$$

where: $\boldsymbol{h_n^2}$ is the narrow-sense heritability; $\boldsymbol{\sigma_f^2}$ is the HS family additive genetic variance component; $\boldsymbol{\sigma_\varepsilon^2}$ is the error variance component, $\boldsymbol{n_r}$ is the number of replicates and $\boldsymbol{n_s}$ is the number of plants sampled within a HS family within a replicate. The estimates of narrow-sense heritability ($\boldsymbol{h_n^2}$) on a HS family mean basis, and the associated standard errors were generated using the software DeltaGen (Jahufer & Luo, 2018).

The statistical significance of the variance components was assessed using deviance of log-likelihood (Galwey, 2006; Holland et al., 2003).

4.2.4.2.2 Genetic coefficient of variance (CVA)

The genetic coefficient of variance ($\boldsymbol{CVA}$) for each trait was calculated manually using the following equation from Cheung (2020):

$$\boldsymbol{CVA} = \frac{\sqrt{\boldsymbol{V_A}}}{\bar{\boldsymbol{X}}} \times \mathbf{100} \tag{3}$$

where: $\boldsymbol{V_A}$ is the additive genetic variance component of the trait; $\bar{\boldsymbol{X}}$ refers to the phenotypic mean of the trait before selection.

4.2.4.2.3 Analysis using HS families and commercial check cultivars as fixed effects.

To investigate if there were significant differences among the HS families and commercial checks, HS families and check cultivars were considered as fixed effects in the linear mix model analysis using the DeltaGen software (Jahufer & Luo, 2018). This enabled the estimation of the best linear estimates (BLUEs) and the quantification of differences among them (Smith et al., 2005).

4.2.4.3 Multivariate analysis

4.2.4.3.1 Genetic correlation coefficients and Pearson's correlation coefficients

Pairwise Pearson correlation analysis and genetic correlation analysis were carried out to determine the type and strength of the relationship between traits using HS family BLUP-adjusted means. The genetic correlation coefficients, Pearson's correlation coefficients, and the estimation of significance for Pearson's correlation coefficient were calculated using the DeltaGen software (Jahufer & Luo, 2018). The estimation of genetic correlation (r_A) between pairs of traits involved utilizing the multivariate analysis of variance (MANOVA) feature within the DeltaGen software (Jahufer & Luo, 2018), that generates a matrix encompassing variances and covariances. The equation used to estimate genetic correlation between traits (Falconer, 1960) is:

$$\boldsymbol{r_A} = \frac{\boldsymbol{CO_{XY}}}{\sqrt{\boldsymbol{\sigma^2_{A_x}\sigma^2_{A_y}}}} \tag{4}$$

where: CO_{XY} additive genetic covariance between traits X and Y; $\boldsymbol{\sigma^2_{A_x}}$ is the additive genetic variance of trait X; and $\boldsymbol{\sigma^2_{A_y}}$ is the additive genetic variance of trait Y.

4.2.4.3.2 Principal component analysis (PCA) and cluster analysis

A graphical summary illustrating the association among the 50 HS families based on the traits measured was generated using pattern analysis. This analysis involved the combination of Principal Component Analysis (PCA) and Cluster Analysis, conducted using DeltaGen software (Jahufer & Luo, 2018), to summarise information from the HS family × trait data matrix (Gabriel, 1971; Kroonenberg, 1994; Watson et al., 1995). Only traits with significant ($P < 0.05$) additive genetic variation were considered in the analyses. The data were standardized to eliminate scaling effects, with a mean of zero and a variance of one (Cooper & DeLacy, 1994; Fox & Rosielle, 1982). Cluster analysis was performed on the standardized data using a hierarchical agglomerative classification method. Squared Euclidean distance was utilized as a measure of dissimilarity in the analysis. To determine the optimal truncation level for the cluster analysis hierarchy, the increase in the sum of squares among HS family groups as the number of groups increased was investigated (DeLacy, 1981). The selection of the group level was based on the point where there was no significant improvement in the percentage of the HS family sum of squares among groups as the number of groups increased.

4.2.4.4 Estimation of genetic gain ($\boldsymbol{\Delta G}$).

The predicted percentage genetic gain per cycle ($\%\Delta G_c$) relative to the mean of the 50 HS families evaluated was calculated using the DeltaGen software programme (Jahufer & Luo, 2018), using the breeder's equations below.

The two breeding strategies assessed in this study were:

(i) Among family HS family selection (AF-HS): This breeding method is based on the selection of superior HS families based on their phenotypic performance. The selected individuals become the parents of the subsequent generation. The prediction equation for genetic gain (Casler & Brummer, 2008) used in DeltaGen is:

$$\Delta \mathbf{G}_{\mathbf{AF-HS}} = \mathbf{k_f c} \frac{\mathbf{1/_4\ \sigma_A^2}}{\mathbf{\sigma_{PF}}} \tag{5}$$

where: $\Delta \mathbf{G}_{\mathbf{AF-HS}}$ is the genetic gain based on selection and random mating of the top performing HS families; k_f is the among family selection intensity; $\boldsymbol{c}$ is the parental control, taken as 0.5 for HS family selection; $\boldsymbol{\sigma_A^2}$ is the additive genetic variance; and $\boldsymbol{\sigma_{PF}}$ is the among-family phenotypic standard deviation.

(ii) Among and within HS family selection (AWF-HS): This breeding method involves two main steps: First, the selection of best HS families using different selection pressures based on standard phenotypic measurements, and second, the selection of the best individual plants within these families based on individual plant data. The selected individuals become the parents of the next generation. The prediction equation for genetic gain (Casler & Brummer, 2008) used in DeltaGen is:

$$\boldsymbol{G_{AWF-HS}} = \boldsymbol{k_f c} \frac{\mathbf{1/_4\ \sigma_A^2}}{\mathbf{\sigma_{PF}}} + \boldsymbol{k_w c} \frac{\mathbf{3/_4}\, \boldsymbol{\sigma_A^2}}{\boldsymbol{\sigma_{PW}}} \tag{6}$$

where: $\boldsymbol{\Delta G_{AWF-HS}}$ is the genetic gain based on selection and random mating of the top-performing individuals in top-performing HS families; $\boldsymbol{k_f}$ and $\boldsymbol{k_w}$ among- and within-family selection intensity, respectively; $\boldsymbol{c}$ (c=0.5) is the parental control; $\boldsymbol{\sigma_A^2}$ is the additive genetic variance; and $\boldsymbol{\sigma_{PF}}$ is the among-family phenotypic standard deviation; $\boldsymbol{\sigma_{PW}}$ is the within-family phenotypic standard deviation.

Percentage genetic gain relative to the mean of the best-performing check in the trial, cultivar Férimon 12, was also calculated. This is referred to as (%ΔG_s). This analysis was also conducted using the DeltaGen software programme.

4.2.4.5 Deterministic simulation of genetic gain

Deterministic simulation was conducted to compare the effect of changing the replicate number on the predicted percentage genetic gain per cycle (%$\boldsymbol{\Delta G_c}$) for traits: stem diameter, plant height, number of internodes, seed yield, and plant biomass under among-family breeding strategy at different selection pressures; 20%, 10%, and 5%. The deterministic genetic gain simulation analyses were conducted using DeltaGen (Jahufer & Luo, 2018).

4.3 Results

4.3.1 Meteorological conditions

Meteorological data were collected from The National Climate Database, specifically from the nearest observing authority, "NIWA/AgResearch," in Palmerston North (NIWA, 2022). The daily observations were combined to calculate the weekly total rainfall and average values of maximum and minimum air temperatures for each week. Figure 4.3 illustrates the graphical representation of the weekly mean maximum and minimum temperatures, as well as the total rainfall recorded throughout the duration of the field experiment.

4.3.1.1 Rainfall

In the years 2021 and 2022, the annual total rainfall was 1083.8 mm and 1135.6 mm, respectively (NIWA, 2022). Throughout the trial period, the cumulative rainfall amounted to 254.8 mm. The highest recorded rainfall occurred during the seventh week after planting, reaching a maximum of 100.6 mm. No rainfall was observed during the second, sixth-, and eleventh weeks following sowing.

4.3.1.2 Temperature

The annual mean daily maximum air temperature for 2021 and 2022 was recorded as 18.8°C and 19.2°C, respectively. The annual mean daily minimum air temperature for those years was reported as 9.7°C and 9.9°C, respectively. Throughout the trial period, the maximum air temperature ranged from 21.19°C to 26.30°C, while the weekly minimum air temperature varied from 11.16°C to 16.34°C.

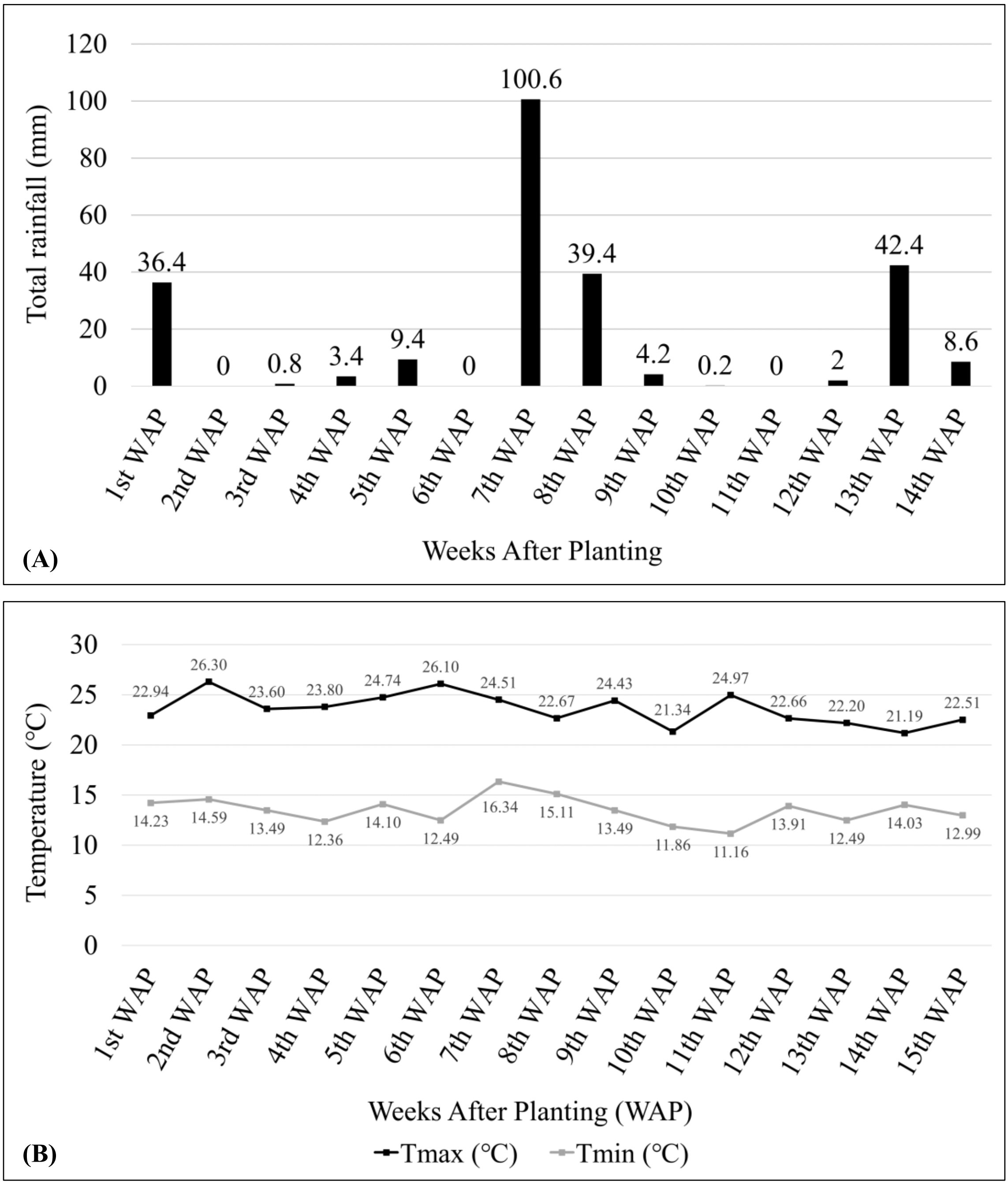

Figure 4.3. Total weekly rainfall (A) and weekly mean maximum and minimum temperatures (B) in Palmerston North (NIWA/AgResearch) during the trial period (NIWA, 2022).

4.3.2 Univariate analysis

4.3.2.1 Variance components and heritability

Results from variance component analysis, estimates of narrow-sense heritability (h_n^2) on a family mean basis and genetic coefficient of variance (CVA), for the traits measured from the 50 HS families are presented in Table 4.3. There was significant (P < 0.05) additive genetic variation (σ_f^2) among the 50 HS families evaluated at Palmerston North for all the traits except for thousand seed weight (TSW). The estimated within HS family phenotypic variance (σ_{PW}^2) components, not presented in the table, are given in Appendix 4, for the traits SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; and BM, biomass.

Additive genetic variance (σ_f^2) and residual error estimates (σ_ε^2) for stem diameter (SD) were 0.68 ± 0.17 and 3.07 ± 0.14, respectively. Family mean narrow-sense heritability (h_n^2) for stem diameter (SD) across samples was estimated as 0.82 ± 0.04 with a genetic coefficient of variance (CVA) of 11.61%. For plant height (HT), additive genetic variance (σ_f^2) was 0.02 ± 0.00, residual error (σ_ε^2) was 0.07 ± 0.00, narrow-sense heritability (h_n^2) was 0.82 ± 0.04, and the genetic coefficient of variance (CVA) was estimated as 9.41%. For the number of internodes, the additive genetic variance (σ_f^2) and residual error estimates (σ_ε^2) were found to be 5.32 ± 1.37 and 22.53 ± 1.03, respectively, with a narrow-sense heritability (h_n^2) of 0.83 ± 0.04 and a genetic coefficient of variation (CVA) of 14.49%. Seed yield (SY) and plant biomass (BM) also exhibited significant additive genetic variation with variances (σ_f^2) of 1.67 ± 0.51 and 18.01 ± 5.10, respectively. The respective residual errors (σ_ε^2) were 14.46 ± 0.67 and 121.52 ± 5.16. The family mean heritabilities (h_n^2) for these traits, seed yield (SY) and plant biomass (BM) were 0.70 ± 0.07 and 0.75 ± 0.05, respectively, and the respective genetic coefficients of variance (CVA) were 23.57% and 21.92%.

Family mean narrow sense heritabilities (h_n^2) were high for all traits except thousand seed weight (TSW), ranging from a minimum of 0.12 to a maximum of 0.83. Among all the traits, seed yield (SY) and biomass (BM) exhibited higher magnitudes of the coefficient of additive genetic variance (CVA), indicating a greater potential for response to selection. Despite their higher narrow-sense heritabilities (h_n^2), the stem diameter (SD), plant height (HT), and number of internodes (IN) showed a low coefficient of additive genetic variance, suggesting a comparatively lower capacity for response to selection. Overall, the results indicate high potential to make genetic gain through selection due to significant additive genetic variance (σ_f^2) and high family mean narrow-sense heritability (h_n^2).

Table 4.3. Among half-sib family (σ_f^2) variance components and associated standard error (± SE) estimated using REML analysis of 50 half-sib families of industrial hemp, evaluated at Palmerston North, for the traits: SD, stem diameter (mm); HT, plant height (m); IN, number of internodes; SY, seed yield (g); TSW, thousand seed weight (g); BM, biomass (g). Family mean narrow-sense heritability (${h^2}_n$) and genetic coefficient of variance (CVA) were also estimated.

Traits†	σ_f^2	(σ_ε^2)	h_n^2	CVA (%)
SD	0.68 ± 0.17*	3.07 ± 0.14	0.82 ± 0.04	11.61
HT	0.02 ± 0.00*	0.07 ± 0.00	0.82 ± 0.04	9.41
IN	5.32 ± 1.37*	22.53 ± 1.03	0.83 ± 0.04	14.49
SY	1.67 ± 0.51*	14.46 ± 0.67	0.70 ± 0.07	23.57
TSW	0.37 ± 0.96 n.s.	56.86 ± 2.34	0.12 ± 0.26	3.67
BM	18.01 ± 5.10*	121.52 ± 5.16	0.75 ± 0.05	21.92

† SD, stem diameter (mm); H, plant height (m); IN, number of internodes; SY, seed yield (g); TSW, thousand seed weight (g); BM, biomass (g).
* significant at the 0.05 probability level ($P < 0.05$).
n.s., not significant ($P > 0.05$).

4.3.2.2 Comparison of HS family and check cultivar performance

4.3.2.2.1 Stem diameter

Figures 4.4 (A) and 4.4 (B) depict the performance of the top ten HS families with the thickest stems and the bottom ten HS families with the thinnest stems in comparison to two check cultivars, Férimon 12 and Fasamo, respectively. The comparisons between HS families and the mean of check cultivars showed significant ($P < 0.05$) differences. When observing thicker stems, among the 50 HS families, five HS families and 35 HS families had stems that were significantly ($P < 0.05$) thicker than the commercial check cultivars Férimon 12 and Fasamo, respectively. The HS family with the thickest stem diameter (HS 35) had a 37.15% and 78.12% higher stem diameter compared to Férimon 12 and Fasamo, respectively. In contrast, when comparing thinner stems, only one HS family significantly outperformed Férimon 12, while no significant ($P > 0.05$) differences were observed between the HS families and Fasamo. The HS family with the thinnest stem (HS 50) was 25.6% smaller than Férimon 12.

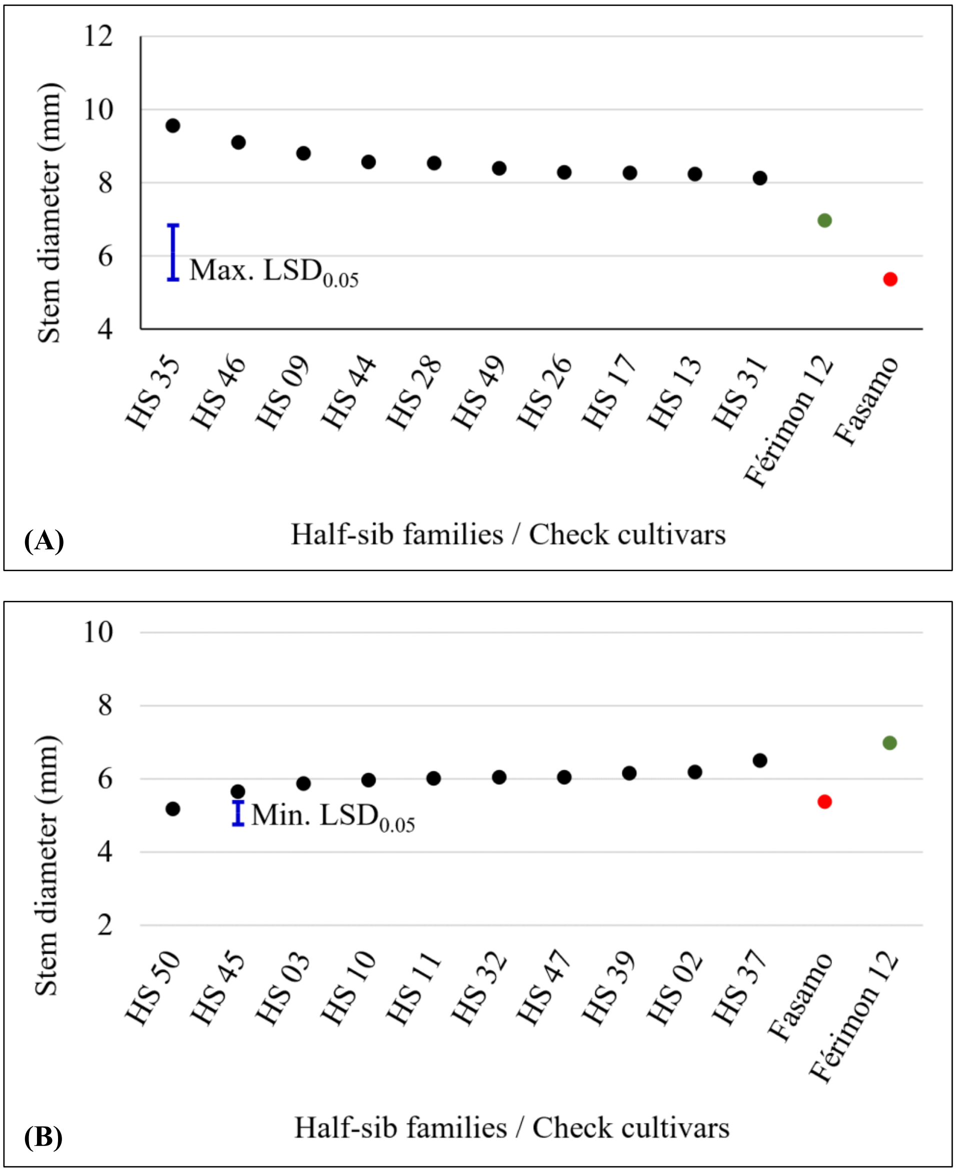

Figure 4.4. Mean stem diameter of the (A) top 20% of 50 half-sib families and (B) bottom 20% of 50 half-sib families, along with the two commercial check cultivars; 'Fasamo' and 'Férimon 12', evaluated at Palmerston North.

4.3.2.2.2 Seed yield

Analysis of variance showed significant ($P < 0.05$) differences among the entries for seed yield when both HS families and check cultivars (Férimon 12 and Fasamo) were included in the analysis. Among all 50 HS families, four HS families significantly ($P < 0.05$) outperformed the commercial check cultivar Férimon 12 and seven HS families significantly ($P < 0.05$) outperformed the commercial check cultivar Fasamo, respectively. The best-performing HS family (HS 44) outyielded the best check cultivar (Férimon 12) by 84.97%. Figure 4.5 illustrates the seed yield performance of the top ten HS families compared to the two commercial check cultivars (Férimon 12 and Fasamo) included in the trial.

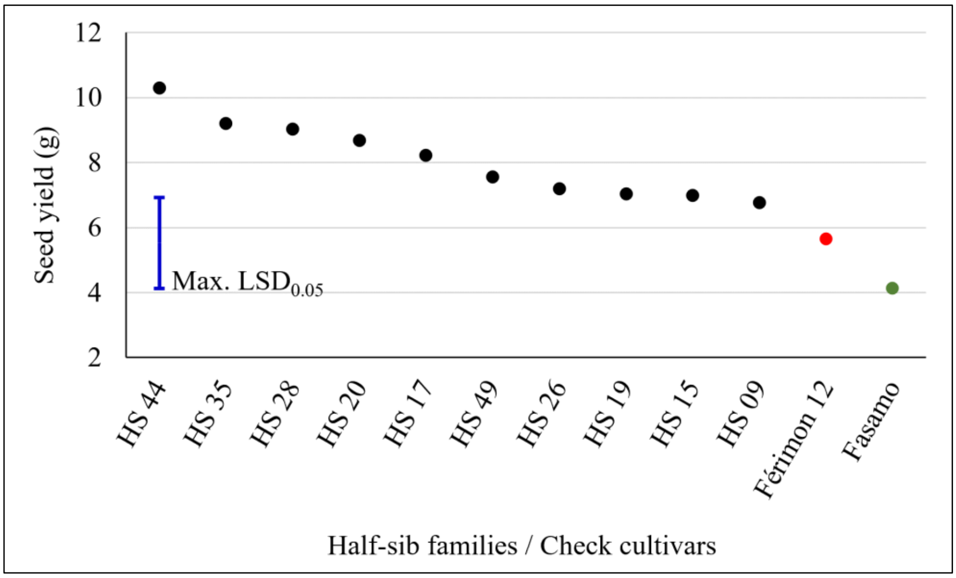

Figure 4.5. Mean seed yield of the top 20% of 50 half-sib families and two commercial check cultivars; 'Fasamo' and 'Férimon 12', evaluated at Palmerston North.

4.3.2.2.3 Plant biomass

When comparing HS family performance to the checks; Férimon 12 and Fasamo, four families significantly ($P < 0.05$) exceeded the biomass yield measured from Férimon 12, and 15 HS families significantly ($P < 0.05$) surpassed the biomass yield from Fasamo. HS 35, the highest-performing HS family produced 84.87% more biomass than Férimon 12, which performed best between the two commercial checks. Figure 4.6 presents the biomass yield performance of the top ten HS families compared to the two commercial check cultivars.

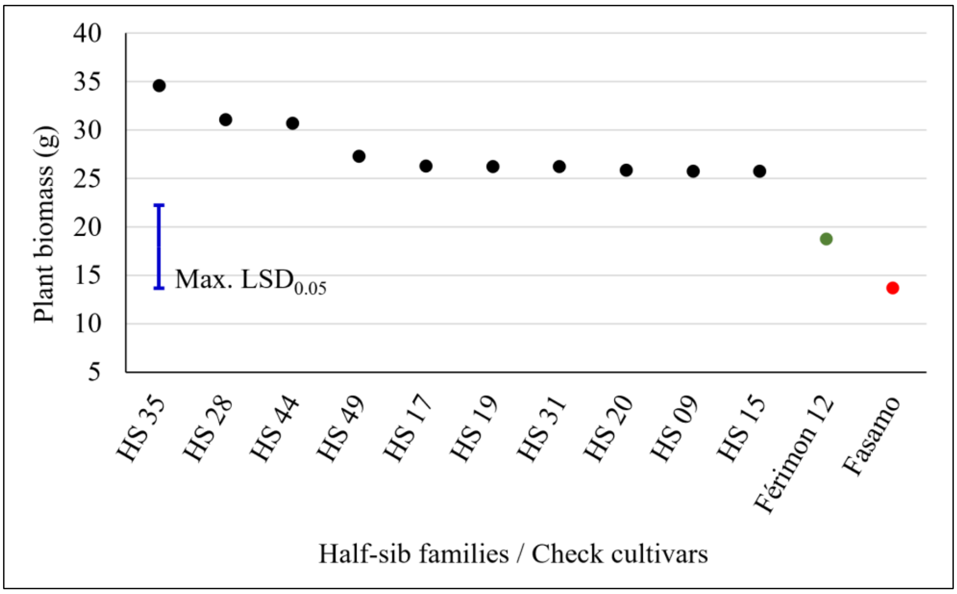

Figure 4.6. Mean plant biomass of the top 20% of 50 half-sib families and two commercial check cultivars; 'Fasamo' and 'Férimon 12', evaluated at Palmerston North.

Plant height and number of internodes were the other two traits that showed significant ($P < 0.05$) differences among the HS families and commercial check cultivars. The performance of the top 10 HS families with higher plant height and number of internodes were compared to the two check cultivars, Férimon 12 and Fasamo, and presented in Appendix 5 and Appendix 6, respectively.

4.3.3 Multivariate analysis

4.3.3.1 Phenotypic and genetic correlation

Table 4.4 presents the pairwise Pearson correlation coefficients (r_p) among traits: SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; BM, biomass, along with the estimates of genetic correlations (r_A) computed for the same traits. For the purpose of discussion, the traits with an estimate of phenotypic and genetic correlation greater than 0.65 are considered to have a very strong association. Likewise, correlation estimates between 0.50 and 0.64 are considered moderately strong, those between 0.30 and 0.49 are considered moderately weak, and those below 0.30 are considered weak (de Souza et al., 1998).

The pairwise Pearson correlation coefficients (r_p) among traits indicated that all the traits were significantly ($P < 0.05$) correlated. Seed yield (SY) had a very strong positive correlation with both biomass (BM) and stem diameter (SD) and a moderately strong positive correlation with plant height (HT) and the number of internodes (IN). Biomass had a very strong positive correlation with seed yield (SY), stem diameter (SD), and plant height (HT) and a moderately weak positive correlation with the number of internodes (IN). The number of internodes showed a moderately strong positive correlation with seed yield (SY) and plant height (HT) and a moderately weak but positive correlation with the stem diameter (SD).

Estimates of genetic correlation (r_A) computed for the traits revealed positive correlations among all traits. Strong associations were found among stem diameter (SD), seed yield (SY), and biomass (BM). Seed yield (SY), biomass (BM), and stem diameter (SD) had very strong positive genetic correlations, while the genetic association among seed yield (SY), number of internodes (IN) and plant height (HT) was positive but moderately strong. Biomass (BM) and plant height (HT) are also strongly associated. The number of internodes had moderately strong genetic relationships with plant height (HT), seed yield (SY), and biomass (BM), while its association with stem diameter (SD) was moderately weak. Plant height positively and moderately strongly correlated with seed yield (SY) and the number of internodes (IN). However, its correlation with stem diameter (SD) was very strong.

Overall, the genetic correlation estimates (r_A) among all the traits were either similar in magnitude or predominantly higher than the phenotypic correlation coefficients (r_P). Phenotypic correlation coefficients (r_P) ranged from 0.38 to 0.91, while the genetic correlation coefficients (r_A) ranged from 0.43 to 0.94. The higher genetic and phenotypic correlation values were obtained between biomass (BM) with stem diameter (SD), plant height (HT), and seed yield (SY). The genetic and phenotypic correlation estimates were also higher between stem diameter (SD) with seed yield (SY) and plant height (HT).

Table 4.4. Pearson correlation coefficients (r_p) and genetic correlation coefficients (r_A) among traits estimated based on the 50 half-sib families for the traits: SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; BM, biomass.

		SD	HT	IN	SY	BM
SD	r_p	1.00	0.80*	0.38*	0.79*	0.89*
	r_A	1.00	0.83	0.43	0.78	0.94
HT	r_p	—	1.00	0.56*	0.64*	0.72*
	r_A	—	1.00	0.57	0.53	0.74
IN	r_p	—	—	1.00	0.52*	0.45*
	r_A	—	—	1.00	0.54	0.54
SY	r_p	—	—	—	1.00	0.91*
	r_A	—	—	—	1.00	0.91
BM	r_p	—	—	—	—	1.00
	r_A	—	—	—	—	1.00

* Significant at 0.05 level of probability.

4.3.3.2 Pattern Analysis

The biplot (Figure 4.7) generated from the Principal Component Analysis (PCA) provides a graphical representation of the correlations between traits as well as the grouping patterns of the 50 HS families based on trait expression. The biplot displays the performance of the 50 HS families (indicated by numbers) relative to the five traits (represented by the directional vectors); stem diameter (SD), plant height (HT), number of internodes (IN), seed yield (SY), and biomass (BM). According to the PCA biplot, principal components 1 and 2 (PC1 and PC2) together account for 88.9% of the total variation and for 74.4% and 14.5% of the total variation, respectively.

All the traits are positively correlated, as evidenced by the angles between the directional vectors that are less than 90 degrees. The angle between the directional vectors indicates the correlation between traits based on the performance of 50 HS families. Seed yield (SY), biomass (BM) and stem diameter (SD) exhibit strong positive correlations, with a particularly pronounced positive association observed between biomass (BM) and stem diameter (SD). The number of internodes (IN) showed a positive but weak relationship with the other traits.

Cluster analysis of the 50 HS families based on the traits: stem diameter (SD), plant height (HT), number of internodes (IN), seed yield (SY), and biomass (BM) generated four entry groups (Figure 4.8 and Table 4.5). The number of HS families within each group ranged from five in group one to 23 in group three. Group one consisted of HS families with the highest group means ($\bar{x}$) for all the traits. Group two has the second-highest group mean ($\bar{x}$) for all the traits except for the number of internodes (IN). Group four has the least mean ($\bar{x}$) for all the traits. Even though group one includes only five HS families, it has the largest variance for stem diameter (SD), indicating the highest diversity for the particular trait among other groups. Likewise, for plant height (HT), the HS families in group four have the highest genetic diversity, and group two have the lowest. Interestingly, group one, which contains the fewest HS families, exhibit the highest levels of genetic diversity. There is more genetic diversity within group one for seed yield (SY) and comparatively low genetic diversity within group two. For biomass (BM), the HS families in group one show the highest genetic diversity and groups two and four show the lowest.

The colours (represented by coloured numbers) in the biplot (Figure 4.7) correspond to the four HS family groups generated through cluster analysis. There was separation among the groups, with some degree of overlapping between groups. Several HS families in group one (red) and group two (green), located far from the vector origin and scattered between the traits represented by the directional vectors, indicate high above-population mean expressions for all the traits. In contrast, groups three and four predominantly contain HS families with below-average expression for all traits except the number of internodes (IN). The results illustrated in the biplot (Figure 4.7) are further supported by the dendrogram generated from the cluster analysis (Figure 4.8, Appendix 7a and 7b) that shows the hierarchical clustering of the traits: stem diameter (SD), plant height (HT), number of internodes (IN), seed yield (SY), and biomass (BM), based on Best Linear Unbiased Predictor (BLUP) values of 50 HS families.

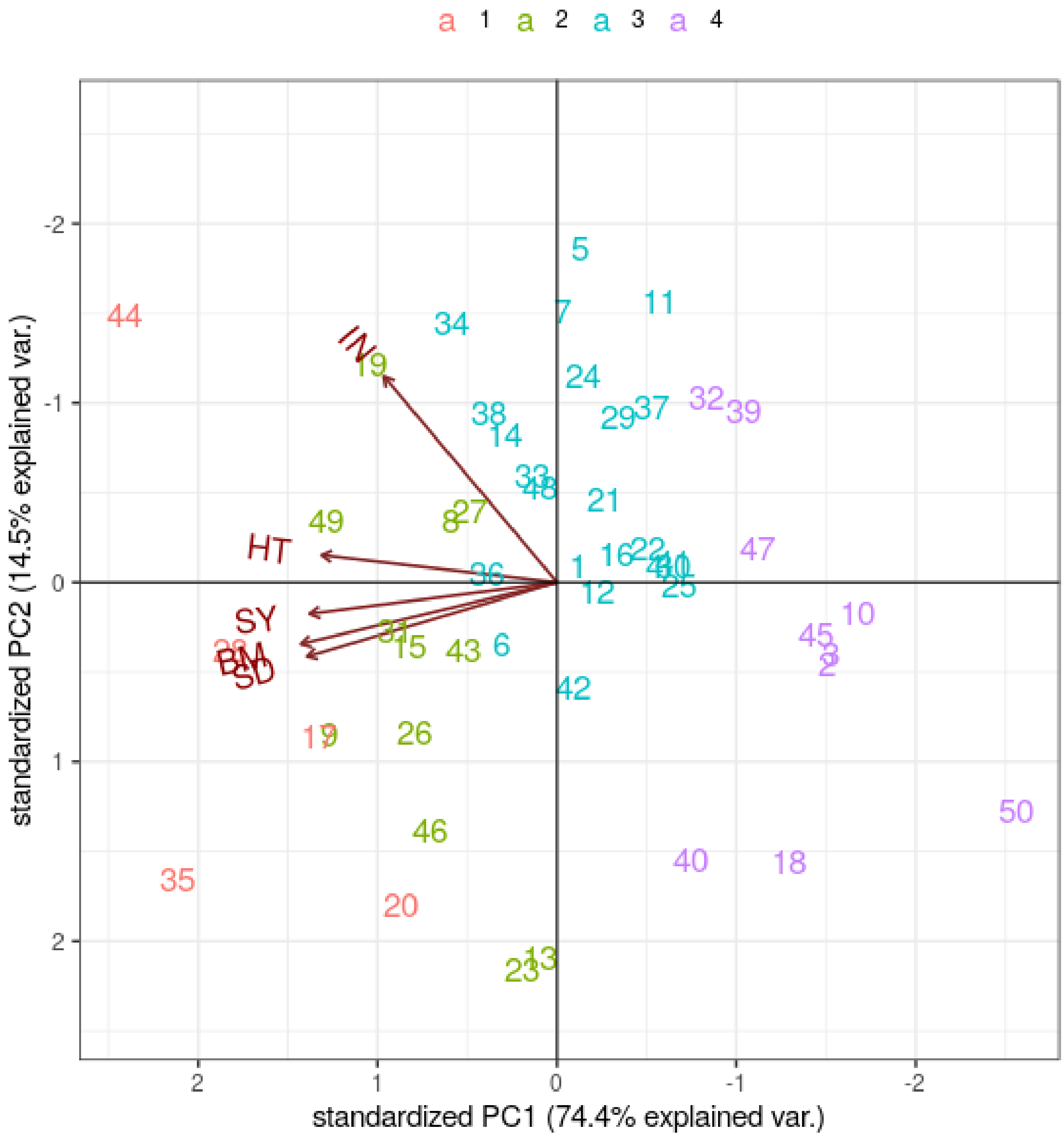

Figure 4.7. Biplot generated from pattern analysis using Best Linear Unbiased Predictor (BLUP) values of 50 half-sib families evaluated at Palmerston North, for the traits: SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; BM, biomass. The colours indicate the four groups generated from cluster analysis.

Table 4.5. The number of within-group Half-sib families and group means ($\bar{x}$) along with standard deviations (σ) generated from cluster analysis of Best Linear Unbiased Predictor (BLUP) values of 50 half-sib families evaluated at Palmerston North, for the traits: SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; BM, biomass.

Group	No of HS families	SD		HT		IN		SY		BM	
		$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
1	5	8.12	0.44	1.52	0.06	17.30	2.84	7.76	0.49	26.22	2.35
2	12	7.77	0.36	1.47	0.05	16.15	1.97	6.12	0.42	22.18	1.38
3	23	6.93	0.31	1.42	0.07	16.45	1.31	5.09	0.47	18.12	1.39
4	10	6.17	0.40	1.22	0.09	13.76	1.72	4.49	0.44	15.40	1.38

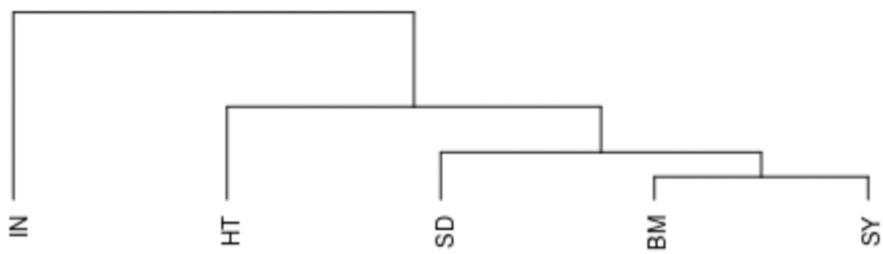

Figure 4.8. Hierarchical clustering of the traits: SD, stem diameter; HT, plant height; IN, number of internodes; SY, seed yield; BM, biomass, based on Best Linear Unbiased Predictor (BLUP) values of 50 half-sib families evaluated at Palmerston North.

4.3.4 Predicted genetic gain per selection cycle

4.3.4.1 Stem diameter

As presented in Table 4.6, the predicted percentage genetic gain per cycle ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) based on among-family selection (AF-HS), was estimated to be 7.17% at a selection pressure of 20% for stem diameter. When the among-family selection pressure was increased to 10% and 5%, the predicted $\%\Delta G_{\bar{x}}$ were increased to 9.01% and 10.55 %, respectively. Likewise, the predicted genetic gain ($\%\Delta G_s$) relative to the industry standard (s) was 11.91% at 20% selection pressure, whilst at a selection pressure of 5%, the predicted $\%\Delta G_s$ per cycle was 15.44% (Table 4.6). The results showed that increasing among-family selection pressure lifted the response to selection. Increasing the selection pressure potentially increased the rate of predicted genetic gains ($\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$). However, it should be noted that increasing the selection pressure reduced the number of parents available for crossing to generate the next cycle of selection. As illustrated in Figure 4.10, when a 20% among-family selection pressure was applied to 50 HS families, the number of parents available would be reduced to 200 (20% of the 50 families (i.e., ten families), and 20 random individuals from each of these families across two replicates, resulting in 200 parents; 10 families × 20 individuals = 200 parents). However, if the selection pressure increased from 20% to 5%, the resulting parental population would be reduced further, and 60 parents would remain available for the next cycle.

Table 4.6 also presented the predicted percentage genetic gains ($\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$) based on among- and within-family selection breeding strategy (AWF-HS). The predicted percentage genetic gains were obtained at different combinations of among- and within-family selection pressures: 20% | 20% (among | within family), 20% | 10%, 10% | 20%, 10% | 10%, 5% | 20%, and 5% | 10%. Here, the predicted $\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$ per cycle increased as both the among-family and within-family selection pressures were raised. The predicted $\%\Delta G_{\bar{x}}$ ranged from 18.35% to 24.6% at an increasing selection pressure of 20% | 20% and 5% | 10%, respectively. At the same 20% of within-family selection pressure, increasing the among-family selection pressure from 20% to 10% and 5% increased the $\%\Delta G_{\bar{x}}$ from 18.35% to 20.19% and then to 21.73%, respectively. Applying higher selection pressures: 20% | 10%, 10% | 10%, and 5% | 10% increased the rate of predicted $\%\Delta G_{\bar{x}}$ to 21.22%, 23.06% and 24.6%, respectively. The raise of $\%\Delta G_{\bar{x}}$ per cycle with increasing within- and among-family selection pressure has been illustrated in Figure 4.9.

Implementing 20% within-family selection pressure with varying among-family selection pressures of 20%, 10%, and 5% estimated the predicted $\%\Delta G_s$ to be 23.58%, 25.51%, and 27.11% per cycle, respectively. Similarly, when 10% of within-family selection pressure together with the same among-family selection pressures (20%, 10%, and 5%) were applied, predicted $\%\Delta G_s$ per cycle were predicted to be 26.58%, 28.51%, and 30.11%, respectively. However, as shown in Figure 4.10, using a combination of among- and within-family selection reduced the resulting polycross sizes compared to using only among-family selection (AF-HS) at different selection pressures. An increase of among- and within-family selection pressures increased the predicted $\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$, but reduced the resulting polycross parent size. For example, selection among and within 50 HS families (AWF-HS) by increasing the selection pressures from 20% | 20% to 5% | 10% increased the predicted $\%\Delta G_{\bar{x}}$ from 18.35% to 24.60%, respectively, but reduced the number of parents identified from 40 to 6, respectively. However, this could be mitigated by planting larger numbers of plants per HS family.

Table 4.6. Predicted percentage genetic gain ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard (s) based on breeding strategies using only among family (AF-HS) and a combination of among family (AF-HS) and within family selection (WF-HS), at different selection pressures for the trait, stem diameter.

		Among family selection (AF-HS)					
		20%		10%		5%	
		$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$
		7.17	11.91	9.01	13.84	10.55	15.44
WF-HS	20%	18.35	23.58	20.19	25.51	21.73	27.11
	10%	21.22	26.58	23.06	28.51	24.60	30.11

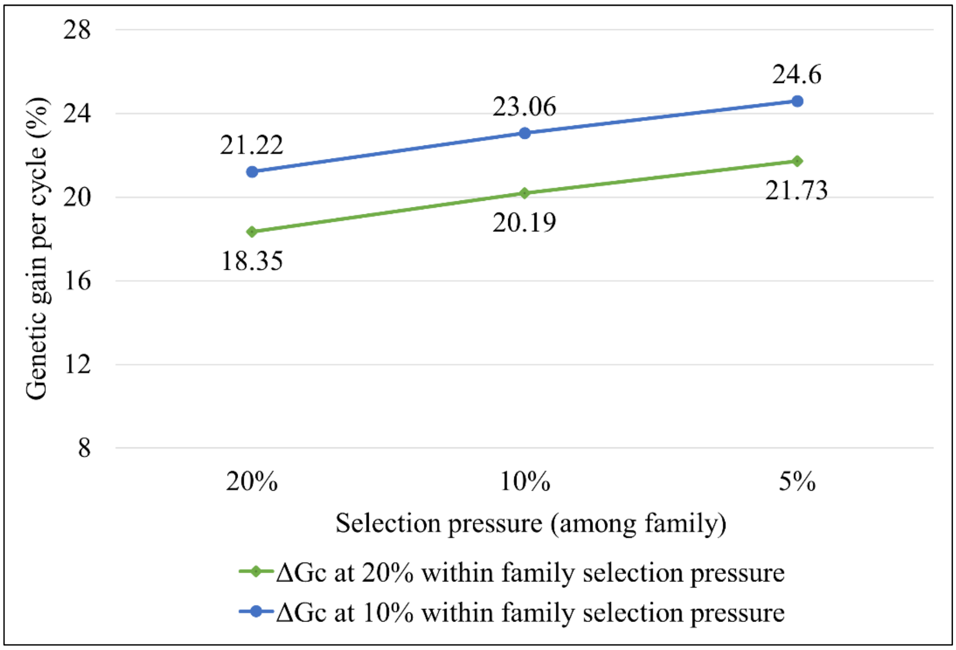

Figure 4.9. Predicted genetic gain per cycle ($\%\Delta G_{\bar{x}}$) for stem diameter compared with a breeding strategy using among-family and within-family selection at different selection pressures.

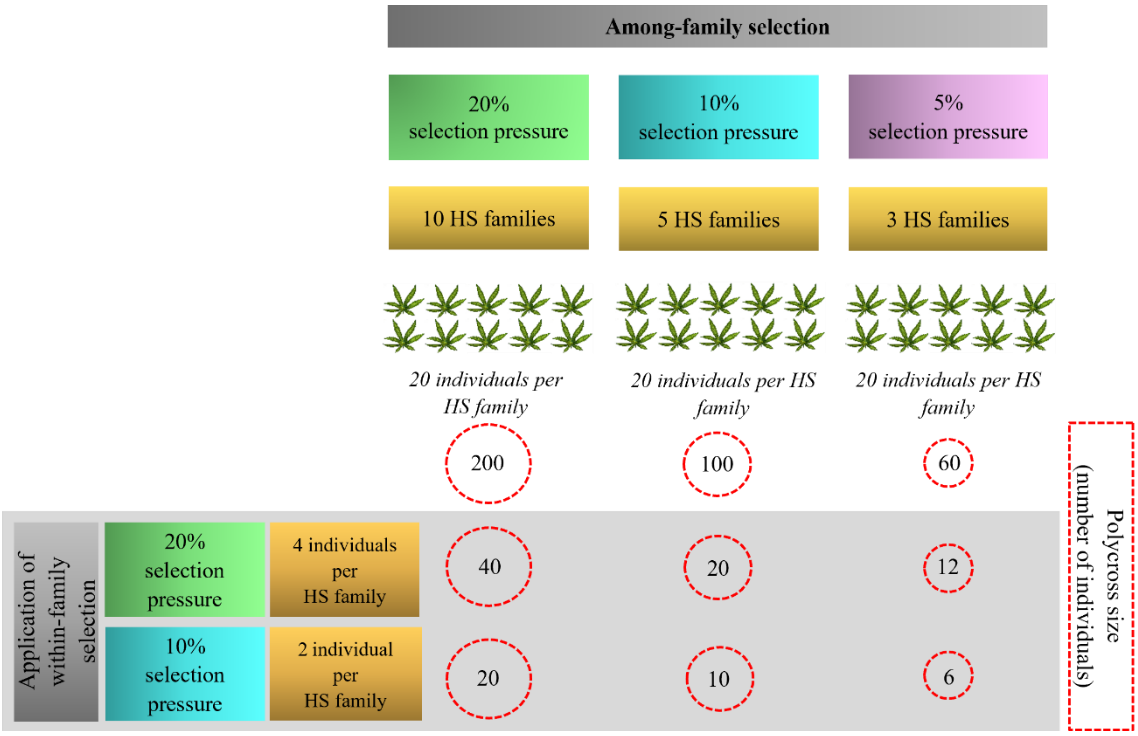

Figure 4.10. Selection scheme showing among and within-family phenotypic selection at different selection pressures for a population of 50 half-sib (HS) families and the resulting polycross sizes. Numbers in the red dashed circles indicate the resulting polycross sizes. For example: with 50 HS families, 5% among-family selection pressure = 3 HS families; total number of plants per HS family phenotyped across two replicates was 20 and the resulting parental population will be 60. Along with 5% among-family selection pressure, an application of within-family selection at 10% selection pressure (2 individuals selected per HS family); resulting in a polycross size of 6.

4.3.4.2 Seed yield

Predicted $\%\Delta G_{\bar{x}}$ estimated for seed yield under among-family (AF-HS) and a combination of among- and within-family selection (AWF-HS) at different selection pressures were presented in Table 4.7. Predicted $\%\Delta G_{\bar{x}}$ under among-family selection (AF-HS) increased with increasing selection pressures. Here, among-family selection pressure of 5% resulted in the highest predicted $\%\Delta G_{\bar{x}}$ (19.90%) and 20% among family selection pressure resulted in the lowest predicted $\%\Delta G_{\bar{x}}$ (13.52%). The predicted $\%\Delta G_{\bar{x}}$ at 15% among-family selection pressure was 17%. Overall, the predicted $\%\Delta G_{\bar{x}}$ rose as among-family selection pressure increased. A parallel trend was observed with the predicted $\%\Delta G_s$, where the $\%\Delta G_s$ increased with an increase in selection pressure. The predicted %ΔG_s increased from 12.5% to 18.45% when among-family selection pressure increased from 20% to 5%.

The application of among-family selection plus within-family selection (AWF-HS) to 50 HS families resulted in a predicted %$\Delta G_{\bar{x}}$ ranging from 29.92% to 40.51% at among | within family selection pressures of 20% | 20% and 5% | 10%, respectively. Similarly, predicted %ΔG_s ranged from 28.34% to 38.80% at the same selection pressures of 20% | 20% and 5% | 10%, respectively. When 20% of within-family selection pressure was applied, the respective predicted %$\Delta G_{\bar{x}}$ at 20%, 10%, and 5% among-family selection pressures increased from 29.92% to 33.39% and 36.29%, respectively (Figure 4.11 and Table 4.7). A comparable pattern was observed among the respective predicted %$\Delta G_{\bar{x}}$ estimated at 10% within-family selection pressure, where the %$\Delta G_{\bar{x}}$ increased with increasing among-family selection pressures from 34.13% (20%) to 37.61% (10%) and 40.51% (5%) (Figure 4.11 and Table 4.7).

A corresponding pattern was noticed with the predicted %ΔG_s. As the within-family selection pressure increased from 20% to 10%, there was a corresponding increase in the predicted %ΔG_s at among-family selection pressures of 20%, 10%, and 5%. At 20% within-family selection pressure with an increasing among-family selection pressure from 20% to 10% and 5%, the predicted %ΔG_s rose from 28.34% to 31.78% and then to 34.64%, respectively, as shown in Table 4.7. A similar trend was noticed when looking at the predicted %ΔG_s at a selection pressure of 10% within families, it increased from 32.51% (at a selection pressure of 20%) to 35.94% (at a selection pressure of 10%) and finally to 38.80% at a selection pressure of 5%.

Table 4.7. Predicted percentage genetic gain ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard (s) based on breeding strategies using only among family (AF-HS) and a combination of among family (AF-HS) and within family selection (WF-HS), at different selection pressures for the trait, seed yield.

		Among family selection (AF-HS)					
		20%		10%		5%	
		$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$
		13.52	12.15	17.00	15.58	19.90	18.45
WF-HS	20%	29.92	28.34	33.39	31.78	36.29	34.64
	10%	34.13	32.51	37.61	35.94	40.51	38.80

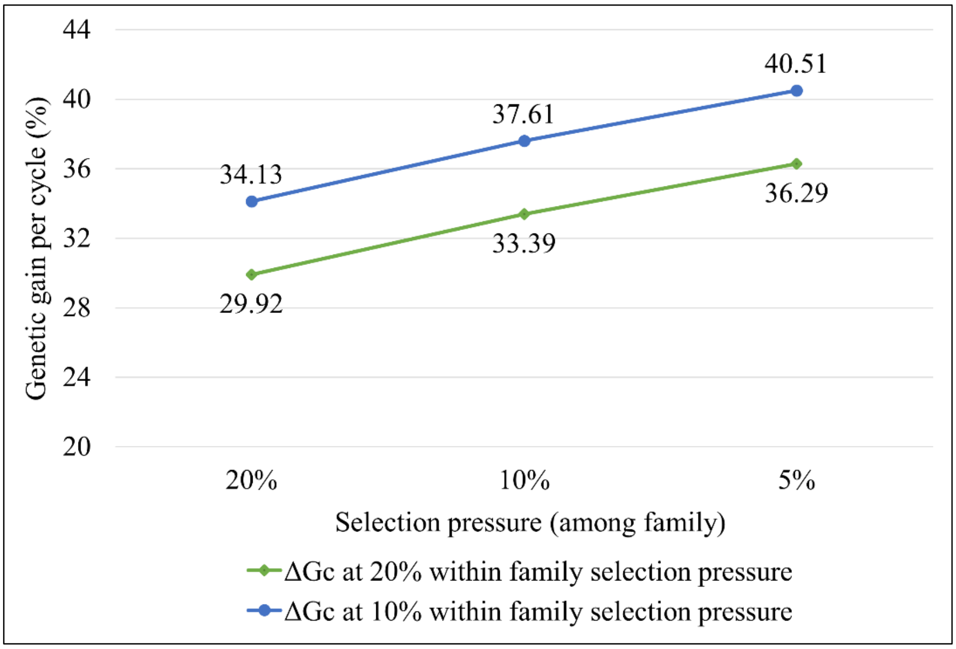

Figure 4.11. Predicted genetic gain per cycle ($\%\Delta G_{\bar{x}}$) for seed yield compared with a breeding strategy using among-family and within-family selection at different selection pressures.

4.3.4.3 Plant biomass

The among-family selection (AF-HS) applied on 50 HS families for the trait, plant biomass at different selection pressures, 20%, 10%, and 5% resulted in predicted $\%\Delta G_{\bar{x}}$ of 12.89%, 16.20%, and 18.96%, respectively (Table 4.8). The respective predicted $\%\Delta G_s$ for the among-family selection pressures; 20%, 10%, and 5% were 20.04%, 23.56%, and

26.50%. As results obtained for the traits, stem diameter and seed yield, the predicted $\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$ rose with increasing selection pressure.

The among- and within-family selection (AWF-HS) applied on 50 HS families for plant biomass at 20% | 20%, 20% | 10%, 10% | 20%, 10% | 10%, 5% | 20%, 5% | 10% among | within family selection pressure combinations resulted on predicted $\%\Delta G_{\bar{x}}$; 30.10%, 34.53%, 33.42%, 37.84%, 36.18%, and 40.60%, respectively (Figure 4.12 and Table 4.8). In the meantime, as presented in the same Table 4.8, applying the among- and within-family selection method (AWF-HS) at increasing among-family selection pressures of 20%, 10% and 5%, and 20% within-family selection pressure increased the predicted $\%\Delta G_s$ by 38.35%, 41.87, and 44.81%, respectively. When the among- and within-family selection method (AWF-HS) was applied with a 10% within-family selection pressure and among-family selection pressures of 20%, 10%, and 5%, there was a corresponding increase in the predicted $\%\Delta G_s$. Specifically, the values rose by 43.05%, 46.58%, and 49.51%, respectively.

Table 4.8. Predicted percentage genetic gain ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard (s) based on breeding strategies using only among family (AF-HS) and a combination of among family (AF-HS) and within family selection (WF-HS), at different selection pressures for the trait, plant biomass.

		Among family selection (AF-HS)					
		20%		10%		5%	
		$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$
		12.89	20.04	16.20	23.56	18.96	26.50
WF-HS	20%	30.10	38.35	33.42	41.87	36.18	44.81
	10%	34.53	43.05	37.84	46.58	40.60	49.51

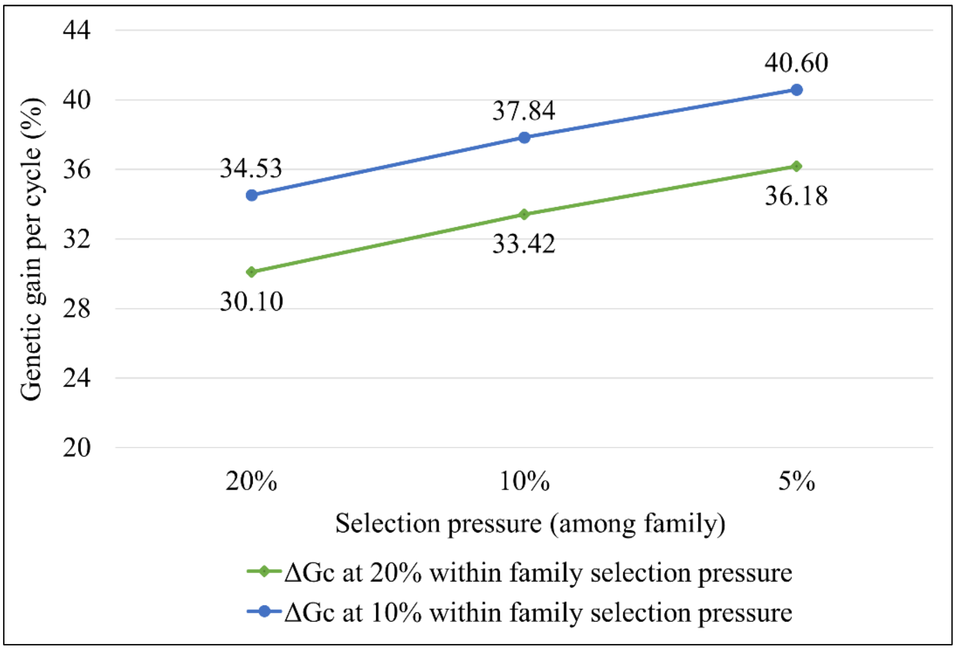

Figure 4.12. Predicted genetic gain per cycle (%$\Delta G_{\bar{x}}$) for biomass compared with a breeding strategy using among-family and within-family selection at different selection pressures.

Overall, applying among-HS family breeding strategy (AF-HS) at increasing selection pressures of 20%, 10%, and 5% increased the predicted percentage genetic gains per cycle (%ΔG_C) for all traits; stem diameter, seed yield, plant biomass (Table 4.6 – Table 4.8), plant height and the number of internodes (Appendix 8 and Appendix 9, respectively). Predicted genetic gain per cycle (%$\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) for plant height and number of internodes compared with a breeding strategy using among-family (AF-HS) and within-HS family (AWF-HS) selection at different selection pressures, were also presented in Appendix 10. Increasing the selection intensity increased the predicted %ΔG_C but reduced the number of parents for selection (Figure 4.10). The genetic responses to the combination of among- and within-HS family selection (AWF-HS) were higher compared to those among-HS family (AF-HS) selections. The explored genetic responses for among-HS family selection (AF-HS) and the combination of within- and among-HS family selection (AWF-HS) indicated that AWF-HS markedly lifted the genetic gain responses. When assessing the predicted genetic gains obtained from among- and within-HS family selection (AWF-HS), the combination of 5% among-family selection pressure with within-family selection pressures showed higher genetic gains compared to the combination of 20% among-family selection pressure with within-family selection pressures, with 5% | 10% among |

within family selection pressure achieving the highest genetic gains for all traits. In contrast, the resulting parent population size under among- and within-HS family selection (AWF-HS) was low compared to among-HS family selection. The resulting parent population size reduced along with increasing among and within-family selection pressures, with 5% | 10% having the lowest number of parents for the next selection.

4.3.5 Genetic gain simulations

The effect of replication on predicted genetic gain per cycle ($\%\Delta G_c$) for traits; stem diameter, seed yield, and plant biomass under among-HS family breeding strategy at different selection pressures; 20%, 10%, and 5% were presented in Figure 4.13 (Other traits presented in Appendix 11). As expected, increasing the number of replicates caused a direct increase in the rate of genetic gain and a positive trend of predicted $\%\Delta G_c$ were apparent for all traits (Figure 4.13 and Appendix 11). The simulation results indicated that doubling the number of replicates from two to four markedly lifted the predicted $\%\Delta G_c$, where similar responses to selection were observed when increasing the number of replicates from four to five.

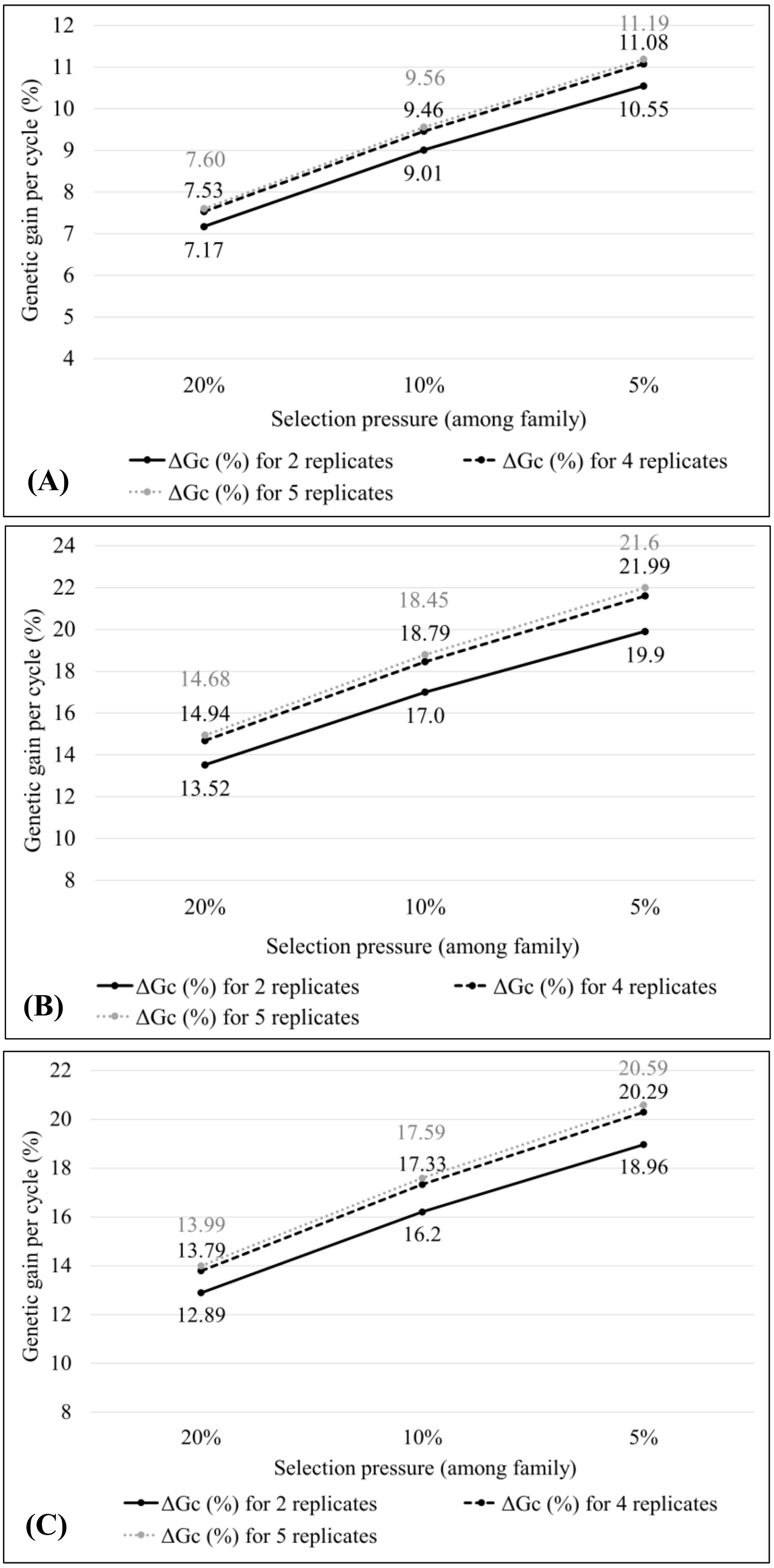

Figure 4.13. The effect of replication on predicted percentage genetic gain per selection cycle ($\%\Delta Gc$) for the traits; (A) stem diameter, (B) seed yield, and (c) plant biomass using the among-family selection breeding strategy at different selection pressures.

4.4 Discussion

4.4.1 Genetic Variation and Heritability

Additive genetic variation is one of the most important factors that influences the rate of genetic improvement (Falconer, 1981; Fehr, 1987; Rutkoski, 2019b; Lin et al., 2023). Estimating the magnitude of additive genetic variance helps to determine the likelihood of achieving genetic gain through selection (Acquaah, 2012; Ehoche et al., 2022; Falconer & Mackay, 1996). In the present study, variance component analysis indicated significant ($P < 0.05$) additive genetic variation for the traits; stem diameter, plant height, number of internodes, seed yield, and biomass yield, among the HS families evaluated in a field experiment. These results quantified the magnitude of additive genetic variation for the different traits among the HS families, which indicated that they are heritable from parents to offspring. In plant breeding, the additive effect of genetic variance underlying a specific trait is of significant importance. Additive effect represents the component of genetic variation transmitted from parents to offspring and is therefore a key determinant of response to selection (Karavolias et al., 2020). The presence of significant additive genetic variation indicates the possibility of genetic improvement in traits through the selection of superior HS families based on their phenotypic expression from the breeding population, as response to selection is directly influenced by the additive genetic variance (Briggs & Goldman, 2006).

Significant ($P < 0.05$) additive genetic variation estimated for the traits, stem diameter, plant height, number of internodes, seed yield and biomass yield indicate the availability of additive genetic variation for increasing or decreasing the expression of these traits based on breeding objectives. Breeding objectives may vary from one region to another and different markets and may be based on the need for specific trait expressions and combinations in a particular environment. Considering the stem diameter as an example, depending on the end-use, the final product would require either thinner or thicker plants (Darby et al., 2018). Thin stems are more suitable for the textile industry because they have higher bast fibre content and low lignin content than thick stems (Amarasinghe et al., 2022). In contrast, thick stems yield a greater amount of hurd (the inner woody core of the hemp stem) (Livingstone et al., 2022) - the woody core fibre (Stevulova et al., 2014) that possesses unique thermal and acoustic insulation properties (Elfordy et al., 2008) and are actively utilized in the construction industry (Ivanovs & Rucins, 2014). The availability of genetic variation can therefore be used to meet the specific needs of each industry, making sure that the best product is available for the target market. Therefore, the high additive genetic variation for stem diameter observed in this study is crucial for breeders since selection can only be effective when sufficient additive genetic variation is available to enable genetic gains to be achieved. Overall, the contribution of an additive genetic effect to the variance of a target trait is a requirement for genetic improvement, and the current research showed that the traits, stem diameter, plant height, number of internodes, seed yield and biomass yield satisfy this requirement. No significant ($P > 0.05$) additive genetic variance was estimated for thousand seed weight. A possible reason for the results may be due to the limited sample size of ten plants per HS family and/or small sample size of 200 seeds per plant.

Heritability is the ratio of genetic variation to the total phenotypic variation among individuals in a population (Dempster & Lerner 1950; Dudley & Moll, 1969; Falconer, 1965; Fehr, 1987; Hopkins et al., 2009; Hughes et al., 2008; McGuigan & Sgro, 2009). In crop improvement programmes breeders use heritability estimates to assess the genetic potential of base populations (Bernardo, 2020; Dudley & Moll, 1969; Fehr, 1987; Falconer, 1965; Falconer & Mackay, 1996; Mofokeng et al., 2019; Yao & Mehlenbacher, 2000). Narrow-sense heritability is a key estimated parameter in quantitative genetics that is part of the breeder's equation (Dudley & Moll, 1969; Jahufer & Luo, 2018; Rutkoski, 2019a; Spitze, 1995) to predict genetic gain resulting from a single-generation of selection (Lynch & Walsh, 1998). Narrow-sense heritability represents the proportion of phenotypic variation in a specific trait within a population that can be attributed to additive genetic variation (Hopkins et al., 2009). More importantly, heritability is one of genetic parameters that breeders should consider before determining the selection method (Kang et al., 2007). In the present study, the narrow-sense heritability was high for the traits stem diameter (0.82), plant height (0.82), and number of internodes (0.83). More complex traits, such as seed yield (0.70) and biomass yield (0.75), estimated to have high yet relatively low narrow-sense heritabilities in comparison to stem diameter, plant height, and number of internodes. These high estimates indicate that selection based on the phenotypic performance of these traits will be reliable and effective (Waldron et al., 1998). Also, these high narrow-sense heritability values suggest that genetic gains can be achieved through phenotypic selection.

Results published by Carlson et al. (2021), based on a study of 23 hemp families evaluated in Geneva, NY, USA, reported narrow-sense heritability values for the traits stem diameter, plant height, and biomass as 0.85, 0.83, and 0.80, respectively. These estimates are similar to those found in the present study. Carlson et al.'s (2021) study examined the inheritance of morphological traits and their relationship with biomass and cannabinoid yields using 23 families derived from genetically diverse parents. Even so, it is surprising how similar the narrow-sense heritability values were, despite the different hemp population under study and the experiments being evaluated in different environments. In contrast, Hennink (1994) reported low narrow-sense heritability values for stem diameter (0.22) and plant height (0.28) based on the parent-offspring regression method. Hennink's (1994) research utilized parent-offspring relationships (HS families) to determine narrow-sense heritability and to assess the direct and indirect impacts of artificial selection. In a strict sense, estimates of genetic parameters are specific to the population undergoing selection (Hoarau et al., 2022; Laperche et al., 2006), environment (Whitaker et al., 2012), and estimation method (Rameez et al., 2022). These factors explain the apparent differences between the heritability estimates obtained in the present study and those reported by Hennink (1994).

The estimate of narrow-sense heritability on a family mean basis for thousand seed weight was low (0.12). The low additive genetic variation estimated for this trait indicates that achieving genetic gain based on phenotypic selection would be limited (Atlin et al., 2001; Bhandari et al., 2010; Dudley & Moll, 1969; Eid, 2009; Klápště et al., 2020). The low narrow sense heritability estimated in this study may be attributed to several plausible factors, such as (1) limited additive genetic diversity within the sampled reference population or (2) non-genetic (environmental) effects (Daoyu et al., 2002). Furthermore, it is worth considering that the small per plant-sample size of 200 seeds used in this study may have contributed to a low result. None of the studies reported by Hennink (1994) and Carlson et al. (2021) referred to thousand seed weight, which therefore restricts the possibility of comparison with results from the present study.

The genetic coefficient of variance (CVA), more specifically, the coefficient of additive genetic variance, is a standardized value based on the trait mean. Since the CVA is a comparison of the size of the standard deviation of genetic variation to the mean of the reference population, it provides an indication of the potential positive or negative response to selection for a particular trait (Cheung, 2020; Houle, 1992). A high coefficient of variance for seed yield (23.57%) followed by biomass (21.92%) not only indicates that selection can be effective for these traits but also reveals the presence of substantial variability, providing ample opportunity for selection to improve them (Virmani & Athwal, 1973). The coefficient of additive genetic variation for thousand seed weight (3.67%), however, was much lower than the other traits, indicating less potential for this trait to respond to selection.

Overall, the estimates of additive genetic variation, narrow-sense heritability, along with the coefficient of additive genetic variation provide a better picture of the potential genetic gain to be expected. However, these estimates may be confounded by the additive genetic variance × environment interaction effects in the present study and might have resulted in inflated heritabilities and expected genetic gains from selection (Nguyen & Sleper, 1983; Talbert et al., 1983). The other possible confounding factors could be insufficient replicates and sample size. As mentioned in the introduction (section 4.1), only a few research reports have been focused on the additive genetic variation and narrow-sense heritability of hemp seed yield and plant morphological traits. As stated above, part of the discrepancies in results obtained in the present study could be due to differences in the reference populations investigated, estimation methods, crosses utilized, and test environments. Furthermore, the estimates of genetic parameters can be affected by sampling errors, and these parameters may vary over time, particularly in smaller populations undergoing selection. These results indicate the importance of studying population-specific genetic parameters in plant breeding programmes, which will provide breeders with information that will enhance decisions on the choice of breeding strategy to improve the efficiency of genetic gain.

4.4.2 Association of traits

Both genetic and phenotypic correlations are important in plant breeding because they can help breeders understand the relationships between different traits and make informed decisions on selection. The genetic correlation is a measure that shows the genetic relationship between two traits (Alvarado et al., 2020; Border et al., 2022; Cheverud, 1988; Van Rheenen et al., 2019; Werme et al., 2022; Zhao et al., 2022). It is a significant quantitative genetic parameter in plant breeding, as it can either improve or impede the response to selection since genetic correlation is contingent upon whether the direction of the correlation is congruent or antithetical to the direction of selection (Etterson &

Shaw, 2001). Information on genetic correlation can help breeders improve multiple traits simultaneously. Many quantitative traits of interest to plant breeders are often genetically correlated, complicating progress from selection on multiple traits (Neyhart et al., 2019). Therefore, identifying the genetic correlations among the traits is an essential step in plant breeding to help breeders make indirect selections (Alvarado et al., 2020). Likewise, phenotypic correlations can help breeders understand how two traits are related in observable ways (Li et al., 2021; Uhlarik et al., 2022; Vasileva & Kosev, 2015). Although phenotypic correlations among traits arise from the combined influence of genetic and environmental factors, they could be served as reliable indicators of underlying genetic correlations (Waitt & Levin, 1998).

Estimates of genetic correlations, reported in Table 4.4, suggested the occurrence of a genetically based positive relationship between the five traits, stem diameter, plant height, number of internodes, seed yield, and biomass. The study found that stem diameter had a strong positive correlation with seed yield and biomass yield. This suggests that selecting genotypes with higher stem diameter could lead to better biomass and seed yield. The genetic relationship of plant height with biomass and seed yield was strong and moderately strong, respectively, and the estimates were comparatively low to the correlation of stem diameter to biomass and seed yield. This is also supported by moderately strong genetic association of number of internodes to biomass and seed yield since number of internodes is one of the major factors that determines the plant height (Zhou et al., 2023). Moreover, the genetic correlation between plant height and stem diameter was strong, too. While plant height was positively correlated with seed yield, it was to a lesser extent than biomass and stem diameter. Overall, the genetic correlations indicate that hemp plants with thick stems yielded more biomass and seed, with stem diameter being stronger indicator of seed yield and biomass yield.

With respect to the traits mentioned above, the estimated phenotypic correlations were good predictors of underlying genetic correlations. As shown in Table 4.4 the phenotypic correlation coefficients for all five traits appear to be comparable and of predominantly lower magnitude than the genetic correlation coefficient. Higher genetic correlation coefficients in comparison to the phenotypic correlation coefficients indicated in this study demonstrate that the association between the traits was mainly due to additive gene actions. Environmental effects appear to have less influence on trait expression (Akhtar et al., 2011; Cheverud, 1988; Kang et al., 1983; Waitt & Levin, 1998). Empirical evidences of the conjecture also have been found across plant species, with genetic correlation coefficients being typically higher than phenotypic correlation coefficients (Akhtar & Chowdhry, 2006; Akhtar et al., 2011; Waitt & Levin, 1998; Yao & Mehlenbacher, 2000). However, this is not always the case and can vary greatly between species and traits. For instance, in the present study, the phenotypic correlation coefficients of seed yield with plant height and number of internodes were higher than the genetic correlation coefficients. This suggests that the relationship between seed yield and those traits is influenced by both genetic and environmental factors working together in a synergistic manner (Tenkouano et al., 2002). Phenotypic correlations are often assumed to reflect genetic correlations in plants (Waitt & Levin, 1998). Cheverud's conjecture asserts that the use of phenotypic correlations as proxies for genetic correlations is appropriate (Cheverud, 1988). In the present study the genetic correlations mirrored the phenotypic correlations: the patterns of genetic and phenotypic correlations are similar suggesting that genetic variation is likely to be the main source of phenotypic variation, and that the two types of variation are closely associated.

As previously mentioned, it was hypothesized that taller plants with thicker stems would accumulate more biomass and consequently produce higher seed yields. This hypothesis was supported by the results of the present study, which found moderate to strong positive correlations between these four traits. These findings are consistent with the study by Carlson et al. (2021), which reported a significant correlation ($r > 0.65$, $P < 0.001$) between stem diameter, plant height, and biomass yield. Furthermore, their study identified basal stem diameter as the single best predictor of biomass yield. Similarly, in the present study, stem diameter stands out as the most reliable indicator of both biomass and seed yield.

While Carlson et al. (2021) focused on the relationship between morphometric traits and biomass and cannabinoid yield in hybrid hemp, other studies have also investigated the relationship between these traits and seed yield. For example, Campbell et al. (2019a) reported significant positive correlations between seed yield and both plant height ($P < 0.05$, $r = 0.33$) and biomass ($P < 0.001$, $r = 0.62$). They found that while plant height was significantly positively correlated ($P < 0.001$, $r = 0.68$) with stem diameter, in contrast to the present study, it was not significantly correlated with biomass or seed yield. Another study by Aubin et al. (2016) found significant positive correlations between plant height and biomass yield as well as between biomass yield and seed yield. These results support the findings of the present study, although the weak correlation observed between plant height and seed yield differs from the results herein. However, note, the first study mentioned here is conducted using 23 families generated from genetically diverse parents to investigate the inheritance of morphological traits and their association with biomass accumulation and cannabinoid yield. The second study was conducted to estimate genotype by environmental interactions of industrial hemp cultivars. The third study was conducted to evaluate eleven hemp cultivars in seven contrasting environments in Canada. It is important to note that all three investigations above reported phenotypic correlation among the traits. No genetic correlations among the traits investigated in the present study have been reported in the literature. Even so, the results of these three studies show partial support for the additive genetic correlation coefficients estimated among the traits in the present study.

The biplot (Figure 4.7) from the Principal Component Analysis (PCA) summarized the association among the traits: stem diameter, plant height, number of internodes, seed yield, and biomass, and supported the genetic and phenotypic correlations by reflecting the relationship pattern among these traits. The PCA and cluster analysis results helped identify several promising high seed and biomass yielding HS families. Even though the ultimate aim is to improve the yield of seed and fibre, breeding objectives may vary, and this will be depending on the genetic improvement of specific associated plants traits. The overall objective is to develop cultivars that provide both agronomic and economic benefits to farmers that also support environmental sustainability. According to the results obtained in this study, the strong positive correlation of stem diameter with plant height, seed yield and biomass, suggests that stem diameter could be used as an indirect selection criterion for seed yield and biomass yield. This is because stem diameter is easy to measure and has higher heritability (Songsri et al., 2008). However, if a breeder aims to reduce stem diameter to develop a cultivar that is easier to harvest and agronomically advantageous, indirect selection based on stem diameter could complicate the selection process since selecting for reduced stem diameter could also reduce seed and biomass yield. Nonetheless, the correlations among the traits in this study generally indicate the potential for breeding for increased seed yield and biomass.

4.4.3 Genetic gain and simulation

Genetic gain is the improvement of average genetic or phenotypic value within a population over breeding cycles due to selection (response to selection) (Falconer, 1960; Fehr, 1987; Hazel & Lush, 1942; Moose & Mumm, 2008; Rutkoski, 2019b; Sun et al., 2011; Xu et al., 2017). When discussing genetic gain, the focus can be on either predicted or realized genetic gain. Predicted genetic gain is an estimation of the change in phenotype due to genetic improvement resulting from a proposed selection or breeding strategy (Burrows, 1972). Predicted genetic gain can be estimated using genetic parameters from breeding experiments under a set of quantitative genetic assumptions (Rutkoski, 2019b). On the other hand, realized genetic gain is the observed gain due to selection over cycles (Rutkoski, 2019a). Genetic parameters, such as additive genetic variance, heritability, and genetic correlation, provide important information about the genetic potential of a trait or set of traits. The benefit of having estimates of these parameters is that they enable the calculation of predicted genetic gain specific to the reference population (Johnson et al., 1955; Najeeb et al., 2009). In the present study, predicted genetic gain was estimated only for those traits with significant (P < 0.05) additive genetic variance. The estimates of significant additive genetic variance for the traits stem diameter, plant height, number of internodes, seed yield, and biomass yield provided an indication of the potential to improve the expression of these traits through genetic improvement.

Deterministic modelling revealed that increasing selection pressure led to an increase in predicted percentage genetic gain per cycle ($\%\Delta G_c$). In the present study, the predicted percentage genetic gain per cycle ($\%\Delta G_c$) was estimated and presented as genetic gain relative to the family population mean ($\%\Delta G_{\bar{x}}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard. Both $\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$ increased with increasing selection pressures. An increase in $\%\Delta G_c$ with increasing among-family selection pressure is generally expected when high selection pressures are applied to a population with large and highly heritable phenotypic variation (Fasoula & Fasoula, 2002). This outcome is a consequence of increased selectiveness in choosing high-performing families as parents, resulting in a greater difference between the average genetic value of the selected families and the average genetic value of the entire population, leading to a greater response to selection. This result has also been observed in rice (Facchinello et al., 2021), maize (Fasoula et al., 2019), barley (Gracia Gimeno et al., 2012), sugar cane (Jackson, 2005) and different forage species (Bajgain et al., 2020; Casler, 2001; Ehoche et al., 2022; Jahufer et al., 2021). In the context of hemp, a selection intensity of 90% applied to parental plants did not yield consistent improvements in the offspring population, regardless of the plant's characteristics. Conversely, selection intensities of 10% or 50% frequently resulted in positive selection responses and led to correlated responses (Hennink, 1994).

The study also estimated the predicted genetic gains ($\%\Delta G_c$) for stem diameter, plant height, number of internodes, seed yield, and biomass yield based on among- and within-HS family selection (AWF-HS). According to Casler and Brummer (2008), AWF-HS grants widespread benefits compared to among-HS family selection (AF-HS) and is frequently superior to progeny-test selection. Additionally, their findings suggest that the AWF-HS method can potentially enhance expected genetic gain. The results in the present study revealed that irrespective of the trait, the genetic responses ($\%\Delta G_{\bar{x}}$ and $\%\Delta G_s$) were significantly greater when using AWF-HS compared to the AF-HS. This is because the AF-HS only uses a quarter of the total available genetic variance ($\frac{1}{4}\sigma_A^2$), while AWF-HS also accesses within HS family variation equal to $\frac{3}{4}\sigma_A^2$, resulting in increased $\%\Delta Gc$ (Hallauer et al., 2010; Jahufer et al., 2021). Using stochastic plant breeding simulation, Hoyos-Villegas et al. (2019) compared the efficiencies of two breeding strategies, AF-HS and AWF-HS, based on; three levels of HS family mean heritability (0.1, 0.5, and 0.9), three initial parental population sizes (10, 50, and 100 individuals), and three genetic effects models (fully additive, a mixture of additive, partial and over dominance, and a mixture of partial dominance and over dominance). In all scenarios, AWF-HS outperformed AF-HS selection, indicating that AWF-HS resulted in higher rates of genetic gain compared to AF-HS.

The study of parent-offspring relationship conducted by Hennink (1994) investigated the direct and indirect effects of artificial selection on various traits, including fibre content, plant height, flowering date, stem diameter, and cannabinoid contents. This study was conducted as part of a hemp breeding programme which was primarily aimed at improving bast fibre production. The results showed that bast fibre content was highly heritable and directly related to bast fibre yield, while the other traits measured were not significantly associated with fibre yield. In contrast to the present study, low heritabilities were observed for stem diameter and plant height. Hennink (1994) studied the effect of selective breeding on stem diameter in a parental population of plants, using different selection intensities (10%, 50%, or 90% of the plants) and found that the average increase in stem diameter due to direct selection was 16.5, 15.9, and 15.7, respectively, for each selection intensity. However, these estimates were not significantly different from the average stem diameter of the population (15.7). This finding is different to the results of the present study. However, in the same study, the mean direct response to selection for plant height in the parental population was analyzed using the same selection intensities. The mean responses for the selection pressures of 10%, 50%, and 90% were 404.9, 388.4, and 384.5, respectively. The means for the 50% and 10% selection intensities were significantly different from the population mean ($P < 0.05$). While the results of the present study do not align directly with the findings reported earlier, some consistency can be noted between the two studies. Specifically, both studies suggest the possibility of improving plant height and also indicate that increasing the selection intensity can lead to a greater response to selection.

Even though the application of high selection pressures increased the rate of genetic gain, the final number of selected parents decreased with increasing selection pressure. Increasing selection pressure in a breeding strategy means that a breeder selects only a subset of individuals from the parental population that possess the desired traits. As a result, the frequency of favourable alleles increases in the population. Over time, this process can reduce genetic diversity in the parental population (Ehoche et al., 2022; Frankham, 2008; Woolliams et al., 2015; Xu et al., 2017). Genetic diversity in a breeding population is important because it provides the raw material for adaptation (Badu-Apraku et al., 2021; Cobb et al., 2019; Frankham, 2008). If the genetic diversity of a population is reduced, it becomes less able to respond to changes in the environment or new selective pressures, limiting the potential for further improvement (Dawson & Goldringer, 2012; Ehoche et al., 2022; Hall & Richards, 2013; Fischer & Edmeades, 2010; Xu et al., 2017). In cross-pollinated crops like hemp, reducing genetic diversity due to increasing selection pressure can have negative consequences. In hemp, genetic diversity is crucial for maintaining vigour within a population (Clarke & Merlin, 2016). Reducing genetic diversity due to selection pressure can increase the likelihood of inbreeding depression, leading to reduced fitness and productivity (Govindaraj et al., 2015). Furthermore, in this present study, the final number of selected parents was higher based on the AF-HS breeding method, whereas the application of the AWF-HS method resulted in a smaller number of parents being selected. However, the predicted genetic gain achieved was higher in the letter method. In light of the foregoing discussion, it is clear that when planning breeding strategies, plant breeders must consider both the percentage genetic gain per selection cycle and the maintenance of genetic diversity within the resulting progeny population. When applying AWF-HS, the final number of selected parents could be increased by having larger within-HS family samples evaluated.

Computer based decision support simulation enables the investigation of the potential impacts of new breeding technologies, including the rate of genetic gain, other genetic outcomes, and cost-efficiency. This will enable breeders to make informed decisions about which breeding strategies to pursue (Barrett et al., 2021). The deterministic simulation used to assess the impact of replication on predicted genetic gain ($\%\Delta G_c$) for stem diameter, plant height, number of internodes, seed yield, and biomass showed that doubling the number of replicates from two to four substantially increased predicted genetic gain and a similar response to selection was observed when increasing the number of replicates from four to five. Despite this, it is necessary to recognize that each additional replicate incurs costs associated with establishing and managing breeding trials and phenotypic evaluation. Specifically, the costs and time required for phenotyping hinder the efficiency of screening traits in hemp. Hence, the breeder should make the final decision based on the associated cost per percentage increase in genetic gain ($\%\Delta G_c$).

4.4.4 Check cultivar performance

In plant breeding trials, check cultivars serve as benchmarks to compare newly bred material and assess realized genetic gain (Bull et al., 1992; Smith et al., 2005). In this study commercial check cultivars enabled identification of HS families with superior performance. Fasamo and Férimon 12 were the two commercial check cultivars included in the field trial. Fasamo and Férimon 12 are monoecious, dual-purpose cultivars bred in Germany and France, respectively. Both cultivars are commonly grown throughout Canterbury (New Zealand's main grain and seed production is based in the Canterbury region) and other hemp-growing regions of New Zealand (Townshend & Boleyn, 2008; Scott, 2023; Wulff et al., 2021).

Linear mix model analysis with checks and HS families modelled as fixed effects, was conducted to determine the relative performance of the HS families and checks. The results indicated significant ($P < 0.05$) differences among the entries. Several HS families outperformed the check cultivars for the traits; stem diameter, plant height, number of internodes, seed yield and biomass yield. To provide further clarification, the trait seed yield is used as an example. The check cultivars Fasamo and Férimon 12 are high seed-producing cultivars (Deleuran & Flengmark, 2006; Höppner & Menge-Hartmann, 2007; Miyan, 2022). Of the 50 HS families evaluated, four outyielded cultivar Férimon 12 and seven outyielded cultivar Fasamo. Seed yield of the best HS family (HS 44) was 85% higher than the best-performing commercial check in this trial (Férimon 12). These results demonstrated the potential use of these HS families to be used as elite parents for developing breeding material to increase the seed yield in modern breeding programmes. Likewise, HS families performed significantly better than the commercial checks for each trait; stem diameter, plant height, number of internodes and biomass were identified.

It is important to note that the reported study was conducted in a single environment. It is crucial to evaluate a larger sample of HS families across the target population of hemp production environments in New Zealand. Results from multi-location trials will be significant in the development of broadly adapted cultivars for New Zealand. Evaluation of material across a broader range of environmental conditions (years and contrasting locations) will improve selection accuracy as the confounding effects of Genotype by Environment interaction (G × E interaction) will be reduced (Brown & Caligari, 2008; Byth, 1981; Cooper & Byth, 1996; Elias et al., 2016; Gage et al., 2017; Jahufer et al., 2013; Jahufer & Luo, 2018; Moose & Mumm, 2008; Richards et al., 2010; Smith et al., 2021; Yue et al., 2022). Conducting multi-environment testing of breeding material, across multiple locations and years, will also enable characterization of the magnitude and significance of G × E interaction, resulting in more informed decisions being made when selecting for specific or broad adaptation.

4.5 Conclusion

Results from this study indicated that:

- There was significant ($P < 0.05$) additive genetic variation among the 50 HS families for the traits; seed yield, biomass yield, stem diameter, plant height, and the number of internodes, indicating the potential to achieve genetic gain through selective breeding.
- Both AF-HS and AWF-HS breeding strategies resulted in achieving genetic gain for the traits measured. However, the AWF-HS strategy showed the highest genetic gains for the traits in comparison to AF-HS. This was due to the fact that

the AWF-HS breeding strategy included both the among HS family additive genetic variation ($\frac{1}{4}\sigma_A^2$) and the within HS family additive genetic variation ($\frac{3}{4}\sigma_A^2$). Even though increasing selection pressures increased genetic gain from both AF-HS and AWF-HS, it was clear that the number of selected parents decreased as selection pressure increased. This would reduce the genetic diversity available in the parental sources by reducing the pool of potential parental lines for the next breeding cycle. These results emphasize the importance of determining the optimal selection pressure that maximizes genetic gain and maintaining genetic diversity, especially for cross-pollinating species such as hemp.

- Deterministic simulation revealed that increasing the number of replicates in the field trial increased predicted genetic gain for each trait. However, increasing replication resulted in augmenting trial costs, which decreased cost-efficiency of the breeding strategies, increasing the cost of achieving 1% genetic gain.
- Moderate to strong positive genetic correlations were estimated among all the traits and were generally higher than the phenotypic estimates. A key point to consider is that genetic correlation coefficients can be confounded by environmental correlation effects.
- The genetic correlation coefficients estimated indicate that genotypes with higher seed and biomass yields can be developed from the reference population, represented by the random sample of 50 HS families.
- There were significant differences among the 50 HS families and two commercial checks, Fasamo and Férimon 12. For each trait, HS families that significantly outperformed the commercial checks were identified.

This study provides important information on estimating genetic gain based on two breeding strategies for a range of morphological traits that were measured. The data for genetic gain analysis were generated from field evaluation of 50 hemp half-sib families. Although quantitative genetic estimates are population-specific, the outcomes from this study provide information for hemp breeders developing cultivars for New Zealand, focused on improving seed and biomass yield. It is important that a similar experiment be conducted across multiple years and locations to identify HS families with specific and broad adaptation across the target population of hemp production environments in New Zealand. Further evaluation over years and locations is crucial to quantify the magnitude and significance of genotype-by-environment effects, which will be useful when planning future hemp breeding programs for New Zealand.

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CHAPTER 5

Estimation of genetic diversity and the degree of variability within and among a selection of hemp varieties using microsatellite (SSR) markers

Abstract

Industrial hemp (*Cannabis sativa* L.) is a genetically diverse and highly variable crop grown for seed, fibre, and metabolites. Starting with the approval of the cultivation in 2001, hemp is being revitalized in New Zealand, but cultivation, research and breeding are strictly regulated. Few breeding programmes have been conducted to develop new cultivars adapted to New Zealand environments. Moreover, limited work has been conducted to characterize genetic diversity and the degree of variability within and among the hemp cultivars currently approved for growing in New Zealand. Information on the extent and organization of genetic diversity and the relationship among introduced hemp cultivars will significantly help breeders develop new and superior material adapted to New Zealand. A total of 288 randomly sampled individuals from six New Zealand-approved cultivars were assayed to study genetic diversity and population structure using seven previously developed microsatellites (SSR) markers. STRUCTURE analysis identified two distinct genetic clusters that align with the plant's reproductive biology (monoecious and dioecious) followed by further breakdown according to the plant's end-use (fibre, seed, fibre and seed, and CBD cultivars). The genetic diversity metrics revealed moderate genetic diversity among the six hemp cultivars. The average observed heterozygosity (H_o) and the expected heterozygosity (H_e) were 0.44 and 0.50, respectively. The Wright's fixation index (F_{IS}) varied from 0.26 (Futura 75) to -0.02 (Finola). Analysis of molecular variance (AMOVA) indicated that a large proportion of the total genetic diversity was observed within cultivars. Only 11% of the molecular variation was attributed to differentiation among the cultivars, while the variation among individuals within cultivars was 19%. The genetic variation within individuals (69%) was higher than the variation among individuals and cultivars. Moreover, considerable variation among the male and female individuals of dioecious hemp cultivars was detected. The genetic diversity parameters of female and male groups revealed that female individuals possess more genetic variation than males. The results obtained in this study clarify the genetic diversity and the genetic structure of selected New Zealand-approved hemp cultivars, which have been limited to date. This study provides insights into the

selected hemp cultivars' relationship and diversity. The results of this study also present valuable information for establishing a foundation for planning future breeding programmes, that avoid potential genetic vulnerability.

Keywords: Industrial hemp (*Cannabis sativa* L.), SSR, microsatellites, genotyping, and genetic diversity

5.1 Introduction

Industrial hemp (*Cannabis sativa* L.) is a cross-pollinating crop tailored for a wide array of specific end-use quality traits. Hemp is a highly valued plant, despite its relatively limited acreage worldwide (Onofri & Mandolino, 2017). Over a 12,000-year history of cultivation (Russo, 2011), hemp has been used across civilizations for diverse purposes, including textile fibres, woody pulps, edible seeds and oils and medicinal products (Stansell & Osatuke, 2021). Archaeological evidence for hemp fibre textile production suggests that *Cannabis* was one of the first domesticated fibre plants (Lynch et al., 2016). Central Asia has often been suggested as the origin of cultivation for the plant, and that it was initially grown for fibre and grain (Punja et al., 2017). According to Ethan Russo in Pain (2015), *Cannabis* spread globally quickly once people started growing the plant. In the past two decades, the expansion of hemp's potential across conventional and novel markets has gained traction through an increasing number of public and private entities (Fine, 2014; Mi et al., 2020).

Cannabis, a member of the family Cannabaceae, is a diploid (2n = 2x = 20) outcrossing plant (Goff et al., 2022) that relies on wind-mediated pollination (Small & Antle, 2003). Although the sexual phenotype of *Cannabis* often shows some degree of flexibility that causes the differentiation of hermaphrodite flowers or bisexual inflorescences (monoecious phenotype), it is predominately dioecious (Soler et al., 2017). Dioecious *Cannabis* produces either male (pollen) flowers or female (seed) flowers, and therefore, the plants are obligatory out-crossers (Jones & Monthony, 2022). However, self-pollination occurs in monoecious varieties and hermaphrodite plants (Punja & Holmes, 2020; Zheng, 2022). In *Cannabis*, hermaphroditic inflorescences have been observed spontaneously on occasion (Small, 2017). A few or many anthers may develop within the leaf axils or in pistillate flower buds in these plants but produce predominately female inflorescences (Punja & Holmes, 2020). Renner and Ricklefs (1995) hypothesized that dioecy might often evolve from a hermaphrodite ancestor, with the unisexual plants benefiting from the genetic advantages of guaranteed outcrossing (Benevides et al., 2022; del Castillo & Trujillo-Argueta, 2022; Punja & Holmes, 2020). An individual *Cannabis* plant's sex expression is determined by chromosome heteromorphism, where homogametic (XX) and heterogametic (XY) individuals are female and male, respectively (Moliterni et al., 2004). Despite having XX chromosomic configuration (Razumova et al., 2016), monoecious plants of *Cannabis* produce flowers of both sexes, and they arise only rarely in nature (Clarke & Merlin, 2016). It is relatively rare for plants to be wind pollinated, dioecious, and to have X/Y sexual inheritance, but they are all characteristics of *Cannabis* (Clarke & Merlin, 2016).

Environmental factors and a plant's genotype determine its phenotype. *Cannabis* has been widely distributed and adapted to a broad range of geographical regions with continuous natural selection along with extensive selection and domestication, resulting in elevated genetic and phenotypic variation (Clarke & Merlin, 2016; de Meijer & Keizer, 1996; Kovalchuk et al., 2020; Meijer, 1995; Salentijn et al., 2015). With the course of time, *Cannabis* evolved into genetically and phenotypically diverse varieties under natural selection within the diverse environments into which they were introduced. Humans have made further selections to produce fibre, seed, or medicinal products (Clarke & Merlin, 2016). Salentijn et al. (2015) classified hemp based on the following features: population types (e.g., wild and natural populations, landraces and cultivars), plant end-use (fibre cultivars, seed cultivars, medicinal and ornamental strains), flowering time (early, intermediate and late maturing varieties), reproductive biology (monoecious and dioecious varieties) and geographical origin (e.g., European and Asian type cultivars). The wide range of globally available genetic diversity provides the potential for developing improved and unique cultivars through hemp breeding programmes.

Genetic diversity is essential for breeding programmes and has been the basis for plant genetic improvement (Swarup et al., 2021). The availability of genetic variability is vital to breeding programmes and determines the success of genetic crop improvement programs (Gupta et al., 2011; Gupta et al., 2016; Srivastava et al., 2018a, 2018b). Assessment and effective utilization of genetic diversity are crucial for providing a basis for choosing suitable breeding strategies that ensure sustainable genetic improvement (Chakravarthi & Naravaneni, 2006). In crop breeding programmes, this is the key step for maintaining and increasing the rate of genetic gain (Enyew et al., 2022). Landraces, obsolete cultivars, currently grown cultivars, advanced breeding lines, undomesticated plants, mutants, special genetic stocks, and plant introductions are key plant genetic resources of useful variation (Singh et al., 2021). Overall, existing genetic diversity is a crucial foundation for plant genetic improvement. Evaluating this genetic diversity, structure, and lineage relationships of genetic resources available is essential for sustainable breeding programs to develop new and productive cultivars.

Many techniques are being used to explore genetic variation at both genotypic and phenotypic levels. One of the most accurate methods for investigating diversity in hemp is through DNA-based molecular marker technology, which reveals differences among the genetic resources at the DNA-level. This provides comprehensive data for regional genetic improvement, genetic characterization, and breeding strategies' improvement and development (Borna et al., 2017; Fabbri et al., 2019). The most commonly used molecular markers in *Cannabis* research to date are inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), sequence-characterized amplified regions (SCAR), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeat (SSR), chloroplast genome spacer regions, internal transcribed spacer (ITS) of the nuclear genome, single-nucleotide polymorphisms (SNP), whole genome shotgun sequencing (WGS), and genotyping-by-sequencing (GBS) analysis (Hesami et al., 2020). Among the extensive range of molecular markers available, microsatellites are widely and frequently used in *Cannabis* varieties and genotypes to explore genetic diversity (Borin et al., 2021). Unlike other markers such as SNPs, microsatellites can be employed using both low and high-throughput genotyping approaches (Vieira et al., 2016). Microsatellite markers are considered the most capable markers for plant genetics and breeding programmes due to their codominant heredity, high level of reproducibility, multiallelic nature and excellent genome coverage (Mason, 2015). Microsatellites continue to be used in the genetic analysis of cultivated plants as a high-relevance molecular marker (Vieira et al., 2016).

The New Zealand industrial hemp market is growing rapidly and is projected to be a 2-billion-dollar industry by 2030 (Marsh, 2020). Hemp has had to face 80 years of prohibition, is still facing regulatory challenges in New Zealand, and as an industry, it is still in its infancy (Marsh, 2020; Moore et al., 2021). New Zealand is not exporting hemp products yet, but increasing local demands offer immediate opportunities for the growth of the New Zealand hemp industry. There are barriers to research and breeding of *Cannabis*, such as limited research funding for newly established industries and difficulties working within a recently legalized sector, closely related to an illegitimate industry (Newcomb, 2021). Nonetheless, an enormous amount of work is required (Marsh, 2020), especially for breeding programmes, to develop cultivars best suited to New Zealand's environmental conditions (AbacusBio, 2022; Hemp New Zealand, 2017; Marsh, 2020; McPartland et al., 2008; Moore et al., 2021; Venture Taranaki, 2022). Despite increasing interest in and demand for its use in a range of products, the genetic improvement of hemp is almost stagnant in New Zealand. Among the 20 cultivars currently approved to grow in New Zealand, five are New Zealand hemp cultivars (MoH, 2023). Under the ambit of Hempseed Holdings Ltd, Donald J. McIntosh created these five acclimatized generic New Zealand hemp cultivars: Aotearoa 1 (dioecious), A1 Monopurp (monoecious), A1 MACMONO (monoecious), A1 COMSEED (dioecious), and A1 HCFX (dioecious). Aotearoa 1 is the base seed stock for all of the series of subsequent cultivars developed (McIntosh, 2022). According to the breeder McIntosh (2022), Aotearoa 1 and the A1 series of generic landrace Aotearoa New Zealand hemp cultivars are "very different", and the genetics are not available anywhere globally, except for within the A1 series.

Just after the New Zealand government approved hemp cultivation in 2001, McPartland et al. (2004) conducted variety trials with eleven cultivars from Europe or North America (Anka, Carmen, Fasamo, Felina, Finola, Futura 77, Kompolti, Uniko B, USO 14, USO 31, and Zola) in 11 locations across the North and South Islands. Following the first harvest of this trial, D. J. MacIntosh realized that productivity of these offshore cultivars, in terms of both plant growth and yield, could be improved. This observation led him to develop cultivars for New Zealand (Simmonds, 2021). However, these cultivars are relatively unknown in New Zealand. Even though 25% of approved cultivars are acclimatized generic New Zealand cultivars (trademarked), these cultivars do not make up 25% of the cultivars grown in the country, according to McIntosh (2022). Moreover, low uniformity within offshore hemp cultivars was observed in New Zealand farmers' fields (R. Barge, personal communication, November 6, 2019). Many studies in other countries also extensively observed significant phenotypic variation within a single hemp cultivar in terms of morphological traits and chemical profile and pointed out the need to breed uniform and stable hemp cultivars (Campbell et al., 2019; Chen et al., 2021; Ferfuia et al., 2021; Hesami et al., 2021; Stack et al., 2021).

Generally, hemp can grow in a wide range of environmental conditions, but varieties tend to perform better in their areas of development (Dempsey, 1975). According to Ranalli (1999), "modern" industrial hemp varieties are descendants from very few ancestors. Breeding histories in the literature show that hemp varieties have a narrow genetic base (Onofri & Mandolino, 2017; Ranalli, 1999). For example, most European varieties are developed based on three gene pools: Northern and Central European ecotypes, Southern Europe ecotypes, and East Asian ecotypes (Meijer, 1995). Many of today's dioecious fibre varieties share a common ancestor, 'Carmagnola' (a *Cannabis* landrace from Italy), and nearly all modern European monoecious varieties descended from the same ancestor; variety Fibrimon (Flajšman & Ačko, 2022). Ranalli (1999) pointed out that this narrow genetic base of the hemp cultivars is likely the reason why hemp varieties are poorly suited to growing in regions outside Europe. This also explains D. J. MacIntosh's observation on offshore hemp cultivars in New Zealand.

Given the growing hemp industry in New Zealand, there is a pressing need among hemp breeders to develop knowledge to accelerate the breeding of uniform and stable New Zealand cultivars. This can be achieved by investigating the genetic diversity of available parental resources and broadening the genetic base, which is crucial for ensuring genetic diversity, adaptability, disease resistance, improved yields, and the long-term sustainability of agricultural systems. Furthermore, globally, studies that assess the genetic diversity of hemp cultivars using microsatellite markers are limited, and there is limited research published that reveals the genetic relationships and diversity among New Zealand-approved hemp cultivars. Therefore, as an initial step towards the sustainable development of New Zealand-bred hemp cultivars, this study aimed to characterize the genetic diversity and the degree of variability within and among a set of selected commercial hemp cultivars using microsatellite markers.

5.2 Materials and methods

5.2.1 Plant material

This study used six New Zealand-approved hemp cultivars, namely CFX–2, CRS–1, Férimon 12, Katani, Futura 75, and Finola (Table 5.1). A total of 288 young undamaged leaf samples representing all six cultivars were collected from the multi-environmental field trial (Table 3.2) conducted in 2019/2020. The leaf samples were collected from randomly selected 24 male and 24 female plants in dioecious cultivars (CFX–2, CRS–1, Katani, and Finola) and from 48 plants in monoecious cultivars (Férimon 12 and Futura 75), making a total of 48 leaf samples per cultivar. Leaf samples from each of the sampled plants were placed directly into coffee filters, which were then placed in Ziploc® style bags containing silica gel (Chameleon®) to desiccate and preserve the leaves until extraction of genomic DNA.

Table 5.1. Breeding region, reproductive biology and end use of cultivars selected for the genetic analysis.

Cultivar	Breeding region	Reproductive Biology (Cultivar type)	End use (Cultivars bred specifically for)
CFX–2	Canada	Dioecious	Seed
CRS–1	Canada	Dioecious	Seed
Férimon 12	France	Monoecious	Seed & fibre
Katani	Canada	Dioecious	Seed
Futura 75	France	Monoecious	Fibre
Finola	Finland	Dioecious	Seed

5.2.2 DNA extraction

Genomic DNA was extracted from preserved leaf samples using DNeasy Plant Mini-kits (Qiagen) and their quantity and quality were checked on 0.9% agarose gels with ethidium bromide staining in a Bio-Rad Gel Documentation system (Bio-Rad, Inc., California USA).

5.2.3 SSR markers, PCR amplification, and electrophoresis

Twenty polymorphic SSR primer pairs from Zhang et al. (2020) were initially screened to identify markers that generated clear single bands, stable amplification, polymorphism, and specificity to their target loci using seven random DNA samples. All forward primers of each primer pair were 5'-tailed with an M13 tag sequence (5′-CACGACGTTGTAAAACGAC-3′) (Boutin-Ganache et al., 2001). After performing the trial genotyping (details below), seven pairs of SSR primers were chosen for further analysis (Table 5.2).

Table 5.2. SSR markers used in this study.

Primer name	ID	Repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Original product size (bp)
SSRhemp-17	Chr 3	(CT)8	ACAAAACCGAAACCAAATCTCTCTC	ACTTCACAACTTCAATCTTCCAGC	189
SSRhemp-26	Chr 4	(TC)7	ACTTATGCCTTCTCTCTCCTCTCT	ATGGACCTTTCTTCACATTAGCCT	265
SSRhemp-33	Chr 5	(AG)7	CGACCGATGATCAACCCTAATACA	TTTGTAAAACCCCATTCTACCCCT	275
SSRhemp-37	Chr 5	(TGT)6	GAGTAATTTGGCTGCTGTTGGTTA	CTTAGTTTTCAAGCCCCTGTCATG	245
SSRhemp-52	Chr 7	(AGA)8	GAAATAGAAAAGCTGAAAGGGTTGG	TCAAAGCACTCAAGTATCTACCCT	178
SSRhemp-66	Chr 9	(TG)10	AAAGAAGAGTGTGTAGTGACGACA	ACATCTTTCTCCAGTCAAGCTCAT	156
SSRhemp-68	Chr 9	(TAT)6	GCGAGCAGTTGAAAATTAAGCCTA	TTCTTCACTCATTCCTAGTCACGG	184

Source: Zhang et al. (2020)

PCR amplifications were carried out in a 10 µL reaction volume containing 1.0 µL template DNA, 3.7 µL double-distilled water (ddH_2O), 0.2 µL (10 mM) dNTPs (Bioline Ltd., United Kingdom), 1.0 µL Reaction Buffer BD (10× FIREPol® reaction buffer), 1.0 µL $MgCl_2$ (25 mM), 1.0 µL each forward and reverse primer (0.2 µM and 4.5µM respectively), 1.0 µL M13 primer (labelled with either FAM, VIC or NED[15]) (4.50 µM) and 0.1 µL (5 U/µL) FIREPol® DNA polymerase (Solis BioDyne, Tartu, Estonia) and amplified using PCR thermal cyclers: Biometra T-Gradient ThermoBlock (Gottingen, Germany) and MaxyGene™ Gradient Thermal Cycler (USA). Three different PCR conditions were performed separately for a set of primers by varying annealing temperature at either 50°C, 52°C, or 55°C to determine the optimum primer annealing temperature that ensures the ideal pairing between primer and complementary sequences (Hwang et al., 2003). The PCR conditions were: initial denaturation at 95℃ for 3 minutes, followed by 30 cycles of denaturation at 95℃ for 30 seconds, annealing at varying temperatures for 30 seconds, extension at 72℃ for 45 seconds, and a final extension at 72℃ for 20 minutes. An annealing temperature of 52℃ was used in the subsequent runs as it produced more prominent bands during electrophoresis trials. The PCR products were checked by resolving 2 µL of amplicon and 2 µL of 3X loading dye in 1.5% agarose gels and electrophoresed for 45 minutes at 75 volts. The Ethidium bromide-stained bands were visualized using the Bio-Rad gel documentation system.

[15] Fluorescence dye primers; 6-carboxy-fluorescein (FAM), 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) or 2′-chloro-5′-fluoro-7′,8′-benzo-1,4-dichloro-6-carboxyfluorescein (NED).

5.2.4 SSR genotyping

5.2.4.1 Dilutions

PCR products were diluted before submission for genotyping based on the intensity of PCR products checked on 1.5% gels and genotyping trials (based on the results of the initial screening of markers) to reduce the number of pull-up peaks (Dimsoski, 2003).

5.2.4.2 Maker pooling

Three markers were poolplexed at a time to reduce the genotyping costs. For that reason, three markers with non-overlapping size ranges were pooled together, each labeled with either FAM, NED, or VIC (Applied Biosystems, Inc.) dyes.

5.2.4.3 Hi-di mix preparation and submission for genotyping

The Hi-Di mix was prepared by adding 10 µL of ROX-labelled CASS ladder (2.5 µL each of 100, 200, 300, and 400 bp) produced in-house (Symonds & Lloyd, 2004) and 90 µL of ddH_2O to 1000 µL of Hi-Di™ Formamide (Applied Biosystems™, California, USA). 9 µL of Hi-Di formamide-size standards mix was mixed with 1.25 µL of pooled PCR products. The prepared barcoded genotyping plates were submitted to the genotyping services at the Massey Genome Service (Palmerston North, New Zealand) for microsatellite genotyping on an ABI 3730 Genetic Analyzer (Applied Biosystems™, California, USA).

5.2.5 Data analysis

5.2.5.1 Allele calling

GeneMapper® Software 5 (Applied Biosystems™, California, USA) was used to visualize and analyse the raw genotype data for initial allele calling. The alleles were scored manually and formatted for GenAlEx v6.5 in Excel (Peakall & Smouse, 2006; Peakall & Smouse, 2012).

5.2.5.2 Genetic diversity analysis

Genetic diversity was analysed within and among the six cultivars in GenAlEx v6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012). The following diversity statistics were calculated for each cultivar: number of samples (N), number of alleles per locus found among the seven loci (N_a), number of private alleles (P), observed (H_o) and expected (H_e) heterozygosity (Nei, 1973), fixation index (F_{IS}), and pairwise F_{ST} among cultivars (Wright, 1965).

5.2.5.3 Genetic structure analysis

Genetic structure and diversity of the hemp cultivars were assessed using three different approaches. The genetic structure of the hemp individuals and cultivars was initially assessed with a model-based Bayesian structure implemented in the software STRUCTURE v2.3.4 (Pritchard et al., 2000). The programme was run ten times each for a continuous series of cluster values (*K*) from one to six, and for each run, the Markov chain Monte Carlo (MCMC) algorithm was run with a burn-in period of 100,000 iterations followed by 1,000,000 replications. The admixture model was used without prior on the sample origin, and allele frequencies were set to be correlated. STRUCTURE output results were imported into the CLUMPAK (Cluster Markov Packager Across *K*) online server tool (Kopelman et al., 2015) to identify the most informative *K* value based on the delta *K* (*ΔK)* statistic (Evanno et al., 2005). In spite of the most informative *K* value identified by the CLUMPAK, STRUCTURE results from $K = 2 – 4$ are presented in the results section. Based on those results, the individuals were grouped hierarchically for subsequent analysis, where genetic groups were obtained from the assignment of admixed samples to their most likely genetic cluster for which they have the highest proportion. CLUMPAK (Kopelman et al., 2015) was also used to find consensuses among the replicates at each *K* value using the Markov clustering algorithm of CLUMPP (Cluster Matching and Permutation Programme) (Jakobsson & Rosenberg, 2007) and to format the output results by aligning single results obtained for different *K* values using DISTRUCT V.1.1 (Rosenberg, 2004).

Besides the STRUCTURE analysis, the distribution of genetic variation and the relationship between the genetic clusters were assessed using two other methods. A Principal Coordinate Analysis (PCoA) was performed as part of the model-based discriminant analysis of principle components (DAPC) analysis in R 3.6.1 (R Core Team, 2022) using the package 'adegent' (Jombart and Ahmed, 2011) to identify genetic differentiation between groups. With DAPC, multiple cluster (*K*) values (1 - 4) were assessed, as with STRUCTURE.

Adding to the assessment of genetic structure, the R package *poppr* (Kamvar et al., 2014) was used to generate cultivar-level pairwise genetic distances. Based on the distance matrix developed, a Neighbor-Net splits-graph was generated in SPLITSTREE v.4.14.6 (Huson and Bryant 2006) to produce a snapshot of the microsatellite data with detailed and informative networks among the six cultivars. Finally, an Analysis of Molecular Variance (AMOVA) was performed using GenAlEx v6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012) to estimate the molecular variance within and between cultivars.

5.3 Results

5.3.1 Genetic structure

STRUCTURE analysis was used to identify genetic groupings in the full microsatellite data set by placing samples into groups whose members share a similar pattern of variation (Porras-Hurtado et al., 2013). Using Evanno's method (Evanno et al., 2005), $K = 2$ was estimated as the best-supported number of clusters for the data set (Figure 5.1), indicating the presence of two genetic groups in the panel of 288 individual plants from six cultivars of hemp. At $K = 2$, a clear partitioning of individuals is evident ($\Delta K_2 = 483.127$). Although it has a much lower ΔK value, the genetic structure at $K = 3$ also makes sense as it seems to be associated with the end-use of the cultivars. Therefore, rather than focusing on a single best value of K to explain the STRUCTURE results, here, the results of STRUCTURE analyses at several K values are explored and interpreted as in McLay et al. (2022).

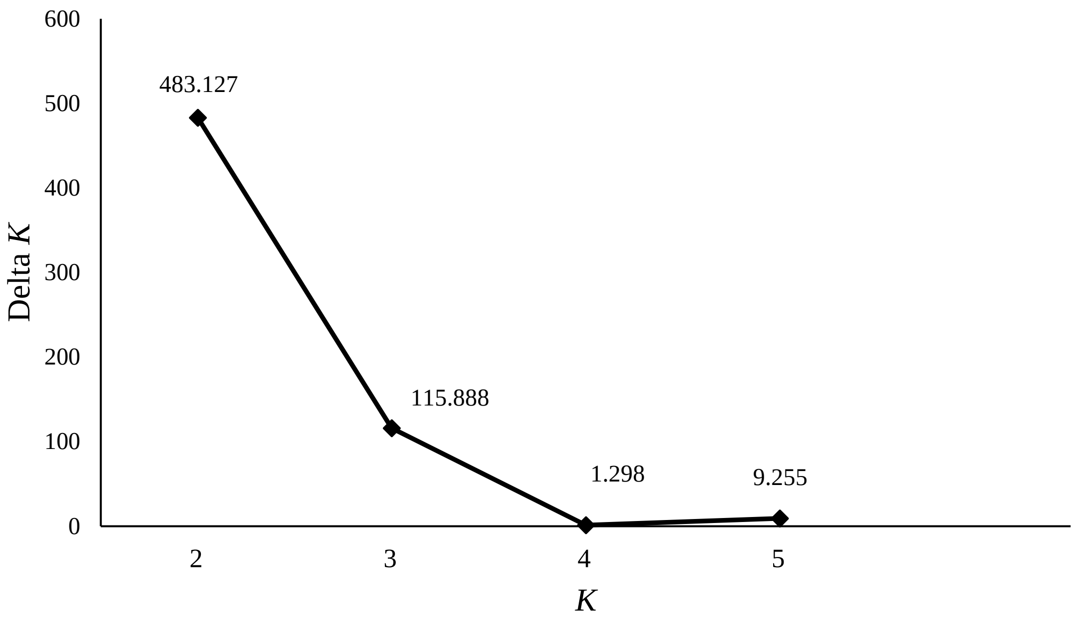

Figure 5.1. Plot of delta-*K* (*ΔK*) results for industrial hemp based on the Evanno et al. (2005) methods for the estimation of the number of genetic clusters in a sample. This method indicates that STRUCTURE results at K=2 have the greatest support.

As indicated previously, in the STRUCTURE plot for $K = 2$, individuals were clustered into two genetic groups. This genetic structure identified at $K = 2$ appears to reflect the groupings either by reproductive biology or the cultivars bred for specific use. One group mainly consisted of all the dioecious/seed cultivars (CFX-2, CRS-1, Katani, and Finola) and the individuals from the monoecious/fibre and dual-purpose cultivars (Futura 75 and Férimon 12) were grouped in another cluster (Figure 5.2). Interestingly at $K = 3$, the CFX-2 and CRS-1 individuals were largely separated from Katani and Finola, potentially reflecting the use of Katani and Finola in CBD (Cannabidiol) production. It should be noted that despite Katani and Finola being traditionally bred for seed production, these cultivars are thought to possess significant levels of CBD and exhibit consistent CBD content (Hammami et al., 2022; Todd et al., 2022). Notably, the second cluster with the two French cultivars clearly separate out from one another: fibre (Futura 75) and dual-purpose (Férimon 12) cultivars. In general, despite the fact that, in some cases, an admixture of individuals from different clusters was found in the same cultivar, most of the individuals of a given cultivar were found to be genetically similar (particularly for the French and Finnish cultivars). To reveal more internal structure, the genetic structure at $K = 4$ is also illustrated in Figure 5.2. However, individual membership coefficients splintered in structure results at $K > 3$, producing progressively less consistent results ($K > 4$ not shown).

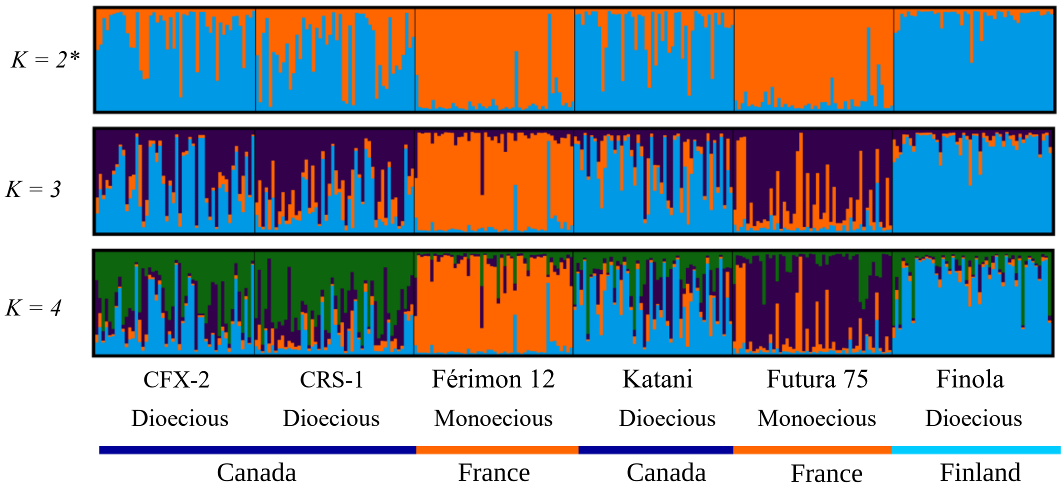

Figure 5.2. Estimated genetic structure for 288 individuals of six industrial hemp cultivars from $K = 2$ to $K = 4$. Each row represents the number of clusters (K). Vertical lines correspond to a different individual, with each colour representing a relative proportion of association with each inferred cluster (membership coefficient), assigned by distinct colours. * The Ideal number of clusters according to Evanno's method.

Principal Coordinate Analysis (PCoA) is a multivariate analysis, generally applied in population genetics to characterize population subdivisions based on the degree of divergence between individuals and interpret the underlying genetic structure, providing assignment of individuals to groups (Tesfaye, 2022; Wang, 2022). The PCoA was performed as an alternative method for identifying and describing genetic clusters within the six hemp cultivars. The PCoA results reveal similar but partially contrasting output patterns compared with the STRUCTURE results (Figure 5.3). The genetic structure pattern yielded was similar to that of the $K = 2$ STRUCTURE results, mostly separating the dioecious cultivars from the monoecious cultivars. It also showed that Katani and Finola roughly clustered together, which was in agreement with the STRUCTURE results at $K = 3$. The PCoA did not differentiate the cultivars based on end-use (dual-purpose, fibre, seed and flower/CBD) as STRUCTURE identified. In STRUCTURE, the CFX-2 and CRS-1 showed a high percentage of admixture among the six cultivars (Figure 5.2). This admixture pattern is evident in PCoA, where no systemic clustering was observed among the individuals belonging to these two cultivars. Overall, PCoA presented somewhat discordant but complementary results to the STRUCTURE.

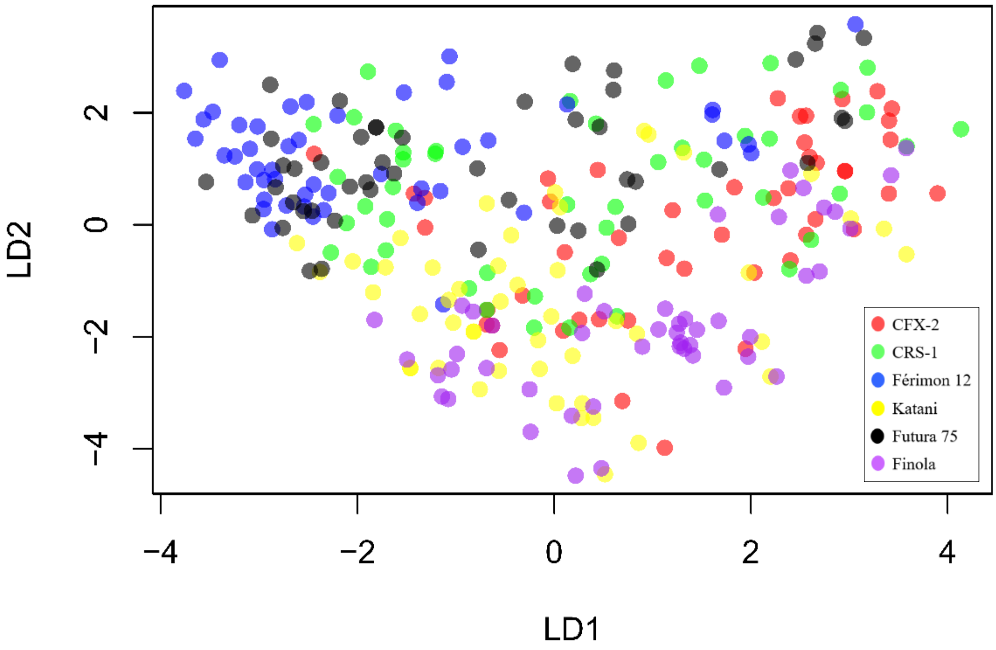

Figure 5.3. Principal Coordinate Analysis (PCoA) for industrial hemp at *K* = 3, where individuals from cultivars CFX-2, CRS-1, Férimon 12, Katani, Futura 75, and Finola colour-coded as red, green, blue, yellow, black, and purple.

Neighbor-Net is a distance-based method for constructing phylogenetic networks that is based on the Neighbor-Joining algorithm of Saitou and Nei (Bryant & Moulton, 2004). Neighbour-Net graphs are used to visualise the genetic structure and provide a detailed snapshot of the data (Bryant & Moulton, 2004). The Neighbor-Net analysis was performed with the six hemp cultivars to explore the similarities and dissimilarities among the cultivars, instead of individuals. The results showed that the monoecious or the French cultivars bred for fibre and dual-purpose (Férimon 12 and Futura 75) clustered together, isolated from the dioecious/seed cultivars (CFX-2, CRS-1, Katani and Finola) by a long split (Figure 5.4). This result is consistent with the STRUCTURE results at $K = 2$ (Figure 5.2) and PCoA output (Figure 5.3). In most of the splits, the Canadian cultivars CFX-2 and CRS-1 tend to cluster together, whereas the other Canadian cultivar Katani shows similarity with the Finnish cultivar Finola.

Férimon 12 and Finola appear to be the least similar genetically, a pattern consistent with the STRUCTURE results at $K = 3$ and $K= 4$ (Figure 5.2) and PCoA results (Figure 5.3). Despite slight differences, Neighbor-Net results supported the STRUCTURE ($K = 2$ and K

= 3) and PCoA results as a fair summary of the distribution of genetic variation obtained herein.

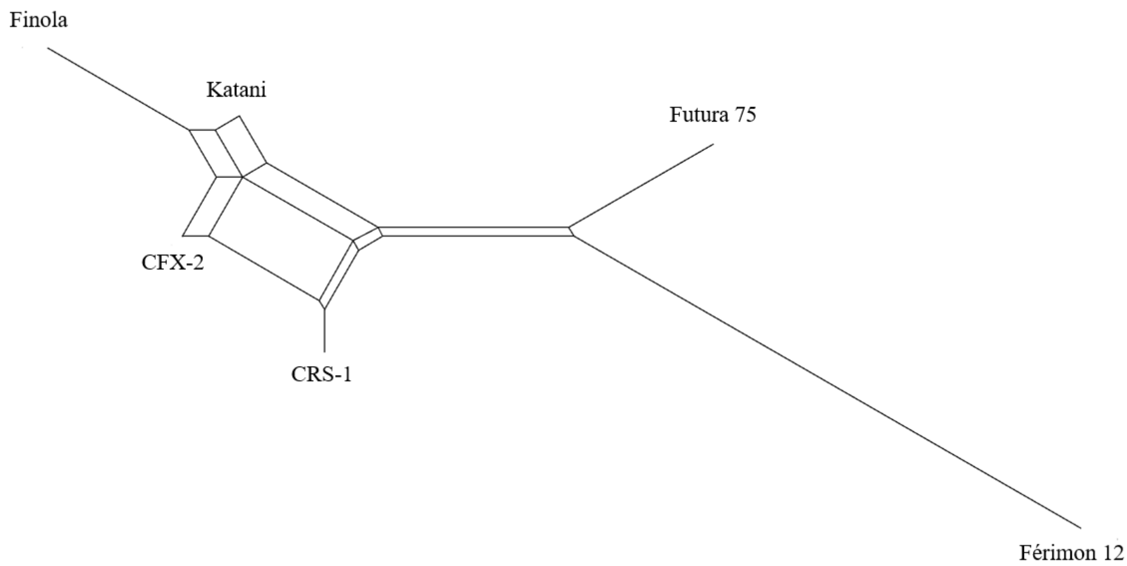

Figure 5.4. Cultivar level Neighbor-Net splitsgraph from a pairwise (Provesti) distance matrix for six industrial hemp cultivars.

5.3.2 Genetic diversity among cultivars

Genetic diversity was analysed among the six cultivars, and the summary statistics are shown in Table 5.3. Although all cultivars were found to have very similar values for the total number of alleles and the average number of alleles per locus, CRS-1 had the highest (N_a) per cultivar (4.71), and Férimon 12 had the lowest number of alleles per cultivar (4.14). The average number of alleles per locus per cultivar was 4.48. Sixteen private alleles (*P*) were identified in total (an average of 2.67 per cultivar), and half of them were found in just two cultivars: five in the Futura 75 and three in the CRS-1. The observed heterozygosity (H_o) for individual cultivars ranged between 0.40 in Férimon 12 and 0.48 in CRS-1, with an average value of 0.44, while the expected heterozygosity (H_e) varied between 0.43 for Finola and 0.54 for Futura 75 and had an average value of 0.50. CFX-2, CRS-1, Férimon 12, and Futura 75 had more than 50% of loci in the heterozygous state ($H_o \geq 0.5$). In cultivars CFX-2, CRS-1, Férimon 12, and Futura 75, the H_o values were slightly lower than those of *He*; in the other two cultivars, Katani and Finola, both values were very similar. Among the six cultivars, Futura 75 had the highest level of genetic diversity (H_e = 0.54). The Wright's fixation index (F_{IS}) of the six cultivars ranged from (-0.02) to 0.26. Finola had a negative Wright's fixation index (F_{IS}) value (-0.02), suggesting a low inbreeding level in the cultivar. The rest had mild to moderate homozygote excess. Compared with other cultivars, Katani and Finola had lower F_{IS} values. Interestingly, the two monoecious cultivars (Férimon 12 and Futura 75) had the lowest H_o and the highest F_{IS}.

Table 5.3. Genetic diversity metrics and their associated standard errors (± SE) among the six cultivars of industrial hemp analyzed.

Cultivar	N	N_a	N_p	H_o	H_e	F_{IS}
CFX-2	46.00 ± 1.35	4.43 ± 0.48	2	0.45 ± 0.10	0.51 ± 0.08	0.20 ± 0.14
CRS-1	44.57 ± 2.49	4.71 ± 0.61	3	0.48 ± 0.11	0.52 ± 0.09	0.21 ± 0.15
Férimon 12	45.71 ± 1.81	4.14 ± 0.34	2	0.40 ± 0.07	0.51 ± 0.04	0.24 ± 0.11
Katani	46.29 ± 0.99	4.57 ± 0.57	2	0.46 ± 0.11	0.47 ± 0.10	0.08 ± 0.08
Futura 75	44.43 ± 3.08	4.43 ± 0.65	5	0.41 ± 0.08	0.54 ± 0.05	0.26 ± 0.12
Finola	45.29 ± 2.55	4.57 ± 0.69	2	0.43 ± 0.09	0.43 ± 0.09	-0.02 ± 0.02
Mean	45.38 ± 0.84	4.48 ± 0.22		0.44 ± 0.04	0.50 ± 0.03	

N: sample size; N_a: number of alleles; N_p: number of private alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: Wright's fixation index.

5.3.3 Genetic diversity in genetic clusters

To evaluate the genetic diversity within and among different genetic groups inferred from the structure results, the same genetic metrics were calculated using data partitions aligning with genetic structure at $K = 2$ and $K = 3$ (Table 5.4). Each genetic group was considered as a single genetic cluster. Among the two clusters at $K = 2$ (group 1 and group 2), group 1, which mostly (98%) consisted of individuals from the dioecious cultivars (CFX-2, CRS-1, Katani and Finola), displayed the higher number of alleles per locus (N_a) (5.57). Group 1 also showed the higher observed heterozygosity (0.45) and the lower expected heterozygosity (0.48), along with the lower Wright's fixation index (0.17). In group 2, which consisted of ~90% individuals from the monoecious cultivars, the expected (H_e) and observed (H_o) heterozygosities were 0.41 and 0.57, respectively. Group 2 had the lower observed heterozygosity, the higher expected heterozygosity and Wright's fixation index (0.31).

Among the three genetic clusters at $K = 3$, the highest number of alleles per locus (N_a) was found in group 3 (4.71), and the lowest was found in group 1 (3.71). The highest observed heterozygosity (H_o) was found in group 3 (0.45), and the lowest was in group 1 (0.38). Expected heterozygosity (H_e) ranged from 0.43 for group 2 to 0.53 for group 3.

Group 2 showed equal heterozygosity (H_e) to observed heterozygosity (H_o). The highest Wright's fixation index (F_{IS}) was in group 1 (0.27), whereas the lowest was in group 2 (-0.02). In brief, group 3, which included ~56% of French-fibre cultivar and ~ 44% of Canadian-seed cultivars, had the highest number of alleles per locus (N_a) per cluster, observed heterozygosity (H_o) and expected heterozygosity (H_e), whereas group 1, which included ~98% of French dual-purpose and fibre cultivars (monoecious), had the lowest alleles per locus (*Na*), the lowest observed heterozygosity (H_o) and the highest Wright's fixation index (F_{IS}). Group 2, which comprised ~99% of seed cultivars (dioecious), showed the lowest expected heterozygosity (H_e) and Wright's fixation index (F_{IS}).

These matrices were also calculated on groups considering the STRUCTURE results at K = 4 to better understand the diversity within and among the genetic clusters at the finest level of genetic structure. The highest number of alleles per locus (N_a) was in group 2, and the lowest was in group 3. The observed heterozygosity (H_o) ranged from 0.38 (group 4) to 0.51 (group 2), and the expected heterozygosity (H_e) ranged from 0.39 (group 1) to 0.61 (group 2). The greatest difference across the K = 4 group was for F_{IS}, which ranged from -0.12 (group 1) to 0.22 (group 4).

At all levels of K (K = 2 – 4), the groups consisting of mostly monoecious cultivars (Férimon 12 and Futura 75) showed the lowest observed heterozygosity (H_o) and the highest Wright's fixation index (F_{IS}), and this result is in accordance with the genetic diversity metrics obtained for each cultivar separately (Table 5.3).

Table 5.4. Genetic diversity metrics and their associated standard errors (± SE) for industrial hemp data partitions based on STRUCTURE results.

	N_{ind}*	N	N_a	H_o	H_e	N_{cul}	F_{IS}
Original total		45.38 ± 0.84	4.48 ± 0.22	0.44 ± 0.04	0.50 ± 0.03		0.17 ± 0.05
***K* = 2 (groups)**†							
Group 1	126	119.43 ± 4.44	5.57 ± 0.65	0.45 ± 0.09	0.48 ± 0.09	6	0.17 ± 0.14
Group 2	96	90.43 ± 5.08	4.86 ± 0.74	0.41 ± 0.07	0.57 ± 0.04	5	0.31 ± 0.10
***K* = 3 (groups)** †							
Group 1	44	41.71 ± 1.81	3.71 ± 0.29	0.38 ± 0.07	0.50 ± 0.04	3	0.27 ± 0.10
Group 2	73	69.00 ± 2.72	4.43 ± 0.72	0.43 ± 0.09	0.43 ± 0.10	5	-0.02 ± 0.03
Group 3	43	41.00 ± 2.00	4.71 ± 1.02	0.45 ± 0.10	0.53 ± 0.08	4	0.24 ± 0.15
***K* = 4 (groups)** †							
Group 1	42	39.57 ± 1.78	3.86 ± 0.63	0.43 ± 0.11	0.39 ± 0.10	3	-0.12 ± 0.03
Group 2	10	9.71 ± 0.18	4.14 ± 0.67	0.51 ± 0.11	0.61 ± 0.04	3	0.20 ± 0.15
Group 3	26	24.29 ± 1.71	3.00 ± 0.72	0.39 ± 0.09	0.42 ± 0.09	2	0.08 ± 0.07
Group 4	36	34.29 ± 1.39	3.43 ± 0.37	0.38 ± 0.07	0.48 ± 0.06	2	0.22 ± 0.10

N_{ind}: number of individuals; N: sample size; N_a: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; N_{cul}: number of cultivars and F_{IS}: Wright's fixation index. Data partitions: Original: all six cultivars, providing average per cultivar metrics; K = 2 (groups) treats all cultivars assigned to one of the genetic clusters defined by STRUCTURE results as a single group; K = 3 (groups) treats all cultivars belonging to a cluster as a single group; K = 4 (groups) treats all cultivars assigned to a cluster as a single group.

*The genetics metrics were calculated on groups considering the STRUCTURE results observed at K = 2, K = 3 and K = 4, but limited to individuals that are ≥80% of one parental type.

† Information on data partitions is in Appendix 12.

5.3.4 Genetic diversity among monoecious and dioecious cultivars

The summary of genetic parameters based on the reproductive biology of the hemp cultivars (monoecious and dioecious), is shown in Table 5.5. Among the dioecious and monoecious cultivars, the dioecious cultivars displayed a higher number of alleles per locus (N_a). The observed heterozygosity (H_o) values were lower than the corresponding expected heterozygosity (H_e) for both genetic groups. The monoecious cultivars have lower observed heterozygosity (H_o) and higher Wright's fixation index (F_{IS}).

Table 5.5. Genetic diversity metrics and their associated standard errors (± SE) for monoecious and dioecious industrial hemp cultivar data partitions.

Cultivar	N	N_a	H_o	H_e	F_{IS}
Original total	45.38 ± 0.84	4.48 ± 0.22	0.44 ± 0.04	0.50 ± 0.03	0.17 ± 0.05
Monoecious and dioecious cultivars (groups)					
Dioecious cultivars / Seed cultivars	45.54 ± 0.94	4.57 ± 0.28	0.46 ± 0.05	0.48 ± 0.04	0.12 ± 0.06
Monoecious cultivars / Fibre and dual-purpose cultivars	45.07 ± 1.73	4.29 ± 0.35	0.40 ± 0.05	0.52 ± 0.03	0.25 ± 0.08

N: sample size; N_a: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: Wright's fixation index. Data partitions: Original: all six cultivars, providing average per cultivar metrics; Monoecious and dioecious (groups) treat all cultivars belonging to one of the two groups as a single cluster.

5.3.5 Genetic diversity of male and female individuals in dioecious cultivars

The summarized genetic diversity statistics for male and female individuals of each cultivar can be found in Table 5.6. The genetic diversity metrics calculated showed considerable genetic diversity among each cultivar's male and female individuals. Among all dioecious cultivars except Finola, females had a higher number of alleles (N_a) than males. Female individuals from CRS-1 and Katani showed the highest (4.43) number of alleles (N_a), and male individuals of CRS-1 showed the lowest (3.86), which is similar to the number of alleles (N_a) in female individuals of Finola. The observed heterozygosity (H_o) ranged from 0.46 (CFX-2) to 0.50 (CRS-1) in female individuals and from 0.38 (Finola) to 0.46 (CRS-1) in male individuals, with a mean value of 0.46. The expected heterozygosity (H_e) in female individuals varied from 0.45 (Finola) to 0.55 (CRS-1) and from 0.40 (Finola) to 0.49 (CFX-2) in male individuals, with a mean value of 0.48. Observed and expected heterozygosities (H_o and H_e) of females were higher than the males in all cultivars. The male individuals of all cultivars had higher expected heterozygosity (H_e) and lower observed heterozygosity (H_o). Among the female individuals, only CFX-2 and CRS-1 had higher expected heterozygosity (H_e) and lower observed heterozygosity (H_o). The highest Wright's fixation index (F_{IS}) was observed in female individuals of CFX-2 and CRS-1 (0.21), and the lowest was in female individuals of Finola (-0.09). It is interesting to note the substantial differences in Wright's fixation index (F_{IS}) between male and female individuals within the cultivars; CRS-1, Katani, and Finola. Among all male and female individuals of all cultivars, female individuals of CRS-1 had the highest expected heterozygosity (H_e), observed heterozygosity (H_o) and Wright's fixation index (F_{IS}). The male individuals of Finola had the lowest expected and observed heterozygosities (H_o and H_e). The general observation on lower observed heterozygosity (H_o) and higher expected heterozygosity (H_e) indicates a deficit in heterozygotes, which is also supported by positive Wright's fixation index (F_{IS}).

Table 5.6. Genetic diversity metrics and their associated standard errors (± SE) for female and male individuals among four dioecious industrial hemp cultivars analyzed.

Cultivars (Dioecious)	Female / Male (groups)	N	N_a	H_o	H_e	F_{IS}
CFX-2	Female	23.00 ± 0.69	4.29 ± 0.47	0.46 ± 0.09	0.52 ± 0.07	0.21 ± 0.13
	Male	23.00 ± 0.69	3.86 ± 0.40	0.45 ± 0.10	0.49 ± 0.09	0.17 ± 0.16
CRS-1	Female	22.57 ± 1.27	4.43 ±0.53	0.50 ± 0.11	0.55 ± 0.08	0.21 ± 0.15
	Male	22.00 ± 1.29	4.14 ± 0.59	0.46 ± 0.11	0.48 ± 0.10	0.04 ± 0.09
Katani	Female	23.14 ± 0.59	4.43 ± 0.61	0.48 ± 0.12	0.47 ± 0.11	-0.03 ± 0.05
	Male	23.14 ± 0.46	4.14 ± 0.51	0.45 ± 0.12	0.46 ± 0.09	0.14 ± 0.14
Finola	Female	22.43 ± 1.57	3.86 ± 0.59	0.49 ± 0.11	0.45 ± 0.09	-0.09 ± 0.05
	Male	22.86 ± 0.99	4.00 ± 0.65	0.38 ± 0.08	0.40 ± 0.09	0.03 ± 0.05
Mean		22.77 ± 0.34	4.14 ± 0.18	0.46 ± 0.03	0.48 ± 0.03	0.09 ± 0.04

N: sample size; N_a: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: Wright's fixation index. Data partitions: Male and female groups: treat all individuals from each cultivar belonging to one of the two groups, as a cluster.

5.3.6 Partitioning of genetic variation among the industrial hemp cultivars

The partitioning of genetic variation was computed among the six cultivars using AMOVA. The results indicated that 11% and 19% of variations were attributed to differentiation among and within the six cultivars, respectively (Table 5.7). The results also showed significantly higher genetic variation within individuals (69%) than among individuals and cultivars.

Table 5.7. AMOVA results comparing the six industrial hemp cultivars analyzed.

Comparison	df	SS	% var	*p*
Among cultivars	5	131.57	11%	< 0.001
Among individuals	282	664.14	19%	< 0.001
Within individuals	288	435.50	69%	< 0.001

df: degrees of freedom; SS: sum of squares; %var: per cent variation that resides within a level; *p*: *p*-value for a given test.

5.3.7 Genetic relationships

Pairwise comparisons between cultivars confirmed the genetic differentiation among cultivars (Table 5.8). Between cultivars, pairwise F_{ST} varied from 0.02 to 0.12. Finola showed the largest genetic distance with the cultivar Férimon 12 (0.14) and the smallest genetic distance with Katani (0.02). CRS-1 and Katani displayed the same genetic distance (0.02) with CFX-2. Genetic distances between the dioecious cultivars (seed cultivars) and the monoecious cultivars (fibre and dual-purpose cultivars) seemed to be larger than comparisons within each group, and the pairwise genetic distance differentiation among all the seed cultivars was low.

Table 5.8. Pairwise genetic differentiation (F_{ST}) among the cultivars

CFX-2	CRS-1	Férimon 12	Katani	Futura 75	Finola	
0.00						CFX-2
0.02	0.00					CRS-1
0.12	0.10	0.00				Férimon 12
0.02	0.04	0.11	0.00			Katani
0.06	0.06	0.08	0.06	0.00		Futura 75
0.04	0.06	0.14	0.02	0.10	0.00	Finola

5.4 Discussion

5.4.1 Effectiveness of microsatellite markers in industrial hemp cultivars

The development of molecular markers in *Cannabis sativa* has significantly contributed to the evaluation of the genetic diversity of *Cannabis* (Hesami et al., 2020). Over the last twenty years, molecular markers in *Cannabis* have been mainly used to explore genetic diversity and population structure (Alghanim & Almirall, 2003; Datwyler & Weiblen, 2006; Gao et al., 2014; Gilmore et al., 2003; Soler et al., 2017; Soorni et al., 2017), evolutionary origins and relationships among cultivated genotypes (Forapani et al., 2001; Hillig, 2005; Soorni et al., 2017), classification and identification of varieties and genotypes (Mendoza et al., 2009; Sawler et al. 2015; Soler et al., 2017), sex determination (Mandolino et al., 2002; Sakamoto et al., 2000; Sakamoto et al., 2005), chemotype associated markers (de Meijer et al., 2003; Kojoma et al., 2006; Toth et al., 2020) and building genetic linkage maps (Faux et al., 2016).

As in many plant genetic diversity studies, microsatellite markers were the markers of choice in the current study. Microsatellites have proved to be more polymorphic than other molecular markers and have been used to reveal the genetic variation in *Cannabis* (Allgeier et al., 2011; Gao et al., 2014; Gilmore et al., 2003; Howard et al., 2008; Shirley et al., 2013; Soler et al., 2017; Zhang et al., 2020). This indicates the reliability and effectiveness of these markers in *Cannabis* cultivars. Soler et al. (2017) have confirmed that among the molecular markers used to study the genetic diversity in *Cannabis*, microsatellite markers are more informative. Even though only a small scale of validated microsatellite markers has been published in *Cannabis* (Alghanim & Almirall, 2003; Chandra et al., 2017; Gilmore & Peakall, 2003), as compared with the most widely used genetic markers in *Cannabis* research, the use of microsatellites is more promising for analysing the genetic variability in *Cannabis* cultivars (Bazanov et al., 2020).

Despite its high value and the multibillion-dollar market, industrial hemp lacks research on genetic diversity, and biotechnology techniques are still in an early stage. This is largely due to the complex evolutionary past of the plant with Marijuana (drug-type) and a long history of prohibition. Genetic diversity studies in *Cannabis* have focused more on the discrimination between drug-type and fibre-type (hemp), and very few studies have focused solely on variety-level genetic variation in hemp. The current study has investigated the genetic variation of six hemp cultivars with different breeding origins, reproductive biology and end-use using seven microsatellite markers. Interesting initial findings of this study prompt the experimental curiosity to explore more about the genetic diversity and population structure among hemp genotypes with more varieties/ accessions and microsatellite markers.

5.4.2 Genetic differentiation and structure

The selected microsatellite markers concurrently revealed the genetic structure of the six hemp cultivars and the genetic relationships among the individuals. The STRUCTURE analysis and the Neighbour-Net genetic clustering exposed clear genetic groups based on the reproductive biology (monoecious or dioecious) and their intended end-use, and the PCoA results further supported this distinction. STRUCTURE at $K = 2$ highlighted congruence between the genetic diversity and the reproductive biology of the cultivars: a clear separation between the monoecious and the dioecious cultivars. The results of pairwise genetic differentiation (F_{ST}) among the cultivars are also consistent with these results. The genetic distinctiveness observed here is consistent with the results observed by Dufresnes et al. (2017), where hemp varieties clustered based on their reproductive biology (monoecious versus dioecious). Likewise, similar results were reported by Borin et al. (2021), where 104 individuals from 11 distinct hemp varieties were genotyped with 20 microsatellite markers. Here, they successfully clustered individuals based on reproductive biology (dioecious and monoecious), further confirming the results obtained at $K = 2$.

Hemp cultivars exhibit varying degrees of genetic variability, due to their reproductive biology (dioecious or monoecious), commercial value, intended end use, and the specific breeding criteria employed (Forapani et al., 2001). A number of monoecious varieties have been developed that provide agronomic advantages over dioecious cultivars (Onofri & Mandolino, 2017). However, *Cannabis* is, in fact, a predominantly dioecious plant in nature, marked by the presence of distinct male and female plants (Chandra et al., 2017). Modern monoecious varieties have been obtained by selecting naturally occurring variants (Bócsa, 1958). To maintain a monoecious population, it is necessary to maintain strict isolation (according to the Canadian Seed Growers' Association (2018), the minimum isolation distance required from a dioecious variety of hemp or non-pedigreed hemp should be 4.8 km) during the seed multiplication since the monoecious pollen is less competitive compared with contaminating dioecious-male individuals, which leads to the return to their dioecious state (Bócsa & Karus, 1998). The original monoecious trait arose spontaneously from a material from Central Russia (Meijer et al., 1995). Grassi and McPartland (2017) indicated that Grishko (1935) in the Soviet Union initiated work that led to monoecious hemp. Neuer and Sengbusch (1943) from Germany fixed the monoecious trait, along with increasing the fibre content (Flajšman & Ačko, 2022). Their efforts resulted in the development of 'Fibrimon,' which was later improved to create modern cultivars. Fibrimon served as a parent cultivar for varieties from various countries: Férimon, Fédora, Félina, and Futura in France; Juso 11 in Ukraine; Beniko and

Białobrzeskie in Poland; Uniko B in Hungary; and Secuieni 1 in Romania (Grassi & McPartland, 2017). In the present study, Férimon 12 and Futura 75 tended to cluster together, a result that agrees with the genetic background of these cultivars, which share a common ancestor, Fibrimon.

The dioecious-seed cultivar Finola is a stable cross between two Northern Russian landraces from the Vavilov Institute, originally made by Sam_Skunkman and Dr. J.C. Callaway by open pollination in 1995 (The hemp seed hub, 2013). Finola has long been a primary model system for plant genetics and cultivar development (Grassi & McPartland, 2017). This Finnish cultivar is mainly grown in Europe and Canada (Golimowski et al., 2022). Canadians have bred several dioecious seed cultivars such as ESTA-1, CanMa, Petera, CFX-1, CFX-2, CRS-1 and Katani. However, Finola dominated hemp production in Canada, accounting for nearly 48% of the national acreage in 2020 (Stockford, 2021) despite their own seed varieties. It is interesting to note that the Canadian cultivar CanMa is a cross between Finola and ESTA-1 (Grassi & McPartland, 2017). The breeding histories of the other Canadian cultivars, especially those used in the present study (CFX-2, CRS-1, and Katani), have not been reported elsewhere (or the information is confidential and has restricted access). In the present study, the genetic structure similarity among the Canadian and Finnish cultivars could indicate that the Canadian breeders might have used a common parent or gene pool in developing the cultivars: CFX-2, CRS-1, and Katani. Indeed, besides the breeding history of the selected cultivars, their reproductive biology (monoecious and dioecious) better explains the genetic structure revealed at $K = 2$.

STRUCTURE ($K = 3$) results and the Neighbour-Net analysis revealed further patterns. Four separations were detected: the seed cultivars, which comprises CFX-2 and CRS-1; CBD cultivars (Katani and Finola); fibre cultivar (Futura 75); and finally, dual-purpose cultivar (Férimon 12). At $K = 2$, the cultivars bred for seed purpose CRS-1, CFX-2, Katani, and Finola were clearly clustered together, so their separation at $K = 3$ into two clusters despite the end-use for which they were bred, in the light of their current use, is striking. Nevertheless, while some varieties are primarily bred for seeds, they are instead highly appreciated as dual-purpose or CBD hemp cultivars. Hemp cultivars are bred for specific end-use (fibre, seed, medicinal, and seed and fibre) (Rupasinghe et al., 2020). However, some of these cultivars would allow a change in end-use according to agronomic performances because it is a well-known fact that in breeding for fibre, food or medicinal uses, the selection is applied for several characters simultaneously and not just to one, since multiple characteristics contribute the economic value. In the same way, although Katani and Finola are generally grown for seed, they are also used for inflorescence production to extract CBD (Canapuglia, 2022; HGI, 2022). Previous studies have showed that these cultivars produce appreciable levels of cannabinoids (Hammami et al., 2022; Pavlovic et al., 2019; Todd et al., 2022). It seems most likely that the specific use of Finola and Katani as CBD hemp invokes the similarity observed among these two cultivars. Also, it is noteworthy that both breeders and growers had recognized CFX-2 and CRS-1 as cultivars for fibre and seed, and the Hemp Genetics International has categorized these two cultivars under grain and fibre (HGI, 2022), which may reflect a meaningful possible explanation to their genetic distinctiveness and similarity as dioecious-dual-purpose cultivars, among the other cultivars in this study. As mentioned previously, the marginal genetic similarity observed among the four seed cultivars in the structure results at K = 2 and K = 3 might be revealing that they contain a subset of variation present in Finola. A similar observation was reported by Borin et al. (2021), where among three French varieties, two varieties grouped apart with a substantial proportion of admixed samples, and a consistent representation of the Polish ancestor. Despite the majority of the individuals of a single cultivar belonging to the same genetic cluster, the genetic structure analysis also indicated that a few cultivars exhibit a high degree of genetic admixture, suggesting gene flow as reported elsewhere (Soler et al., 2017). The absence of strong differentiation and clear evidence of admixture among the Canadian dioecious cultivars is not surprising, given that hemp cultivars are wind pollinated and highly heterozygous. This phenomenon is similar to that found by Soorni et al. (2017). In both STRUCTURE runs of K = 3 and the cultivar-level Neighbour-Net analysis, the two monoecious French cultivars clearly separate out from one another, identifying as fibre and dual-purpose cultivars. Even though these two cultivars have a common genetic ancestor, the results obviously show the response to selection for specific end-use. It is worth noting that a PCA on marijuana varieties showed that their genetic diversity weakly correlated with the documented breeding history (Dufresnes et al., 2017). So, it appears that this is another possible explanation for the results obtained. A study conducted by Carlson et al. (2021), assessed the genetic relatedness of 190 genotypes, which included seed, fibre, and cannabinoid cultivars, and United States-feral accessions. They identified seven primary clusters, grouping the genotypes based on market class (grain, fibre, and cannabinoid), geographical origin, and/or a common founder. However, in contrast to the results in the present study, the cultivars CRS-1, CFX-2, Ferimon, Futura 75, and Finola clustered together, and Carlson et al. classified the group as grain or dual-purpose cultivars. Generally, compared to SNPs, SSR markers provide more information on genetic diversity and population structure (Hamblin et al., 2007; Yang et al., 2011), which might explain the difference in results where the SSR markers used in the present study may have greater resolving power than the SNPs used by Carlson et al. (2021). Also, with Carlson's greater sample size, they might be describing a higher genetic structure level than the present study.

5.4.3 Geographic origin and genetic similarity

Previous studies have reported population structure in hemp (or in *Cannabis*) in terms of geographical origin. Gao et al. (2014) found that, in a panel of 115 *Cannabis* genotypes, the population was structured primarily based on geographical origin. Likewise, Borin et al. (2021) tested 104 individuals belonging to 11 hemp varieties using 20 SSR markers and identified eight genetic clusters, grouped based on reproductive biology (monoecious and dioecious) and geographical origin. Further, Soorni et al. (2017) observed geographical partitioning across 98 *Cannabis* genotypes and 24,710 SNPs. Even though the genetic structure found on hemp most likely correlated with the geographical origin, the present study did not reveal a relationship between cultivars and their breeding region. This output corresponds to the results obtained by Zhang et al. (2020), where population structure analysis divided 199 *Cannabis* germplasms (mainly collected from 20 regions across China) into two groups but not based on the region of origin. Here, they also indicated that geographic origin may provide an advantage in domesticating certain varieties and introducing new cultivars, but it is not always possible to clearly distinguish *Cannabis* germplasm.

Previous genetic diversity studies on *Cannabis* have mainly concerned with differentiating so-called “fibre-type” from “drug-type” plants, monoecious and dioecious cultivars and male and female plants of dioecious varieties and identifying the genetic clusters based on known common ancestors and their geographical origin (Borin et al., 2021; Dufresnes et al., 2017; Faeti et al., 1996; Onofri & Mandolino, 2017; Sawler et al., 2015). This study provides insight into the population structure of hemp cultivars based on reproductive biology (monoecious and dioecious) as well as different end-use, an area that has been limitedly explored to date. However, more work needs to be done to further evaluate the validity of the results obtained.

5.4.4 Analysis of genetic diversity of industrial hemp

The genetic diversity indices provided insight into the genetic diversity of each of the six cultivars. Although all cultivars had a similar number of alleles per locus (N_a), it ranged from 4.14 to 4.71, with an average value of 4.48. The mean value of the number of alleles per locus is in agreement with previous studies (Alghanim & Almirall, 2003; Dufresnes et al., 2017; Maria et al., 2015; Zhang et al., 2020). However, these studies showed different ranges for the number of alleles per locus. For example, Zhang et al. (2020) examined 30 local Chinese hemp cultivars and 109 wild *Cannabis* germplasm resources using 59 SSR markers. They found an average of 4.13 alleles per locus, with the number of observed alleles varying from two to eight. Similar value ranges were observed by Borin et al. (2021) and Gao et al. (2014), where the values ranged from 2.10 to 6.15 and 2 to 7, respectively. Soler et al. (2017) found the maximum number of alleles per locus across six gSSR markers and 154 individual plants of 20 cultivars of *C. sativa* var. indica (drug-type) and two cultivars of *C. sativa* var. sativa (hemp) ranged from 2 – 7. Here, the two hemp cultivars, Futura and Finola, had the highest maximum number of alleles per locus. A study conducted by Dufresnes et al. (2017) found a comparable average number of alleles per locus (4.0 ± 0.8) to the present study, also highlighted that drug-type varieties showed a low average number of alleles per population (scaled to eight individuals; 2.3 ± 0.9) compared to hemp varieties.

Heterozygosity is an indicator of genetic diversity within the samples. In the present study, the expected heterozygosity (H_e) varied between 0.43 and 0.54, while the observed heterozygosity (H_o) varied between 0.40 and 0.48. These values are comparable with other microsatellite studies of *Cannabis* (Soler et al., 2017; Zhang et al., 2020). As indicated before, Soler et al. (2017) used six genomic SSRs to genotype 154 individual plants of cultivars of *C. sativa* var. indica (marijuana) plus two cultivars of *C. sativa* var. *sativa* (hemp). Here, they found that the observed heterozygosity (H_o) for individual cultivars ranged between 0.125 (BL-57 – a drug-type variety) and 0.602 (Critical – a drug-type variety), with an average value of 0.293, while the expected heterozygosity was detected with a mean of 0.406, and it ranged between 0.175 (NST-75-75 – a drug-type variety) and 0.617 (Futura – hemp variety). The average observed and expected heterozygosities (H_o and H_e) of the two hemp cultivars in their study; Finola and Futura, were 0.405 and 0.603, respectively. These values are comparable with observed and expected heterozygosities (H_o and H_e) in the present study, where Finola showed a lower expected heterozygosity (0.43) than Futura 75 (0.54). Likewise, Zhang et al. (2020) estimated the genetic diversity parameters of hemp with different geographic origins using 80 SSR markers and found the observed and the expected heterozygosity values (H_o and H_e) in the range of 0.49 to 0.55 and 0.54 to 0.56, respectively. These values are similar to the values observed in this research.

In the present study, observed heterozygosity was lower than the expected heterozygosity for cultivars: CFX-2, CRS-1, Férimon 12, Katani and Futura 75. Borin et al. (2021) observed a similar result where observed heterozygosities (H_o) were always lower than the expected heterozygosities (H_e) in all varieties. The results obtained in this study shows that a large proportion of individuals sampled were homozygous at several loci. Fixation (probably due to inbreeding), consanguinity (Soler et al., 2017) and strong selection pressure during the cultivar development (Beissinger et al., 2014; Borin et al., 2021; Forapani et al., 2001; Remington et al., 2001) could be responsible for the low levels of observed heterozygosity observed across the sampled individuals. According to Borin et al. (2021), one of their plausible explanations for this discrepancy was the effect of intense selection pressure and inbreeding on heterozygosity, which has characterized hemp cultivation over the years. Over the years, Hemp has been cultivated on a broad scale, necessitating the stabilization of agronomically important traits in seed stocks and, possibly, reducing heterozygosity (Lynch et al., 2016). Conversely, drug-type varieties are generally characterized by higher heterozygosity levels than hemp (Dufresnes et al., 2017; Lynch et al., 2016; Soler et al., 2017). This seems obvious since most of the *Cannabis* strains (marijuana) are crossbred hybrids (Gloss, 2015) and are mainly propagated via cloning to ensure consistency in chemical profile and mass-multiplication (Chandra et al., 2017). In contrast, Sawler et al. (2015) found higher heterozygosity levels in hemp than in marijuana and argued that marijuana strains have a more narrow genetic base compared with hemp cultivars and/or that breeding among close relatives is found to be quite common. This finding becomes more evident with the F_{IS} values obtained in this study (0.08 – 0.26), which suggests a considerable degree of inbreeding. It is noteworthy that the two monoecious cultivars in this study, Férimon 12 and Futura 75, had not only the lowest observed heterozygosity but also the highest Wright's fixation index (F_{IS}) as monoecious hemp undergoes 20-25% self-pollination (Berenji et al., 2013), resulting in a reduction in the heterozygotes in the population (Abu Awad & Billiard, 2017; Islam et al., 2020). However, besides the assumptions made above, the results could also be due to the lower number of both cultivars and microsatellite markers used or the occurrence of null alleles.

Dioecious plants display different levels of genetic diversity between male and female populations (Hilfiker et al., 2004; Vandepitte et al., 2010). The present study showed considerable variation among the male and female individuals of dioecious hemp cultivars. Faeti et al. (1996) showed support for grouping *Cannabis* germplasm based on sex phenotype. The study was conducted to assess the genetic diversity of *Cannabis* across 54 samples derived from 13 cultivars or accessions (including hemp) using ten random amplified polymorphic DNA (RAPD) markers and separated female and male individuals into two separate clusters within the same cultivars/accessions. In contrast, Forapani et al. (2001) reported that the contribution of sex to *Cannabis* cultivar diversity was not significant. They analyzed six *Cannabis* cultivars (including three dioecious hemp, a monoecious hemp, a drug strain and a Delta-9-THC and inbred female line) using 102 RAPD markers. The results showed only a small contribution (3.3%) of sex to the total variation, and they concluded that sexual phenotypes and variety sex ratios need not be considered while sampling individuals for *Cannabis* diversity studies. However, none of these two studies estimated the genetic diversity parameters between males and females.

The genetic diversity parameters of female and male groups in the present study indicate that the genetic variation of females was slightly higher than that of males. A similar phenomenon was observed in studies where female subpopulations possess higher genetic diversity than male subpopulations. For example, Jia et al. (2015) observed that female Chinese bayberry (*Myrica rubra*) populations had slightly higher genetic diversity values than males. Likewise, Zhai et al. (2016) found a higher genetic variation in female sea-buckthorn (*Salix viminalis*) subpopulations than in male subpopulations. However, there are some exceptions; for example, in *Hippophae rhamnoides* male individuals present high genetic diversity than female individuals (Chen et al., 2008). The lower observed heterozygosity (H_o) than expected heterozygosity (H_e) and positive Wright's fixation index (F_{IS}) values obtained in the present study indicate a heterozygote deficit that might be explained by crossing between closely related individuals of the opposite sex in hemp

(Zhai et al., 2016). Much work have been performed to develop tools to differentiate male and female *Cannabis* (Balant et al., 2022). However, there is limited research on the genetic diversity parameters of male and female individuals in dioecious hemp cultivars. Nevertheless, with few exceptions, it is critical to understand better the role of sex phenotype in genetic diversity in hemp.

In cross-pollinating plants, the majority of the genetic variation is distributed between individuals within a population, while only a small proportion of it is attributed to the variation among populations (Clegg et al., 1989; Szczecińska et al., 2016). *Cannabis*, compared with self-pollinating and perennial plants (though monoecious forms exist), should be able to maintain higher levels of genetic diversity within cultivars and a lower level of genetic diversity between cultivars due to its breeding system (outcrossing via wind pollination) and short lifespan. In agreement with this, AMOVA test in the present study shows that only 11% of the genetic variation was observed among cultivars. Thus, most genetic variation is within rather than between cultivars. These results were consistent with previous findings obtained by Gilmore et al. (2003), where the genetic diversity of 93 *Cannabis* plants representing nine drug and six fibre accessions were profiled using five SSR markers. Here, they revealed that only 6% of the total variation resided between drug and fibre accessions, 21% among accessions and 73% within accessions. Another study, where the genetic diversity was analyzed in three populations of hemp (fibre) and a potent drug-type *Cannabis* using ten AFLP primer pairs found that 20.9% of the genetic variation is attributed to the difference among hemp varieties, 27.2% to differences between marijuana and hemp and 51.9% to variation within populations (Datwyler & Weiblen, 2006). Also, the results of the present study are consistent Soler et al.'s (2017) findings on 22 *Cannabis sativa* cultivars (20 drug-type and two hemp), which indicated that a large proportion of the molecular variation observed among (37.11%) and within individuals (30.96%). The magnitude of genetic variation found among and within cultivars in this study and previous studies appears to be a typical feature of an obligate outbreeding species (Onofri & Mandolino, 2017). This study also reveals that a large proportion of the molecular differentiation observed is within-individual variation due to heterozygosis (Sawler et al., 2015), which reflects the fact that as a wind-pollinated plant, *Cannabis* is highly allogamous in nature (Chandra et al., 2017).

Pairwise comparisons between cultivars in this study confirmed genetic differentiation among the cultivars. Between-cultivar F_{ST} varied from 0.02 to 0.14 (Table 5.8) and indicated that the cultivars were genetically differentiated. The lower F_{ST} values indicate a low genetic diversity between cultivars, a pattern consistent with the estimates derived from AMOVA. The overall pairwise genetic differentiation among cultivars (F_{ST} = 0.12 ± 0.013) was similar to the overall differentiation among hemps (F_{ST} = 0.15 ± 0.07) reported by Dufresnes et al. (2017). A similar result has been noted by Soorni et al. (2017), where the F_{ST} values ranged from 0.05 to 0.26 among *Cannabis* samples from 36 Iranian locations genotyped using 24,710 SNPs. Even though these two studies reported similar F_{ST} values, their observation on genetic differentiation between drug-type and hemp was contradicting. Dufresnes et al. (2017) quantified the genetic differentiation between marijuana and hemp and found substantially higher genetic distances among marijuana varieties (F_{ST} = 0.39) compared with hemp varieties, while Soorni et al. (2017) observed higher F_{ST} across hemp (0.086) than marijuana (0.039). It is also interesting to note that a comparison of hemp and marijuana in previous studies conducted by Lynch et al. (2016); Sawler et al. (2015) showed that they are genetically more similar, with low average F_{ST} values of 0.156 and 0.098, respectively. Therefore, it is not surprising that the hemp cultivars in this study are closely related with a maximum F_{ST} = 0.14. Even though *Cannabis* breeding has led to distinct genetic variation based on use, hemp cultivars still appear to share a common gene pool. As mentioned in the introduction (5.1), the available literature regarding the breeding histories indicates that hemp varieties are descendants from very few ancestors (Ranalli, 1999) and have a narrow genetic base (Onofri & Mandolino, 2017; Ranalli, 1999). According to Ranalli (1999), combinations of Italian landraces with the single monoecious line Fibrimon and a single Chinese landrace together with a couple of other Central and Southern Eropean accessions were used for breeding all of the hemp varieties (developed before 1999). A recent study conducted by Woods et al. (2022) to examine the genomic variation of *Cannabis sativa* accessions, including hemp varieties, feral populations in the United States, and accessions from the IPK Gatersleben seed bank revealed that the US feral samples and European hemp varieties likely share a recent common ancestor. A study conducted by Pamplaniyil (2016) revealed that the hemp cultivar (*Cannabis sativa*) 'Finola' and marijuana strain (a hypothesized *Cannabis sativa-indica* hybrid) 'Purple Kush' share a common gene pool. This narrow genetic base possibly explains the close relationship among the hemp cultivars in this study. Overall, most measures of genetic diversity indicate that majority of the genetic variation observed in hemp is ascribed to differences between individuals within cultivars. Different genetic diversity indices, such as the average number of alleles per locus (N_a), observed and expected heterozygosities (H_o and H_e), Wright's fixation index (F_{IS}) pairwise genetic distances (F_{ST}) along with the analysis of molecular variance have correspondingly reached this conclusion. However, the explanatory hypotheses and postulations suggested in the discussion need to be followed up by additional research with more markers and cultivars (with known breeding history).

Genetic diversity plays a critical role in plant breeding (Swarup et al., 2021). The present existence of substantial genetic variation is crucial to develop varieties with maximum genetic potential (Pickersgill, 2005). However, literature on *Cannabis* genetic diversity, breeding histories along with the results obtained in this present study shows that breeding has reduced the genetic variation available among the hemp cultivars due to the limited preferences of parents (selection of cultivars that perform best and have the desired characteristics) for further improvements. This is becoming a serious issue in developing new and superior varieties (Begna, 2021). Besides, the narrow genetic base restricts the cultivars' potential for adapting to new environments and makes them susceptible to increased environmental threats associated with climate change (Mousavi-Derazmahalleh et al., 2018). It is becoming common for plant breeders to use genetic materials (introduced non-adapted, introduced adapted and existing genetic material as a source of new alleles) without knowing their genetic history in order to maintain and substantially increase genetic gain through selection (Begna, 2021) which eventually leads to the loss of genetic diversity. Knowledge of genetic variability present in the exotic hemp cultivars will make it possible to broaden the genetic variation, further improve the cultivars by incorporating novel genes or alleles from wild relatives and landraces and release new-New Zealand-adapted cultivars.

5.5 Conclusion

The study reported in this chapter represents the assessment of genetic variation among and within New Zealand-approved hemp cultivars using microsatellite markers. This study indicated that the population structure of hemp reflects the plant's reproductive biology (monoecious and dioecious), followed by further differentiation based on the end-use (fibre, seed, fibre and seed, and CBD cultivars). Evidence from the current and previous studies suggests a moderate level of genetic diversity among the hemp cultivars, where a large proportion of the total genetic diversity was observed within cultivars. The present study revealed considerable genetic diversity among the female and male individuals and showed that female individuals possess slightly higher genetic variation than males. This study has established a foundation for genetic improvement in hemp in New Zealand, and the results will be useful for future breeding, such as for maintaining or increasing the diversity of breeding populations. Even though extensive research must be conducted with more hemp cultivars and microsatellite markers to broaden the results, the findings of this study are well worth the effort.

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CHAPTER 6

General discussion and future recommendations

Information of the magnitude and type of Genotype × Environment interaction (G × E interactions) associated with the performance of cultivars and breeding material is vital when selecting for specific and broad adaptation, across the target population of environments. In hemp, many economic traits, such as biomass and seed yield, are complex and are influenced by G × E interactions. Conducting multi-environment trials across different sites and years is essential for breeders to determine and reduce the confounding effects of G × E interactions. Multisite trials help breeders understand the underlying causes of these complex G × E systems and identify elite genotypes with stable performance across environments. The evaluation of imported cultivars using multisite and multi-year trials is a crucial first step in selecting material that is suited to New Zealand's environmental conditions across the target population of hemp production environments.

In breeding programmes, understanding the genetic architecture of key traits will enhance the selection of superior genotypes. Traits with economic value typically guide both the selection of parents for hybridisation and the identification of elite progeny. The goal is to either improve the expression of key traits or introduce new traits that are climate-resilient and meet the growing demands of the food and fibre industries. Quantifying the magnitude of additive genetic variation, the portion of genotypic variation that is inherited from parents to progeny, and other genetic parameters is essential for choosing appropriate breeding methods that improve the efficiency of achieving maximum genetic gain. In this context, it is essential to undertake research focused on the estimation of quantitative genetic parameters to develop high-performing hemp cultivars specifically adapted to New Zealand's climatic conditions, thereby providing economic and agronomic benefits to the industry.

Assessing genetic diversity using molecular markers plays a fundamental role in plant breeding, serving as the foundation for developing cultivars with maximised genetic potential. Research on the genetic diversity and breeding history of *Cannabis* reveals that breeding practices have led to reduced genetic variation among hemp cultivars. This narrowing of genetic diversity is largely a result of selective breeding focused on specific parental strains for further improvements. To maintain or increase genetic diversity, plant breeders often use a variety of genetic resources, including adapted cultivars as well as existing genetic pools, without fully understanding their genetic backgrounds. Characterising the genetic diversity within and among imported hemp cultivars (genetic resources currently available in New Zealand) will enable the identification of potential genetic diversity for integration into breeding programmes through generation of new breeding pools. In turn, this could lead to the development of new cultivars specifically adapted to New Zealand climatic conditions through efficient breeding programmes.

Recognising the need to advance and strategically improve hemp breeding efforts in New Zealand, experiments were conducted with the aim of

5. analysing and comparing key traits, including yield components (biomass and seed), seed quality, and other agronomic traits, within a set of hemp cultivars from Canada, Finland, and France,
6. investigating how genotype and environment affect hemp seed and biomass production by characterising the type and magnitude of G × E interaction estimated from multisite hemp trials over two separate growing seasons in the North Island of New Zealand,
7. investigating the magnitude of additive genetic variation within a breeding population for key traits based on half-sib families (HS families) and predicting the rate of genetic gain by applying the estimated quantitative genetic parameters in the breeder's equation,
8. characterising the genetic diversity and the degree of variability within and among the set of six commercial hemp cultivars using microsatellite markers.

To address the research questions that outline the above-mentioned objectives of this study, a series of experiments were carried out from 2019 to 2022. Key findings and future research recommendations, organised by each chapter, along with the extent to which the research questions have been addressed, are:

[Chapter 3] What was the yield potential of the selected cultivars in different environments in the North Island and was there a change in their relative performance across the environments?

To answer these questions, field trials were conducted for two years during the 2019/2020 and 2020/2021 growing seasons using six hemp cultivars approved to be grown in New Zealand: CFX-2, CRS-1, Ferimon 12, Katani, Futura 75, and Finola. The 2019/2020 trials were carried out in Palmerston North and Wairarapa, while the 2020/2021 trials were conducted at two separate but nearby sites in Palmerston North, one irrigated and one rainfed, each with different soil properties. The key findings of this study respond to the research questions mentioned above.

Key findings of this experiment are:

- The yield potential of the selected hemp cultivars in North Island locations exhibited substantial variability. Biomass yields extend over a broad range from 1.43 to 28.41 t/ha, and seed yields ranged between 0.018 and 3.78 t/ha. Additionally, seed oil content ranged from 30.66% to 34.02%, while seed protein content was between 22.19% and 27.52%. The thousand seed weight varied from 8.10 g to 19.26 g. Mostly, these findings align with the ranges documented in studies from various countries and on diverse hemp cultivars.
- The monoecious cultivars, Futura 75 and Ferimon 12, consistently outperformed other cultivars across diverse environments and appear well-suited for dual-purpose production.
- The G × E interactions observed in this study, based on the performance of the six evaluated cultivars, present an ambiguous scenario and diverge from findings in previous studies. While most traits showed genotypic variation, only a few demonstrated significant G × E interactions, indicating consistent responses across different sites.
- High line mean broad sense heritability estimates for traits like plant height, stem diameter, biomass yield, seed yield, and thousand seed weight indicate exploitable genetic variation for improving hemp. This holds potential for developing new cultivars suited to New Zealand's climate.

Overall, there were significant differences among the six cultivars for biomass and seed yield potential across North Island locations. The observed yield ranges mostly correspond with those reported in international studies based on various hemp cultivars evaluated. Most traits showed significant genotypic variation, but only a few demonstrated significant G × E interactions. It is important to note that, with only two sites, the environmental diversity may not have been large enough to detect G × E interactions. Although this study was conducted across a limited range of environments and did not fully characterise G × E interactions, its results offer valuable insights for future hemp breeding programs in New Zealand. These findings will serve as a basis for planning hemp breeding strategies to develop new cultivars with adaptation to the local target population of environments.

Recommendations for future research:

For a more in-depth understanding of the effects of G × E interaction on hemp performance in New Zealand, more comprehensive trials must be conducted across a broader range of production environments. This should encompass multi-year, multisite trials featuring a diverse set of cultivars, including those bred locally. Such an approach will enhance the understanding of how environmental factors impact key traits like seed and biomass yield, facilitating the identification of materials with both specific and broad adaptability. It is also important to explore the effect of genotype, environment, and G × E interactions on other traits not assessed in this study: traits associated with floral phenology, physiological traits, biochemical traits (such as Cannabinoids and Terpenes), as well as traits that have primary economic importance such as fibre yield and fibre quality. Also, it is vital that the biotic and abiotic characteristics of each trial site and year are recorded, as this will enhance the interpretation of G × E interactions. A comprehensive understanding of the G × E interaction on hemp performance in New Zealand will allow plant breeders to identify stable, well-adapted hemp cultivars and enable the more efficient use of breeding resources in future breeding programmes.

[Chapter 4] What was the genetic diversity among HS families representing a breeding pool, and could we predict genetic gain for key traits?

To be able to answer this question, a quantitative genetic analysis was conducted using 50 HS families focused on six key traits: stem diameter, plant height, number of internodes, seed yield, thousand seed weight, and biomass yield.

Key research findings of this experiment are:

- Significant additive genetic variation ($P < 0.05$) was observed among the 50 HS families for all traits except thousand seed weight, suggesting that all other traits have the potential for genetic improvement through selective breeding.
- Family mean narrow-sense heritability for all traits ranged from low (0.12; thousand seed weight) to high (0.83; the number of internodes).

- Of the six traits measured, only the five with statistically significant additive genetic variance ($P < 0.05$) were used in further analysis. These five traits (stem diameter, plant height, number of internodes, seed yield, and biomass yield) exhibited moderate to strong positive genetic correlations, generally higher than the corresponding phenotypic estimates.
- The estimated positive genetic correlation coefficients suggest that genotypes with higher seed and biomass yield can be developed.
- Both among-HS family and combined among- and within-HS family breeding strategies resulted genetic gains for the measured traits. However, the combined strategy achieved higher gains compared with the among-HS family approach. As expected, with higher selection pressure, genetic gain increased. However, the number of parental selections decreased. Therefore, it is important to balance genetic gain with selection pressure, the number of parents selected. This is especially important for outcrossing species, as a small number of selected parents could result in inbreeding depression.
- Deterministic simulation revealed that increasing the number of replicates in the field trial increased the predicted genetic gain for each trait. However, this increased trial costs, reducing cost-efficiency of the breeding strategy, increasing the cost of achieving a 1% genetic gain.
- Significant differences were observed among the 50 HS families and two commercial checks, Fasamo and Férimon 12, for all traits except thousand seed weight. HS families that significantly outperformed the commercial checks were identified for each trait. These HS families should be incorporated future breeding programs.

The additive genetic variation among HS families in the breeding pool was significant, as shown by this study, and yes, it was possible to predict genetic gain for key traits due to the presence of significant additive genetic variation among these families. Overall, this experiment revealed the potential of using these HS families as elite parents in modern breeding programs for breeding material development. New Zealand lacks quantitative genetic research on hemp. To develop suitable cultivars for New Zealand environments, breeders must assess the availability of genetic variation. It is crucial to model genetic gain rates for decision-making, as demonstrated in this study. Understanding quantitative genetics within breeding populations becomes even more vital for future genomic selection approaches. Furthermore, there is a need to create New Zealand-specific breeding populations by interbreeding wild germplasm with cultivars or high-performing materials, developing new genetic variations for the traits related to industrial applications such as seed protein content, oil content and fibre quality. However, it is important to note that this study was conducted in one environment, so the quantitative genetic parameter estimates could be confounded by additive genetic × environment interactions. This could have led to biased heritabilities and expected genetic gains.

Recommendations for future research:

It is strongly recommended that similar experiments be conducted over multiple years and across different locations to identify HS families with both specific and broad adaptability within New Zealand's target hemp production environments. In-depth evaluation across years and locations is essential to quantify G × E effects, providing valuable insights for planning future hemp breeding programs in New Zealand.

[Chapter 5] How is genetic variation partitioned within and among hemp cultivars – specifically, how different are the levels of genetic variation among cultivars, and how is this useful for future hemp breeding?

To address this research question, a laboratory experiment examined the genetic diversity and population structure of six New Zealand-approved cultivars using seven previously developed microsatellite (SSR) markers. The following key findings of this study address the aforementioned research question.

- Two genetic clusters, corresponding to the plant's reproductive biology (monoecious and dioecious), were identified through STRUCTURE analysis and further subdivided based on end-use (fibre, seed, fibre and seed, and CBD cultivars).
- Moderate genetic diversity was identified among the six hemp cultivars. The average observed heterozygosity (H_o) and the expected heterozygosity (H_e) were 0.44 and 0.50, respectively. The Wright's fixation index (F_{IS}) varied from 0.26 (Futura 75) to -0.02 (Finola).
- A large proportion of the genetic diversity was found within cultivars. Only 11% of the molecular variation was due to differentiation among cultivars, while variation among individuals within cultivars was 19%. The genetic variation

within individuals (69%) was higher than the variation among individuals and cultivars.

- Considerable variation was detected among male and female individuals of dioecious hemp cultivars. Genetic diversity parameters indicated that female individuals have greater genetic variation than males.

Overall, this experiment provides insights into the relationships and diversity of selected hemp cultivars. This study finds a moderate level of genetic diversity among hemp cultivars, potentially due to selective breeding limitations. The limited range of parent plants for further improvement is a critical concern, narrowing the genetic base of new cultivars. This, in turn, makes them susceptible to environmental threats associated with climate change and limits their adaptability. Thus, understanding the genetic variability in the available genetic material is crucial for developing cultivars suited to New Zealand climatic conditions. This knowledge will enable the expansion of genetic diversity and allow for the improvement of new cultivars by integrating novel genes or alleles from wild relatives and landraces. In this context, this study has established a foundation for genetic improvement in hemp in New Zealand, and these results, with further extensive research, will be helpful for future breeding for maintaining or increasing the diversity of breeding populations. However, it should be noted that this study was conducted only with a small set of offshore cultivars and seven microsatellite markers, and further research must be conducted with more hemp cultivars and microsatellite markers to broaden these results.

Recommendations for future research:

Further extensive research is necessary to expand upon the existing findings, incorporating more NZ-approved hemp cultivars, especially New Zealand-bred, and using additional microsatellite markers. Including these elements will contribute to a more comprehensive and robust set of results.

Collectively, this Ph.D. study lays the groundwork for future hemp breeding research and programmes in New Zealand. It serves as an initial resource, offering insights into genetic diversity, genotypic variation, and the impact of G × E interactions on key traits evaluated among six introduced offshore cultivars. Furthermore, the provided estimates of genetic parameters enrich the existing knowledge base, facilitating the development of hemp cultivars suited to New Zealand environments.

APPENDICES

Appendix 1. History of Cannabis sativa in New Zealand.

Year	Major event
From 1883 on	Sister Mary Joseph or Mother Aubert, was the first person known to grow *Cannabis* in New Zealand at Jerusalem (near Wanganui) as part of her stock of medicinal drugs (McIntosh et al., 1998).
1892	Hemp seed was first imported to New Zealand (McIntosh et al., 1998).
1927	Dangerous drugs and poisons regulations were announced. Restrictions were made to prevent the sale of Indian hemp to anyone in New Zealand (McIntosh et al., 1998).
1941	Department of Scientific and Industrial Research cultivated 1 ha of Hungarian hemp in the central North Island (McIntosh et al., 1998; McPartland et al., 2004).
1941	Ministry of Agriculture and Fisheries experimented with four hectares of hemp near Foxton (McIntosh et al., 1998; McPartland et al., 2004).
1948	The trials were abruptly terminated upon discovering that the variety being tested was Indian hemp, commonly used in the production of hashish (McIntosh et al., 1998).
1960	Regulations that made it an offence to possess or cultivate *Cannabis*. It also banned hempseed in birdfeed and veterinary supplies. This provision was initially incorporated into the Misuse of Drugs Act of 1961 and is now part of the updated Misuse of Drugs Act of 1975 (McIntosh et al., 1998).
1961	The Misuse of Drugs Act of 1961 prohibited hemp cultivation in New Zealand (McIntosh et al., 1998; McPartland et al., 2004).
1990	New Zealand Hemp Industries Association (NZHIA) formation (McIntosh et al., 1998).
1994	The National government placed a Moratorium on issuing licenses to grow hemp (NZHIA, n.d.).
2000	The prohibition on issuing licenses for hemp cultivation, imposed by the National government in 1994, was lifted on June 20, 2000 (NZHIA, n.d.).
2001	The rebirth of commercial production of hemp. Legislation that previously prohibited hemp cultivation has been amended. The Minister of Health declared that the Ministry had begun accepting applications for hemp cultivation licenses (Kerckhoffs et al., 2015; McPartland et al., 2004; NZHIA, n.d.).

2001	The first license to legally deal in *Cannabis* was approved in New Zealand, and the initial 20 kg of seeds, Kompolti and Uniko B varieties, were imported from Hungary (NZHIA, n.d.).
2001	The first legal hemp seeds were planted in North Island soil in New Zealand (NZHIA, n.d.).
2001	Field trials were initiated by Midland Seed Ltd. and Plant Research (NZ) in order to establish best management practices for oil seed hemp (*cv*. Fasamo) production in Canterbury (Townshend & Boleyn 2008).
2002	The Empowering Communities with Industrial Hemp Project (ECIHemp) facilitated plantings at eleven locations across the country, including a site at Massey University, covering a total area of 55 hectares (McPartland et al., 2004; NZHIA, n.d.). The Food (Safety) Regulations 2002 set out the conditions for producing and selling hemp seed oil for human consumption in New Zealand (NZHIA, n.d.).
2004	First New Zealand generic cultivar announced – Aotearoa 1 (NZHIA, n.d.).
2004	The government extended the legislative timeline and the duration of the trials until January 1, 2005 (NZHIA, n.d.).
2006	The Misuse of Drugs (Industrial Hemp) Regulations of 2006 were established to authorize the issuing of licenses for legal industrial hemp cultivation (NZHIA, n.d.).
2006	Anka, Finola, Fasamo, Kompolti and USO 31 hemp cultivars approved following regulation 5 (1) of the Misuse of Drugs (Industrial Hemp) Regulations 2006 (MoH, 2023).
2008	Aotearoa 1 cultivar of hemp approved (MoH, 2023).
2009	Ferimon 12 cultivar approved (MoH, 2023).
2010	Fedora 17 cultivar approved (MoH, 2023).
2014	Futura 75, CRS-1, CFX-2, and CFX-1 cultivars approved (MoH, 2023).
2018	Following a review by the Ministry of Health, regulations were updated to permit cultivating, manufacturing and selling low-THC hemp seeds for human consumption (MoH, 2020).
2018	Katani cultivar approved (MoH, 2023).
2019	Sirius and A1 Monopurp (second New Zealand generic cultivar) cultivars approved (MoH, 2023).

2020	A1 MACMONO and A1 COMSEED - New Zealand's third and fourth generic cultivar) cultivars approved (MoH, 2023).
2021	Fifth New Zealand generic cultivar, A1 HCFX approved (MoH, 2023).

Appendix 2. Physical properties of soil types; (1) Ahikouka silt loam (Wairarapa site) (2) Manawatu silt loam (Palmerston North or irrigated site), and (3) Tokomaru silt loam (rainfed site).

Soil properties	Ahikouka silt loam	Manawatu silt loam	Tokomaru silt loam
Depth class (diggability)	Deep (> 1 m)	Deep (> 1 m)	Moderately deep (69 - 71 cm)
Texture profile	Silt over clay	Loam	Silt
Potential rooting depth	Unlimited	Unlimited	69 - 71 (cm)
Rooting barrier	No significant barrier within 1 m	No significant barrier within 1 m	Low penetration soil material
Topsoil clay range	25 - 35 %	19 - 21 %	19 - 21 %
Topsoil sand range	0 - 10 %	8 – 20 %	8 -12 %
Topsoil silt range	55 – 75 %	59 – 63 %	67 – 73 %
Drainage class	Poorly drained	Imperfectly drained	Poorly drained
Aeration in root zone	Very limited	Moderately limited	Limited
Permeability profile	Moderate over slow	Moderate	Moderate over slow
Profile available water	0 - 30cm - High (57 mm) 0 - 60cm - High (102 mm) 0 - 100cm - High (161 mm)	0-30 cm - High (66 mm) 0-60 cm - Very high (130 mm) 0-100 cm - High (222 mm)	0-30 cm - High (55mm) 0-60 cm - High (99 mm) 0-100 cm - Moderate (114 mm)
Topsoil P retention	Medium (38%)	Medium (33%)	Low (22%)
Water logging vulnerability	High	Moderate	High
Drought vulnerability - if not irrigated	Low	Low	Moderate

(Manaaki Whenua, 2022)

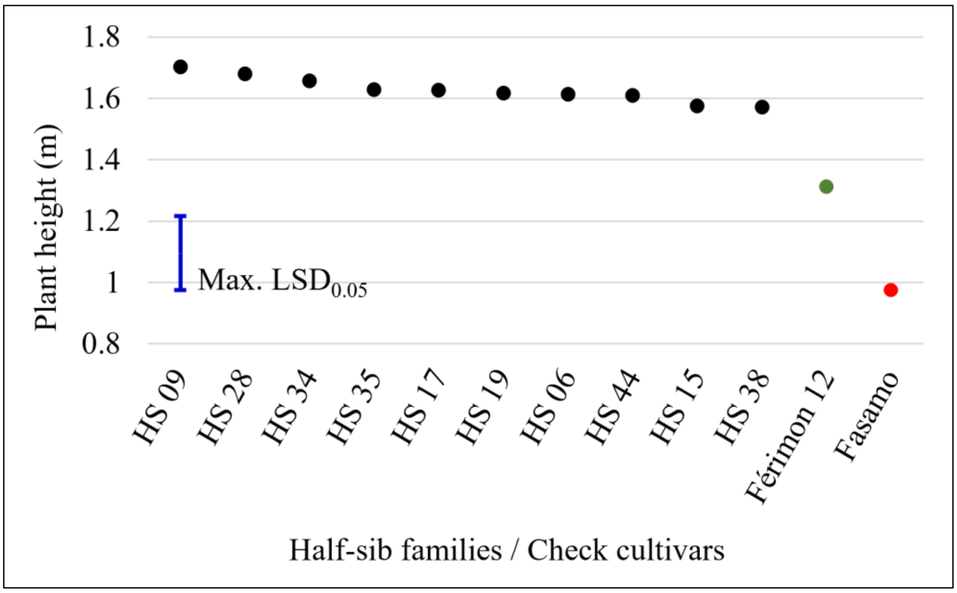
[The references cited within the appendices correspond to the reference lists provided in each respective chapter to which the appendix belongs]

Appendix 3. Germination test results conducted at the laboratory before sowing in growing seasons 2019/2020 and 2020/2021

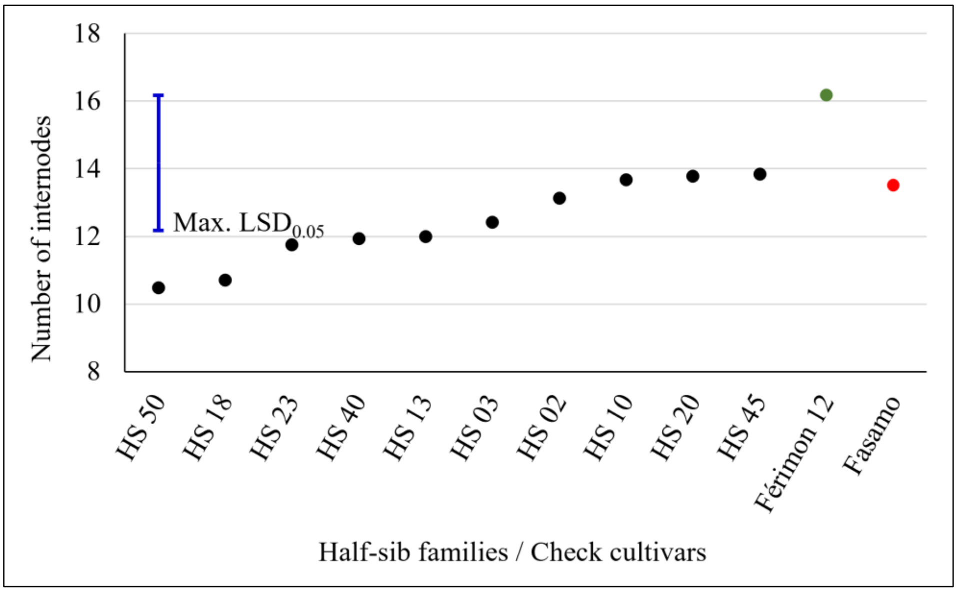
Cultivar	Normal seedling percentage (%)	
	Growing season 2019/2020	Growing season 2020/2021
CFX – 2	94	93
CRS – 1	87	87
Ferimon 12	91	89
Katani	86	84
Futura 75	85	84
Finola	95	95

Appendix 4. The estimated within HS family phenotypic variance (σ^2_{PW}) components of 50 half-sib families of industrial hemp, evaluated at Palmerston North, for the traits: SD, stem diameter (mm); HT, plant height (m); IN, number of internodes; SY, seed yield (g); BM, biomass (g).

Traits	σ^2_{PW}
Stem diameter	3.09
Plant height	0.07
Number of internodes	22.65
Seed yield	14.67
Biomass	121.52



Appendix 5. Mean plant height of the top 20% of 50 HS families and two commercial check cultivars; 'Fasamo' and 'Férimon 12' evaluated at Palmerston North.



Appendix 6. Mean number of internodes of the top 20% of 50 HS families and two commercial check cultivars; 'Fasamo' and 'Férimon 12' evaluated at Palmerston North.

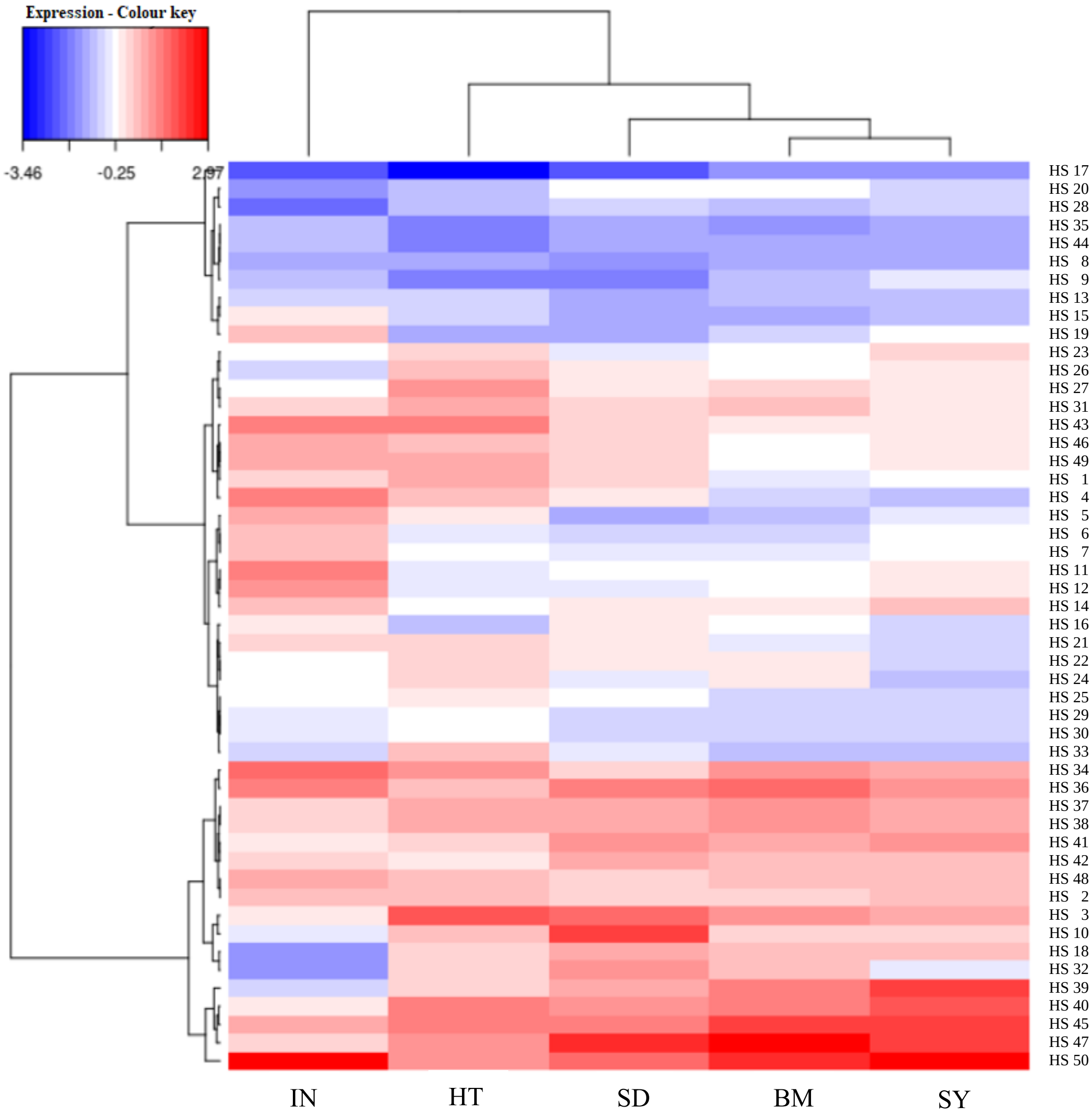
Appendix 7a. Dendrogram based on cluster analysis of the 50 HS families for the traits: stem diameter, plant height, number of internodes, seed yield, and biomass.

Appendix 7b illustrates the heat-map and dendrogram overview of the two-way hierarchical cluster analysis based on cluster analysis of the 50 HS families evaluated at Palmerston North, for the traits: stem diameter, plant height, number of internodes, seed yield, and biomass. The dendrogram effectively illustrates the relationships between these traits and how they correlate with each other, as well as how the HS families are grouped based on their trait expressions.

In the horizontal aspect of the dendrogram, there is a clear representation of the relationships and order in which the traits are correlated to each other. For instance, the initial split in the dendrogram distinctly separates the number of internodes from the other traits, indicating its weak correlation with them. Subsequent splits further isolate traits, eventually highlighting a strong correlation between stem diameter and seed yield, while emphasizing a weak correlation of the number of internodes with seed yield.

Vertically, the dendrogram reveals how the HS families are grouped into various clusters based on the collective expression of the five traits. Families within the same cluster show similar trait expressions, indicated by colour coding – for example, families with high trait expression are marked in red, while those with low expression are in blue.

The bi-plot derived from the Principal Component Analysis (PCA), using Best Linear Unbiased Predictor (BLUP) values of the 50 HS families (referenced in Figure 4.7 of Chapter 4), offers a clearer perspective of the correlations between these traits and the grouping patterns of the HS families based on their trait expression.



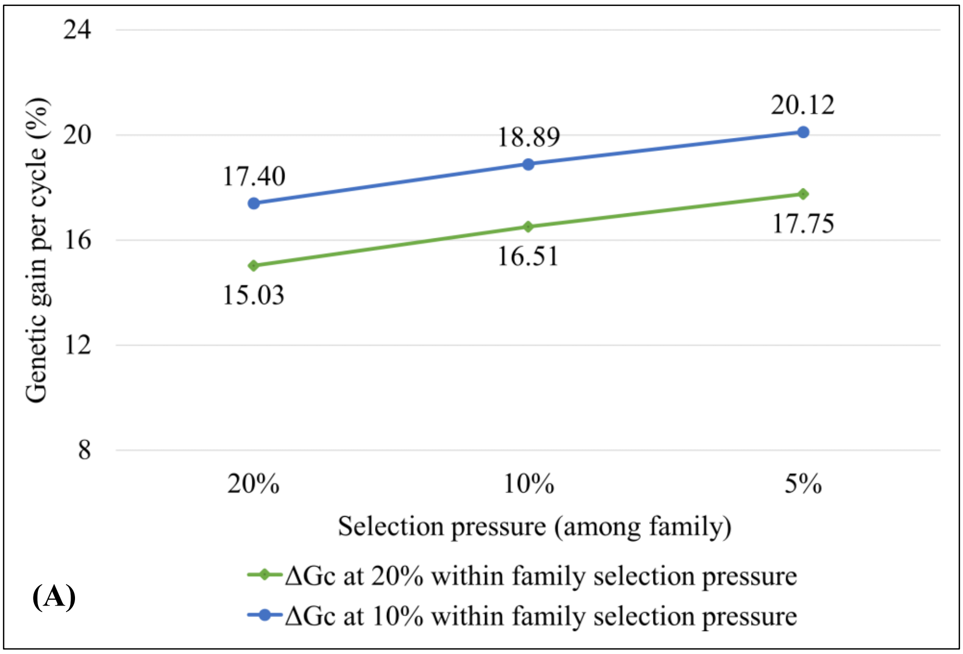
Appendix 7b. Dendrogram based on cluster analysis of the 50 HS families evaluated at Palmerston North, for the traits: SD, stem diameter; H, plant height; IN, number of internodes; SY, seed yield; BM, biomass.

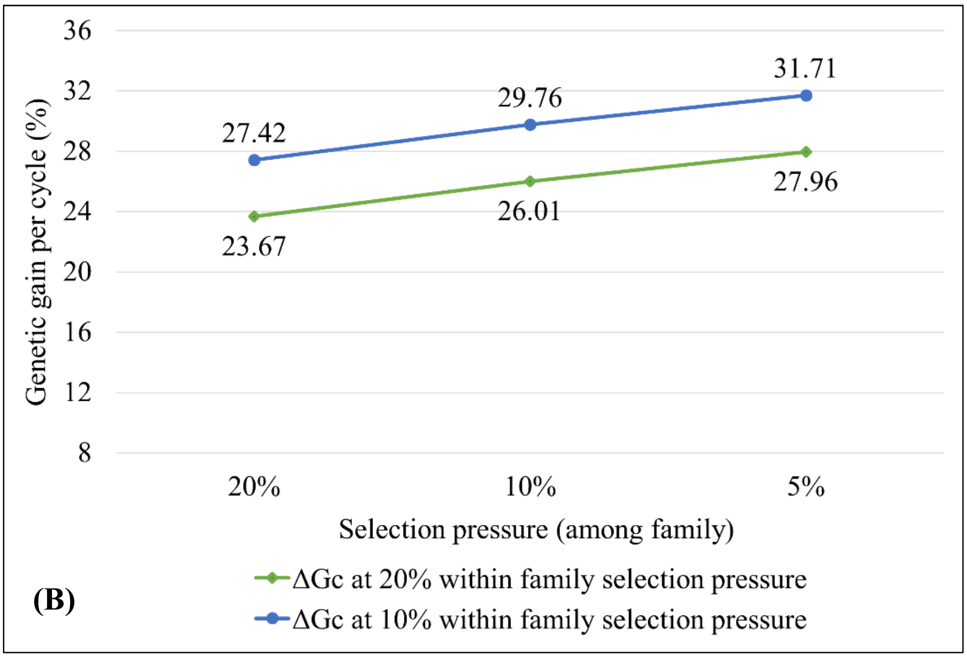
Appendix 8. Predicted percentage genetic gain ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard (s) based on breeding strategies using only among family (AF-HS) and a combination of among family (AF-HS) and within family selection (WF-HS), at different selection pressures for the trait, plant height.

		Among family selection (AF-HS)					
		20%		10%		5%	
		$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$
		5.78	16.86	7.27	18.51	8.51	19.87
WF-HS	20%	15.03	27.08	16.51	28.72	17.75	30.08
	10%	17.40	29.7	18.89	31.34	20.12	32.71

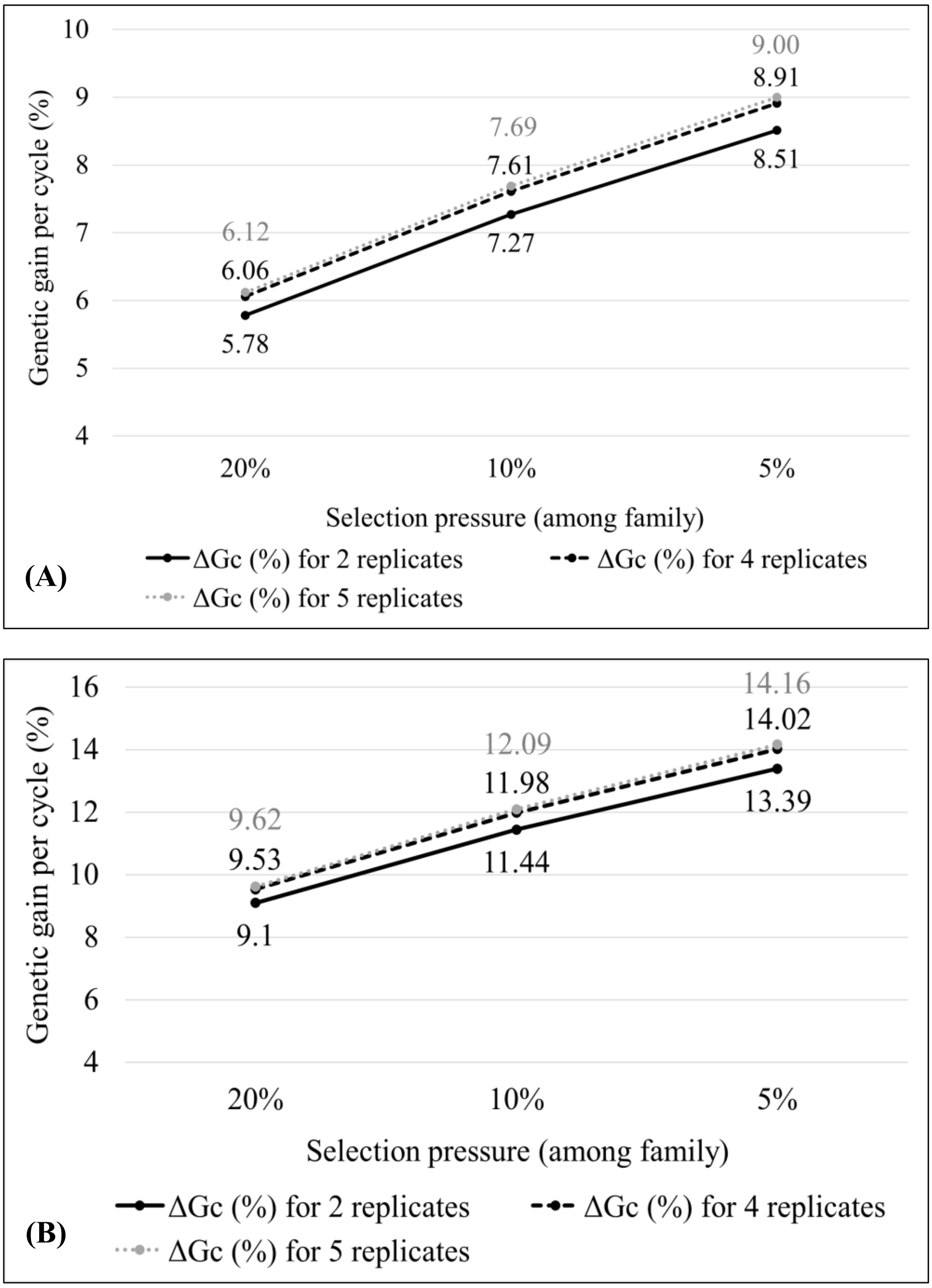
Appendix 9. Predicted percentage genetic gain ($\%\Delta G_{\bar{x}}$) relative to the family population mean ($\bar{x}$) and genetic gain ($\%\Delta G_s$) relative to the industry standard (s) based on breeding strategies using only among family (AF-HS) and a combination of among family (AF-HS) and within family selection (WF-HS), at different selection pressures for the trait, number of internodes.

		Among family selection (AF-HS)					
		20%		10%		5%	
		$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$	$\%\Delta G_{\bar{x}}$	$\%\Delta G_s$
		9.10	8.72	11.44	11.05	13.39	12.99
WF-HS	20%	23.67	23.24	26.01	25.57	27.96	27.51
	10%	27.42	26.97	29.76	29.30	31.71	31.25





Appendix 10. Predicted genetic gain per cycle (%$\Delta G_{\bar{x}}$) for (A) HT, plant height and (B) IN, number of internodes compared with a breeding strategy using among-family and within-family selection at different selection pressures.



Appendix 11. The effect of replication on predicted percentage genetic gain per selection cycle (%ΔGc) for the traits; (A) plant height and (B) number of internodes using the among-family selection breeding strategy at different selection pressures.

Appendix 12. Details on industrial hemp data partitions based on structure results.

Data partitions	Information on groupings
***K* = 2 (groups)**	
Group 1	Consisted of Finola, Katani, CFX-2, CRS-1, Futura 75 and Férimon 12 in the percentages of 34.92%, 26.98%, 23.81%, 12.70%, 0.79% and 0.79%, respectively – 98.41% of dioecious cultivars (seed cultivars) and 1.58% of monoecious cultivars (dual-purpose and fibre cultivars).
Group 2	Consisted of Férimon 12, Futura 75, CRS-1, Katani and CFX-2 percentages of 45.83%, 44.79%, 6.25%, 2.08% and 1.04%, respectively – 90.63% of fibre and dual-purpose cultivars and 9.38% of Canadian seed cultivars.
***K* = 3 (groups)**	
Group 1	Consisted of Férimon 12, Futura 75 and Katani in percentages of 88.64%, 9.09% and 2.27%, respectively – 97.73% of monoecious cultivars.
Group 2	Consisted of Finola, Katani, CFX-2, CRS-1 and Férimon 12 in percentages of 52.05%, 27.40%, 15.07%, 4.11% and 1.37%, respectively – 98.63% of seed cultivars and 1.37 of the dual-purpose cultivar.
Group 3	Consisted of Futura 75, CRS-1, CFX-2 and Katani in the percentage of 55.81%, 25.58%, 16.28% and 2.33%, respectively – 55.81% of the French-fibre cultivar and 44.18% of Canadian-seed cultivars.
***K* = 4 (groups)**	
Group 1	Consisted of Finola, Katani and CFX-2 in percentages of 64.29%, 28.57% and 7.14%, respectively.
Group 2	Consisted of CRS-1, CFX-2 and Katani in percentages of 60%, 30% and 10%, respectively.
Group 3	Consisted of Futura 75 and CRS-1 in percentages of 96.15% and 3.85%, respectively.
Group 4	Consisted of Férimon 12 and Futura 75 in percentages of 94.44% and 5.56%, respectively – 100% monoecious cultivars (French-dual-purpose and fibre cultivars).