Exploring the Growth Dynamics of *Moringa oleifera* Lam. and Its Invasive Potential in New Zealand

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Abstract

As global agricultural systems pursue climate resilience and multifunctional crops, *Moringa oleifera* Lam. (moringa) has gained attention as a resource-efficient crop with potential for agricultural diversification, sustainable weed management, and high-value bioproduct development. This thesis assessed the feasibility and environmental implications of introducing moringa into New Zealand (NZ) farming systems through integrated climatic and ecological suitability, invasive risk assessments, and adaptation mechanisms through physiological and metabolomic analyses. Climate suitability and invasive risk assessments indicated that moringa establishment in NZ is limited to warm, frost-free regions, particularly Northland and selected North Island microclimates. Most of the country's locations were classified as unsuitable due to low winter temperatures and frost frequency. Combined with poor cold tolerance and the lack of a persistent soil seed bank, moringa was classified as a low invasive-risk species under current and projected climates. Allelopathic studies showed that moringa root extracts exert species-specific and concentration-dependent effects. White clover (*Trifolium repens* L.), a key pasture species in NZ was strongly inhibited, whereas perennial ryegrass (*Lolium multiflorum* Lam.) and mānuka (*Leptospermum scoparium*) were minimally unaffected. At higher concentrations, it significantly suppressed major weed species i.e., fathen (*Chenopodium album* L.) and crabgrass (*Digitaria sanguinalis* L. Scop.), supported by metabolomic evidence of bioactive phenylpropanoids, flavonoids, and benzenoids. Physiological and biochemical analyses confirmed that although moringa activates stress-defence mechanisms under cold conditions, these responses are insufficient to prevent severe declines in photosynthesis and membrane integrity at low temperatures. Overall, moringa cultivation in NZ is feasible only in frost-free zones or under protected systems, supporting a controlled, site-specific introduction framework. Through science-based

regulation, regional targeting, and adaptive management, moringa could contribute to NZ's transition towards more sustainable and diversified agricultural systems.

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The moto remains the same "*Where there's no vision, the people perish*" Proverbs 29:18.

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Declaration

This thesis is based on publications, and the formatting style of each chapter follows the guidelines for the journal to which it will be submitted or in which it has been published or accepted for publication. Hence, there are inconsistencies in writing style, referencing format and some repetition of methods between the chapters presented in this thesis. Contributions of authors are specified in each chapter where necessary.

Chapter 1

General introduction

Six-week-old Moringa oleifera seedlings at the nursery at Songhai Agriculture Research Centre in Cotonou, Benin, December 2024. Photo credit: ***Blair Moses Kamanga***

1.1 Introducing moringa in New Zealand : An agroecological opportunity or a biosecurity risk?

Moringa oleifera Lam. (moringa) is a fast-growing, perennial deciduous tree belonging to the family Moringaceae and is native to the sub-Himalayan regions of northwestern India (Anwar et al., 2007; Raman et al., 2018; Singh et al., 2020). The species is widely cultivated throughout the tropical and subtropical regions due to its exceptional adaptability to drought, rapid growth, and multiple economic uses (Abdoul-Salam et al., 2021; Benettayeb et al., 2022). Under favourable conditions, moringa reaches heights of 8–12 m and develops a deep taproot that enhances water acquisition under moisture-limited environments. The species is predominantly propagated by seed, although stem cuttings are commonly used for rapid establishment in commercial production systems (Mashamaite et al., 2020; Palada et al., 2019). Moringa flowers within the first year of growth and is primarily pollinated by insects, particularly bees, enabling prolific seed production under suitable environmental conditions (Leone et al., 2015).

Moringa plants possess a wide range of vital antioxidants, antibiotics, and nutrients including vitamins and minerals which play pivotal roles in human health (Bhattacharya et al., 2018; Farooq et al., 2012; Hassan et al., 2021; Meireles et al., 2020). The plant has production benefits when used as feed for livestock such as feed conversion efficiency, improving health status, growth performance, and product quality of various livestock species at dietary inclusion rate of not exceeding 5% of a total dry matter intake (Falowo et al., 2018; Ghimire et al., 2021; Vieira et al., 2023). It is also used as a concentrate to reduce urinary nitrogen loss in livestock (Chandrashekar et al., 2021).

Beyond its nutritional value, moringa has attracted increasing interest as a component of sustainable agricultural systems. Supplementation of ruminant diets with moringa foliage has been shown to improve feed utilisation while reducing enteric methane emissions through the action of secondary metabolites that modify rumen fermentation (Mangar et al., 2022). Given that methane emissions from livestock represent a major component of NZ's agricultural greenhouse gas emissions, moringa has the potential to contribute to national climate-change mitigation strategies while simultaneously improving animal productivity. These attributes align with NZ's commitment to developing resilient and environmentally sustainable agricultural systems (Reynolds and Robinson, 2022). Hence, moringa is recognized worldwide for its various beneficial properties, making it a versatile and valuable plant in most parts of the world (FAO, 2018).

The introduction of moringa into NZ presents opportunities to diversify agricultural production through the cultivation of a high-value multipurpose crop. Climatically suitable regions, particularly Northland and adjacent northern areas, could support commercial production of moringa for fresh vegetables, functional foods, nutraceuticals, pharmaceuticals, cosmetics, and specialty horticultural products. Furthermore, the high protein content and digestibility of moringa foliage make it a promising supplementary feed for grazing livestock, especially during periods when high-quality forage is limited (Leone et al., 2015).

Although moringa is native to tropical and subtropical regions, it has been successfully cultivated across diverse agroecological zones in Asia, Africa, the Americas, and parts of the Mediterranean, where winters are relatively mild and frost is infrequent (Anwar et al., 2007; Leone et al., 2015). Its occurrence in cooler environments is, however, constrained by sensitivity to prolonged chilling and freezing temperatures, which limit growth,

productivity, and geographical distribution (Anwar et al., 2007; Nouman et al., 2014). Consequently, successful establishment in temperate regions depends on identifying climatically suitable areas and improving cold tolerance. In New Zealand, northern regions characterised by relatively mild winter temperatures may therefore provide favourable conditions for cultivation (Kamanga et al., 2025). Henceforth, successful establishment of moringa would therefore contribute to crop diversification while supporting emerging markets for health-promoting plant products (Mangar et al., 2022; Leone et al., 2015).

In addition to nutritive and pharmacological properties, moringa is used in cosmetics industry, biodiesel production, lubrication, and wastewater treatment and purification (Dzuvor et al., 2021), and soil nutrient enhancement when used as green manure (Taiwo et al., 2022). These properties make moringa an attractive option for all farming systems, including temperate farming systems which may be operating in a warmer drier climate in the future. Furthermore, moringa represents a promising nature-based solution for climate change mitigation, as its rapid growth and biomass accumulation can help reduce greenhouse gas emissions while enhancing carbon sequestration, particularly in agroforestry and dairy farming systems (Abdoul-Salam et al., 2021; Abdoun et al., 2023; Palada, 2021). However, while moringa's agronomic, nutritional, and pharmacological attributes are extensively documented in tropical and subtropical regions, its introduction into temperate climates such as New Zealand (NZ), raises critical questions surrounding environmental suitability, growth dynamics, adaptation mechanisms to cold stress, and potential ecological impacts.

As the global agricultural systems seek climate resilience and resource efficient crops, moringa has emerged as a promising candidate for diversification, sustainable weed

management, and high value product development (FAO, 2018). Its rapid growth, high leaf protein content, drought tolerance, and adaptation to various biogeographical locations (Abdoul-Salam et al., 2021; Palada, 2021), have led to the increasing interests for its cultivation beyond its native and tropical range (Arora et al., 2023; Csurhes & Navie, 2016). In NZ, this interest is driven by the need to identify novel crops suited to warming far north climates, enhance land use diversification programs, and reduce dependency on imported plant-derived bioproducts.

The New Zealand biosecurity framework requires robust evaluation of intentionally introduced plant species (Hulme, 2020), particularly those with traits conducive to rapid growth rates, establishment, and spread potential, which is common in moringa species (Amaglo et al., 2017; Arora et al., 2023). Despite moringa's popularity in agroforestry and sustainable agriculture (Palada, 2021), its adaptability to local NZ's climates and potential invasiveness is poorly understood. The species' adaptability to abiotic stressors including cold, drought, and its capacity to modulate local ecosystems through the release of allelochemicals (Sharma et al., 2020; Soares et al., 2023; UF-IFAS, 2021), demands an integrative assessment that extends beyond its agricultural value.

Emerging research using climate niche modelling and invasive risk assessment tools offers a valuable framework for assessing the invasive potential of introduced plant species (Pheloung et al., 1999; Roigé & Phillips, 2021). Some plant protection agencies use weed risk assessment (WRA) tools to evaluate plant species invasiveness before their introduction into new environments. WRA tools play significant roles in preventing the introduction of invasive species that can cause severe damage to the environment and disrupt ecological biodiversity. Invasive species can increase production costs and decrease crop yields thereby causing economic loss to farmers and government at large

(Csurhes & Navie, 2016; PIER, 2017). While moringa is not widely considered as an invasive species, based on its growth characteristics, some countries outside its native range including China, Cuba, South Africa, and the state of Florida in USA have listed moringa as weed, invasive, or environmental escape (Bridgemohan & Bridgemohan, 2014; Mashamaite et al., 2020; Oviedo-Prieto et al., 2012; UF-IFAS, 2021). Thus, exploring moringa's invasive potential using WRA tools in combination with climate suitability models can inform the safe introduction of this species into New Zealand environments.

Preliminary climate suitability models suggest that certain regions in northern and coastal NZ could support the establishment of tropical plant species such as moringa under the current and future climate scenarios, (Bania et al., 2023; Kamanga et al., 2025). However, these models must be complemented by empirical assessments of growth performance and cold stress responses under control, semi-controlled, and field conditions to assess its establishment or productivity. Predicting moringa's expansion range requires an in-depth understanding of how the species adapts to cold environments. Studies have shown that low temperatures induce specific biochemical and metabolic changes that enhance tolerance in various plant species (Aazami et al., 2021; Rácz et al., 2023; Raza et al., 2023).

Low-temperature stress which encompasses both chilling stress (0–15°C) and freezing stress (<0°C), each of which elicits distinct physiological and biochemical responses in plants (Theocharis et al., 2012). Cold-induced metabolites identified through metabolomics can provide valuable markers for developing cold-tolerant cultivars. These markers may support genetic manipulation, marker-assisted selection, and non-conventional phenotyping approaches aimed at improving crop adaptation to low-

temperature stress (Razzaq et al., 2022). Metabolomics, defined as the systematic study of metabolic profiles in biological samples, has gained increasing attention because of its capacity to identify metabolic markers associated with plant performance under stressful environments. Consequently, it has become a powerful tool for guiding crop genetic improvement and selecting genotypes with enhanced stress resilience.

In addition to environmental suitability, the allelopathic potential of moringa introduces another ecological complexity. Allelochemicals synthesised and released by plant roots may suppress or alter the germination and growth of co-occurring plant species, including native flora, pasture, and weed species (Kumar et al., 2024; Tahir et al., 2018). Invasive plant species usually utilize allelopathy as a trait strategy to compete and establish themselves into new ecosystems (Clavijo-McCormick et al., 2023; Langkilde et al., 2017). While allelopathy helps invasive or introduced species to establish in new environments, the majority of them produce allelochemicals that can negatively affect the performance of native and some economically important species (Lorenzo et al., 2013). This ecological threat calls for screening of allelopathic potential of moringa on native, pastoral, and weed species to establish the impacts of incorporating moringa into existing farming systems or managed landscapes.

To sum up, moringa has been proposed as a potential crop for diversification in temperate regions such as NZ due to increasing interest in sustainable multifunctional plants (AgriFutures, 2024; Decker & Kurnik, 2018; Reynolds & Robinson, 2022). However, the ecological, physiological, and biochemical adaptation mechanisms pertaining to its survival and ability to naturalise in cooler temperate environments remain insufficiently understood. Moreover, the invasive risks and allelopathic interactions with native or economically important pastoral species need to be systematically evaluated prior to its

introduction. Hence, this study responds to a clear gap in knowledge on how moringa would behave in novel temperate niche/environments, considering its cold resistance, competitive (allelochemical) and invasive traits.

1.2 Problem statement, research gap, and justification

Moringa is globally valued for its nutritional, medicinal, and agroforestry benefits (FAO, 2018; Palada, 2021), yet its climatic suitability and ecological implications in temperate climates are poorly understood. In the context of NZ where environmental control and strict biosecurity measures are national priorities (Hulme, 2020), the potential risks associated with moringa introduction are currently unassessed. Moringa's fast growth habits, stress tolerance, and known allelopathic properties (Palada et al., 2019; Soares et al., 2023; Tahir et al., 2020), may contribute to unintended ecological impacts such as competing or suppressing the growth of native or pasture species. Currently, few studies have been conducted to investigate moringa's environmental suitability, invasiveness, and allelopathic behaviour under NZ's unique climatic and ecological conditions.

Despite the species considerable nutritional, medicinal, and agricultural importance, the successful expansion of moringa into temperate regions remains constrained by limited understanding of the physiological and metabolic mechanisms underlying cold stress adaptation. Previous studies have primarily focused on the nutritional composition, pharmacological properties, and agronomic performance of moringa under tropical conditions, whereas comparatively little is known about the invasiveness, allelopathic potential, and metabolic reprogramming that occurs during prolonged exposure to low temperatures. This knowledge gap limits efforts to identify adaptive traits that could improve cold tolerance and support cultivation in cooler environments.

This thesis addresses the critical research gaps by integrating species climatic suitability and distribution modelling, cold stress adaptation mechanisms, and allelopathic assessments to determine its ecological compatibility with NZ's unique environment. While preliminary climate models indicate that some parts of NZ could be suitable for growth and cultivation of tropical and subtropical plant species, these projections have not been validated with physiological or biochemical data of moringa. Additionally, the allelochemical effects of moringa root extracts/exudates on pasture, native, and common weed species in NZ, remain insufficiently explored. Hence, a comprehensive and interdisciplinary evaluation is essential to determine whether moringa can be safely and sustainably introduced in NZ and provide valuable guidance for future land use policies on biosecurity frameworks, breeding programs for cold tolerant cultivars, and cultivation practices.

1.3 Research Aim

The main aim of this research was to explore the growth dynamics and invasive potential of moringa for potential introduction and incorporation into NZ agricultural systems and to understand the impact of moringa on adjacent agricultural and native plants.

1.3.1 Research objectives

1. To evaluate the invasive risk potential of moringa by integrating climate suitability models and invasive risk assessment tools to determine the likelihood of spreading, establishment, and impact potential under current and future climate scenarios in NZ.
2. To evaluate the allelopathic potential and metabolite profiling of moringa through glasshouse and laboratory-based bioassays on selected native, pastoral, and weed species.

3. To investigate the physiological, biochemical, and metabolic responses of moringa to cold stress conditions representative of NZ's template climates.

1.3.2 Research questions

This research is structured to answer the following research questions:

1. Can moringa establish self-sustaining populations and pose an ecological invasion threat under current and future climate scenarios in NZ? If invasion risk is low, what are the potential locations suitable for establishment, growth, and cultivation in NZ?
2. Will moringa exhibit allelopathic effects on selected native, pastoral, and weed species found in NZ? If so, what are the major allelochemicals involved?
3. Does moringa possess any physiological, biochemical, and metabolic adaptation mechanisms to tolerate prolonged cold stress? If so, what are the major primary and secondary metabolites involved and cold-stress responses?

1.5 Thesis structure

This thesis is organised into seven chapters, each designed to contribute progressively towards achieving the research aim of the comprehensive analysis of climatic suitability, invasive and allelopathic risks, and adaptation mechanisms to prolonged cold stress for potential introduction of moringa in NZ. The thesis takes an interdisciplinary approach that integrates climate suitability and invasive risk assessments, screening for allelopathic potential of moringa root extract/exudates, cold stress adaptation mechanisms and metabolomic profiling to determine the potential for introduction of moringa in NZ.

Chapter 1 outlines the background information, context, and rationale for this research. It presents the key research problem statement, identifying the current gaps in knowledge,

and justifying the need for an integrated approach to evaluate the potential for moringa introduction into NZ farming systems. The chapter concludes with clearly defined research objectives, questions, and a detailed summary of thesis structure.

Chapter 2 is a systematic review on the roles of moringa allelochemicals in sustainable agriculture development and provides the current trends on growth dynamics. It analyses the allelochemical composition of moringa and its mechanisms behind allelopathic effects. It further explores the negative and positive effects of moringa allelochemicals on cultivated and non-cultivated plants and its implication on sustainable agriculture production.

Chapter 3 reviews the growth dynamics and adaptation mechanisms of moringa by exploring the growth dynamics, environmental suitability, and adaptation mechanisms of moringa provenances that survive cold stress and explores the coordinated roles of primary and secondary metabolites in response to cold stress in plants. The section also summarises the breeding programs for cold tolerant cultivars of moringa and identifies research gaps for future perspectives.

Chapter 4 investigates the combination of climate suitability models and risk assessment tools to evaluate the invasive potential of intentional introduction of moringa in NZ. Weed risk assessment tools and climate adaptation models were used to assess the invasive potential and climate suitability of moringa in NZ. The invasive risk assessment tools classified moringa as a low-risk species for invasiveness in NZ, with a paucity of information on the formation of permanent soil seed bank and period of seed storability. The results on climate suitability analysis identified Northland and surrounding microclimates as potential sites for moringa growth and cultivation under current climate scenarios.

Chapter 5 screened the allelopathic potential of moringa root extracts on ryegrass, white clover, fathen, crabgrass, and mānuka. It further conducted untargeted metabolomics analysis to identify bioactive compounds responsible for allelopathy. The screening for allelopathic effect was conducted using a root extract method on steel blue germination papers and a pot screening method using plant-plant interference to assess the effect of root exudates. The results revealed dose-dependent and species-specific response effect to root extracts. Moringa exhibited significant allelopathic effects on white clover, fathen and crabgrass, with white clover, a key pasture species in NZ being most negatively affected.

Chapter 6 investigated the adaptation mechanisms by evaluating the physiological and metabolic changes in moringa due to cold stress and assess its implications for cultivation in NZ. This chapter assessed the photosynthetic activities, membrane integrity, and metabolomic profiling under four temperature regimes of 25, 15, 10, and 4°C. The results showed that photosynthesis, stomatal conductance, and transpiration rates declined progressively with decreasing temperature and were almost completely inhibited at 4°C, while water use efficiency was maintained under moderate chilling but collapsed under severe stress. Metabolite profiling revealed strong temperature-dependent clustering, with cold stress inducing the accumulation of osmolytes, amino and organic acids, carbohydrates, and polyamines. Secondary metabolism was strongly altered with enrichment of flavonoids and phenolic acids, alongside elevated phytohormones under severe cold stress.

Chapter 7 is a final chapter that synthesises and discusses the research findings by integrating all the results across research trials and highlights policy guidance regarding moringa introduction into agricultural systems in NZ. It evaluates the implications for

biosecurity, sustainable agriculture through the incorporation of moringa into the existing farming systems and provide policy guidance on intentional plant introduction in NZ. It also offers recommendations for future research and provides a final conclusion regarding exploring the growth dynamics and invasive/allelopathic potential of moringa in NZ.

1.6 Research ethical issues

This study has no human or animal ethical issues.

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Statement of Contribution to Chapter 2

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GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Blair Moses Kamanga
Name and title of main supervisor:	Dr Donita L. Cartmill
In which chapter is the manuscript/published work?	2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

B.M.K. and A.C.M. developed the main idea for the manuscript and wrote the original draft. D.L.C. and C.M., as doctoral supervisors of B.M.K., provided significant input and editing. All authors read and agreed to the published version of the manuscript.

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Chapter 2

Allelopathic Effects of *Moringa oleifera* on Cultivated and Non-Cultivated Plants: Implications for Crop Productivity and Sustainable Agriculture

Moringa plant in glasshouse at the Plant Growth Units. Photo credits: ***Blair Moses Kamanga***

This chapter explores the negative and positive effects of moringa allelochemicals on cultivated and non-cultivated plants and its implication on sustainable agriculture production. It also suggests and defines multiple avenues for future research in this field. This chapter is presented in the style of the journal *Agronomy*, adapted to meet thesis formatting requirements including consistency across chapters. The chapter was published in *Agronomy* as:

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Abstract

Moringa (*Moringa oleifera* Lam.) is widely recognised as a multipurpose crop suitable for human and animal consumption, medicinal, and industrial purposes, making it attractive for introduction into new ranges. Its extracts have been found to have beneficial impacts on various crop species and biological activity against multiple weeds, making their use in agriculture promising. However, concerns have also been raised about moringa's potential to negatively impact the growth and development of other cultivated and non-cultivated plant species, especially in areas where it has been introduced outside its native range. To understand the positive and negative interactions between moringa and other plants, it is essential to investigate its allelopathic potential. Allelopathy is a biological activity by which one plant species produces and releases chemical compounds that influence the reproduction, growth, survival, or behaviour of other plants with either beneficial or detrimental effects on the receiver. Plants produce and release allelochemicals by leaching, volatilisation, or through root exudation. These biochemical compounds can affect critical biological processes such as seed germination, root and shoot elongation, photosynthesis, enzymatic activities, and hormonal balance in neighbouring plants. Therefore, allelopathy is an important driver of plant composition and ecological interactions in an ecosystem. This review explores the positive and negative allelopathic effects of moringa extracts on other plant species, which may help to inform decisions regarding its introduction into new biogeographical regions and incorporation into existing farming systems, as well as the use of moringa plant extracts in agriculture.

Keywords: allelochemicals; biostimulants; hormesis; inhibition; plant competition; productivity

2.1 Introduction

Moringa oleifera Lam. (moringa) is an introduced plant species in most parts of the world and currently cultivated in various tropical and sub-tropical countries due to its health and nutritional benefits (Csurhes & Navie, 2016). Moringa can grow in and adapt to different climatic conditions, making it convenient for cultivation in various biogeographical regions, supporting its worldwide expansion and distribution. Nevertheless, there are concerns about the species becoming invasive when introduced in other areas outside its natural range (Bachheti et al., 2020; Kamanga et al., 2025; Tahir et al., 2018; Vyas & Sharma, 2021). Of particular interest is its allelopathic potential on native and other cultivated species, i.e., its ability to emit allelochemicals that can promote or inhibit the germination, growth, and establishment of other plants in proximity (Ahmed & El-Mahdy, 2022; Alshoaibi, 2021).

Allelochemicals are secondary metabolites that play vital roles for plant communication, competition, and protection or promote tolerance against biotic and abiotic stress (McCormick et al., 2012). These include different classes of compounds such as phenols, terpenoids, alkaloids, and flavonoids (Alshahrani & Suansa, 2020; Ladhari et al., 2020). They are produced and emitted or released into the environment as fluids or gasses through root exudation, leaching, and volatilisation (Effah et al., 2019; Pandey et al., 2023). Plant allelochemicals influence the germination and growth of neighbouring plant species by influencing key biological processes, including cell division, hormonal balance, photosynthesis, and enzymatic activity (Hierro & Callaway, 2021). Hence, allelopathy is considered a critical driver of plant species composition and distribution in terrestrial ecosystems (Farooq et al., 2011; Latif et al., 2017).

While plant allelochemicals can improve germination and growth, they can also exert inhibitory effects depending on concentration levels, target species, environmental conditions, and the specific target plant organs, e.g., seeds, leaves or roots (Danilova et al., 2020; Latif et al., 2017; Mushtaq et al., 2020; Perveen et al., 2021). For instance, bioactive compounds released by invasive plants can adversely affect native plants, aligning with the "new weapon hypothesis" which proposes that exotic species acquire a competitive advantage in new ecosystems because native plants, without a shared coevolutionary history, are more vulnerable to the negative effects of the novel bioactive compounds of exotic plants (Callaway et al., 2008). Although the evidence supports this hypothesis, it also indicates that responses vary among receiving plants and are influenced by allelochemical concentration (Effah & Clavijo McCormick, 2024).

Moringa plant extracts (MPE) are often used to investigate how allelochemicals affect the growth and development of other plants. Results have demonstrated that both positive and negative impacts exist (Buthelezi et al., 2023; Tahir et al., 2018). This dual allelopathic effect of moringa must be taken into consideration before introducing the plant into new environments especially when integrating it into an existing farming system, a new ecological range, or considering its use in cropping systems through extract-based interventions. This review consolidates current knowledge of moringa allelopathy by focusing on the impacts of moringa allelochemicals on various cultivated and non-cultivated plant species. It further seeks to identify knowledge gaps for future research.

2.2 Review Methodology and Literature Search

A thorough information search was performed for the pertinent scientific literature on allelopathy and moringa plant species across scientific databases including Springer

(https://www.springer.com/gp/life-sciences) accessed on 23 May 2025), Google Scholar (https://scholar.google.com/) and CAB Abstracts accessed on 15 April 2025, PubMed (https://pubmed.ncbi.nlm.nih.gov/) accessed on 3 January 2025, and Scopus (https://www.scopus.com/home.uri) accessed on 18 March 2025. The search utilised terms “moringa” and “allelopathy” in conjunction with key concepts or phrases “phytochemistry,” “bioactive,” and “allelochemicals” to encompass the scope of contemporary research on allelochemical or biological activities and its applications. Relevant publications were screened following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework (Page et al., 2021). Duplicate records were removed before titles and abstracts were assessed for relevance. Full-text articles were subsequently evaluated against predefined inclusion criteria, with emphasis placed on studies investigating plant physiological responses, metabolomic adaptations, climatic suitability, and agricultural applications of moringa and related plant species.

To ensure scientific rigor, priority was given to peer-reviewed journal articles published in internationally recognised journals, together with authoritative books and review articles. Studies employing robust experimental designs, appropriate statistical analyses, and reproducible methodologies were preferentially included. Where information regarding moringa was limited, evidence from closely related invasive studies on other plant species was incorporated to provide broader mechanistic context. Government reports and institutional publications were included only where they provided relevant information on invasive species, or agricultural policy in NZ.

The literature search identified 1,856 records across the selected databases, of which 1,515 remained after the removal of duplicates. Following title and abstract screening,

415 full-text articles were assessed for eligibility, resulting in the inclusion of 115 studies in the final review (Figure 1). Of 115 published research articles, 66 offered direct and indirect information on the effects of MPE, and 46 were supported articles on the roles, mechanisms, regulation, and behaviour of allelochemicals. Three (2) additional papers (Tullu 2019; Affengruber et al., 2024, Page et al., 2021) tackled the methodology for the literature search. The literature search concentrated on studies published in the past two decades, with article suitability assessed through the review of titles and abstracts, a method recognised for its efficiency in identifying pertinent research publications (Affengruber et al., 2024; Tullu, 2019).

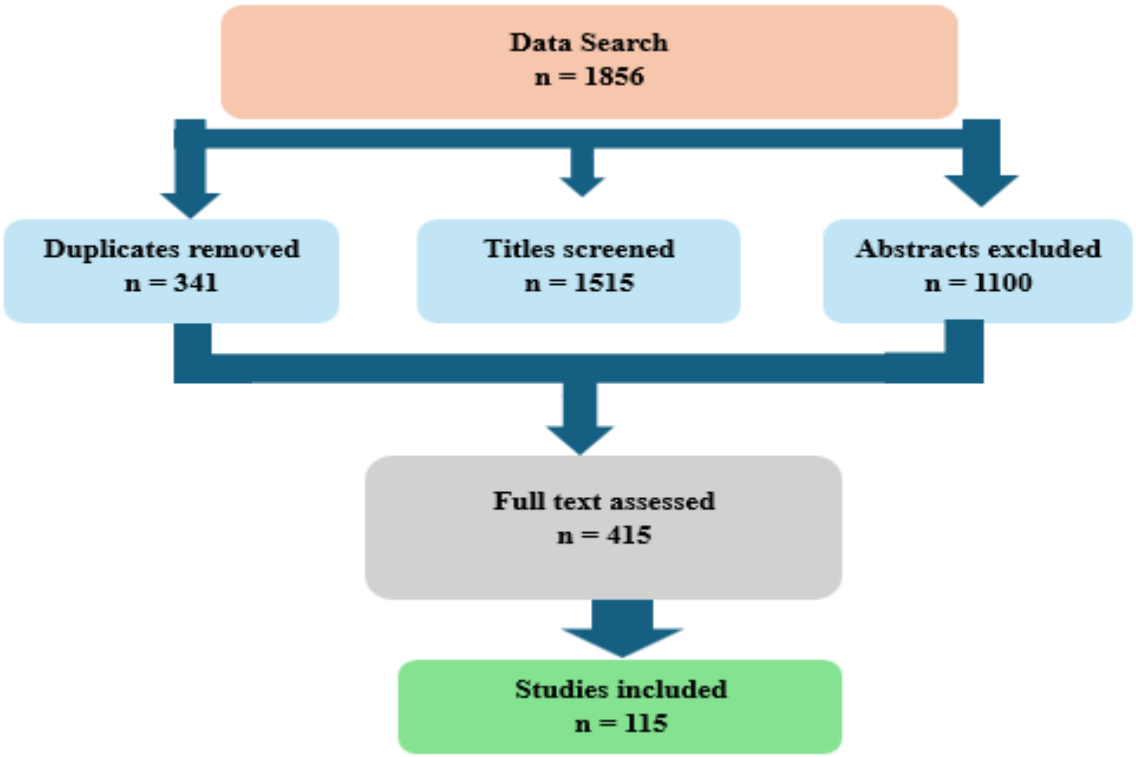

Figure 1. PRISMA 2020 flow diagram illustrating the structured literature search and study selection process adopted for this review. Publications were identified from major scientific databases, screened for relevance, assessed for eligibility, and selected according to predefined inclusion criteria to ensure a comprehensive and reliable synthesis of the available evidence (Page et al., 2021).

A systematic and narrative literature review was conducted to answer the following question: What are the positive and negative effects of moringa allelochemicals on the growth and development of different cultivated and non-cultivated plants? Although we are aware of the multiple pathways through which moringa allelochemicals can come into contact with target plants (through direct root exudation, leaching or volatilisation), most of the available literature focused on the application of plant extracts, highlighting the need for field studies to test moringa allelochemicals under natural conditions (Figure 2). Therefore, this review summarizes findings related to moringa plant extract applications in cereals, legumes, vegetable and fruit crops. We selected these crops because they exemplify a variety of botanical groups and represent an important share of agricultural production systems.

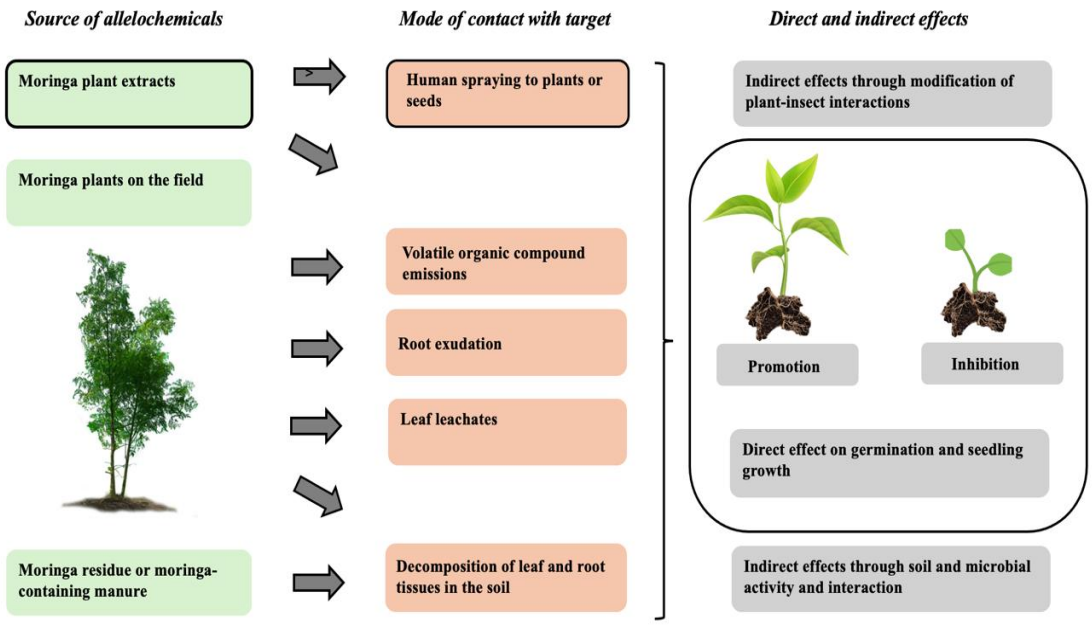

Figure 2. Different pathways through which moringa allelochemicals can reach a target plant and potential effects on the receiving plants. The boxes with black borders are the focus of this review; the other areas require further research.

2.3 Mechanisms Behind Allelopathic Effects

Various mechanisms by which allelochemicals directly affect target plants have been thoroughly reviewed elsewhere, so they will not be covered here in detail (Cheng & Cheng, 2015). These include modifying the cellular structure, influencing cell division and elongation, affecting the antioxidant system or cell permeability, and altering metabolic processes (such as photosynthesis and respiration) and regulatory systems (including several hormones and enzymes).

The action of allelochemicals depends on their chemical identity, concentration, and the sensitivity of the recipient species. Typically, exposure to a low dose can induce a beneficial adaptive response in target plants, while higher doses may have detrimental effects, a phenomenon known as hormesis (Perveen et al., 2021; Vargas-Hernandez et al., 2017). However, different plants may vary in their sensitivity. For instance, as mentioned earlier, native plants may be more sensitive to allelochemicals of exotic plants they did not co-evolve with (Callaway et al., 2008).

There are a variety of compounds involved in negative allelopathy including terpenoids, phenolics, quinones, and nitrogen-containing compounds. At sufficient concentrations, monoterpenes such as eucalyptol, camphor and citral, can induce cellular abnormalities in the nucleus, cell wall and other organelles such as vacuoles and microtubules. Other monoterpenoids (e.g., camphor, 1,8-cineole, β-pinene, α-pinene, and camphene) have been reported to affect cell division and DNA synthesis in plant meristems (Zuzarte et al., 2024). Quinones and phenolics are commonly reported to enhance the production of Reactive Oxygen Species (ROS) or interfere with antioxidant activities in the receiving plants. This imbalance causes degradation of molecules such as proteins, ultimately leading to apoptosis or necrosis (Tak et al., 2023). Other phenolics such as chlorogenic

acid, caffeic acid, and catechol inhibit enzymes involved in seed germination (e.g., λ-phosphorylase), while salicylic acid inhibits the synthesis of the plant hormone ethylene (Mushtaq et al., 2020). Benzoquinones such as sorgoleone, can impact photosynthesis by affecting the normal function of the photosystem II, a crucial protein–pigment complex involved in the light-dependent reactions of photosynthesis (Lacerda et al., 2020). Nitrogen-containing compounds such as cyanogenic glycosides, can release hydrogen cyanide, which inhibits cellular respiration by blocking the enzyme cytochrome c oxidase disrupting electron flow (Cheng & Cheng, 2015).

While at higher concentrations, allelochemicals affect cellular structure and impede vital activities that result in the inhibition of germination and seedling growth in sensitive plants, stimulatory allelopathic effects including enhancement of seed germination and seedling growth is often observed at lower concentrations (Perveen et al., 2021; Vargas-Hernandez et al., 2017). At lower concentrations, most allelochemicals such as phenolics and flavonoids facilitate antioxidant enzyme activities like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), that improve and maintain the cell's redox equilibrium and resistance to abiotic stress (Danilova et al., 2020; Vargas-Hernandez et al., 2017). Additionally, some vitamins such as vitamin C (ascorbic acid), and plant hormones including auxins or cytokinins can facilitate prolific cell division, shoot and root growth, and photosynthetic activities for rapid plant growth and increase productivity (Bibi et al., 2016; Danilova et al., 2020). Some examples of compounds with stimulatory and inhibitory activities found in moringa plants are summarized in Table 1.

2.4 Moringa Plant Extracts as Biostimulants

Moringa leaves, roots, seeds, and bark contain a broad spectrum of phytochemicals capable of altering plant physiology, metabolism, hormonal balance, and gene expression

that influence receiver plant's productivity. Mineral nutrients (Islam et al., 2021), phenolics and flavonoids (Bajwa et al., 2023), and other numerous bioactive compounds (Alam et al., 2020; Förster et al., 2015), make moringa plant extracts (MPE) unique in nature. MPE contains phytohormones such as gibberellin, auxin, and cytokinin (Arif et al., 2023; Brockman et al., 2019; Elzaawely et al., 2017), which are known to affect different parts of plant growth and development (Danilova et al., 2020). Moringa extracts contain important nutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, and other trace elements, i.e., copper, iron, zinc, and manganese. These nutrients are vital for the plant metabolism, structure, and function of different plant cells (Arif et al., 2023; Delvin & Levy, 2020). The plant contains proteins, amino acids, and vitamins, which play important roles in growth, enzyme function and metabolism, stress tolerance, and overall plant health (Kawade et al., 2023; Zulfiqar et al., 2020).

Moringa has phenolic and flavonoid compounds that function as antioxidants. These support the plant to defend against biotic and abiotic stress factors in their environment (Bhuker et al., 2023; Dias et al., 2021; Kumar et al., 2021). The plant has saponins which aid several biological activities and could help to maintain plant health and protect against pests and diseases (Hussain et al., 2019). Previous studies assessed and evaluated moringa leaf extracts (MLE) for bioactive compounds and found zeatin, and its derivatives of plant cytokinin. These substances have beneficial effects on plants, similar to synthetic hormones that promote growth in plants (Zia et al., 2021). When phyto-hormones are applied to plants, they induce positive effect on numerous plant physiological and biochemical processes such as the ascorbate glutathione cycle, transpiration rates, cell division, nitrogen metabolism, and assimilation activities that promote plant growth and yield enhancement (Pal et al., 2023).

Table 1. Chemical and antioxidant constituents identified in moringa plant extracts and their roles in allelopathy.

Compound Class	Key Compounds	Biological Role	Allelopathic Potential	Ref.
Phenolic acids	Chlorogenic, gallic, ferulic, tannic acids, and coumarins.	Antioxidant, allelopathic, signalling molecules.	Stimulatory at low dose (growth promoter); root induction; inhibitory at high doses (inhibit growth).	(Förster et al., 2015; Pervez et al., 2017) ;
Flavonoids	Quercetin, kaempferol, rutin, naringenin.	ROS scavenging, UV protection, auxin transport molecules.	Variable; function as growth enhancers or inhibitors of competing plants via ROS modulation.	(Förster et al., 2015)
Glucosinolates/Isothiocyanates	Benzyl glucosinolate/isothiocyanate.	Allelopathic, detoxifying enzyme induction.	Suppression of seed germination/DNA alkylation, enzyme inhibition.	(El-Rokiek et al., 2022; Lopez-Rodriguez et al., 2020)
Tannins	Catechin, epicatechin.	Defense compounds, metal chelators.	Inhibitory effect: interfere with nutrient uptake and enzyme activity.	(Mushtaq et al., 2020)
Alkaloids	Moringine, moringinine.	Modulation of stress response, antimicrobial.	Allelopathic inhibition: affect cell division and germination in sensitive species.	(Lopez-Rodriguez et al., 2020)
Saponins	Triterpenoid saponin.	Antimicrobial, membrane interaction.	Inhibit growth in some plant species.	(Adekanmi et al., 2020)
Amino acids	Arginine, proline, glutamic acids.	Growth regulators, hormone precursors, osmoprotectants.	Stimulatory effect: promote resilience in intercropping species via root exudation.	(Ahmed et al., 2020; Hanafy, 2017)
Minerals	Fe, Zn, Ca, Mg, K, Mn, B.	Enzyme cofactors, chlorophyll structure, osmoregulation.	Indirect allelopathy through soil nutrient alteration (enhance or inhibit growth).	(Buthelezi et al., 2023; Trigo et al., 2020)
Hormones (Cytokinin)	Zeatin and its derivatives, gibberellins.	Promote cell division, shoot development.	Positive allelopathy: promotes growth of plants in proximity.	(Latif & Mohamed, 2016; Rady & Mohamed, 2015)
Vitamins (antioxidants)	Ascorbic acid (vit. C), α-tocopherol, β-carotene.	Maintain redox balance, protect membrane and enzymes.	Stimulatory to receiving plants through oxidative stress reduction.	(Fraga et al., 2018)

Moringa leaf extracts are known and recognised as an alternative option among biostimulants available because of their numerous bioactive compounds that promote plant growth and their ability to enhance soil nutrients when utilised as green manure (Sakr et al., 2018; Yasmeen et al., 2013). Research has shown that aqueous moringa plant

extracts (Aq. MPE) promote growth and productivity of various crops (Table 2). However, plants respond differently to varying concentrations, with higher application rates exerting inhibitory effects on certain crops (Mona et al., 2017). Thus, it is important to understand the biochemical and physiological processes of receiving plants to successfully use natural biostimulants from plant extracts. Table 2 shows the concentration rates and the positive allelopathic effects of MPE on the growth and development of different agricultural crops.

Table 2. Stimulatory effects of moringa plant extracts on the growth and development of crop species.

Source	Concentration	Observed Effects and Target Crop	Ref.
		Cereal Crops	
Aq. MLE	4:1, 3, 25% (*v*/*v*)	Improve growth, yield, and stress tolerance in maize (*Zea mays*).	(Muneeba et al., 2024; Yasmeen et al., 2013)
Aq. MLE	3% (*w*/*v*)	Improve growth, yield, and photosynthetic activities in wheat (*Triticum aestivum*).	(Khan et al., 2017; Nawaz et al., 2016)
Aq. MLE	100% (*v*/*v*)	Improved growth parameters on sorghum (*Sorghum bicolor*).	(Bashir et al., 2017)
Aq. MLE	3% (*v*/*v*)	Enhanced physiological, biochemical, and yield parameters rice (*Oryza sativa*).	(S. Khan et al., 2022)
		Leguminous Crops	
Aq. MLE	1:30 (*w*/*v*)	Accelerated growth of cowpeas (*Vigna unguiculata*).	(Maishanu et al., 2017)
Aq. MLE	1:20 (*w*/*v*)	Speeded and supported an antioxidant system and tolerance under stress in common/snap beans (*Phaseolus vulgaris*).	(Rady & Mohamed, 2015)(Rady & Mohamed, 2015)
Aq. MLE	1, 2, 3, and 4%	Increased yield and nutrient in pea plants (*Pisum sativum*) and mustard spinach (*Brassica rapa* var. perviridis).	(Merwad, 2018)
		Vegetable Crops	
Aq. WPE	10% (*w*/*v*)	Improved growth parameters and yield of spinach (*Basella alba*) and tomatoes (*Solanum lycopersicum*).	(Hoque et al., 2021)

Aq. MLE	1:30 (*w*/*v*)	Enhanced growth, fruit weight, and nutrient contents of eggplant/brinjal (*Solanum melongen*).	(Hoque et al., 2020)
Aq. MLE	10% (*v*/*v*)	Influenced growth, photosynthetic activity, and nutrient absorption in cabbage (*Brassica oleracea* var. capitata).	(Yaseen et al., 2023)
Aq./Ethanol MLE	1:10 (*v*/*v*)	Influenced vegetative growth and increased yield components in sweet pepper (*Capsicum annum* var. annum).	(Hala et al., 2017; Mehdawe et al., 2023)
Aq. MLE	1:20 (*w*/*v*)	Enhanced the growth and development of cucumber (*Cucumis sativum*).	(Ahmed et al., 2020)
		Fruit Crops	
Aq. MLE	3% (*w*/*v*)	Improved yield and nutrient contents of kinnow mandarin (*Citrus nobilis* x *Citrus deliciosa*).	(Nasir et al., 2020)
Aq. MLE	6% (*w*/*v*)	Enhanced fruit weight and anthocyanin strawberry fruits (*Fragaria x ananassa*) and plums (*Prunus domestica*).	(Ismail et al., 2021)
Aq. MLE	3.5% (*v*/*v*)	Improved quality and nutritional contents of grapevine (*Vitis vinifera*).	(Ali et al., 2020)
		Other Cultivated Plants	
Aq. MLE	3% (*w*/*v*)	Improved growth, biochemical attributes of quinoa (*Chenopodium quinoa*).	(Rashid et al., 2021)
Aq. MLE	50% (*w*/*v*)	Increased the yield and the yield components sunflower (*Helianthus annuus*).	(Iqbal et al., 2020)
Aq. MLE	100% (*v*/*v*)	Promoted growth of nut sedge (*Cyperus rotundus*).	(Ali et al., 2015)
Aq. MLE	30% (*w*/*v*)	Enhancing seed germination and seedling vigour of barnyard grass (*Echinochloa crusgalli*), buffel-grass (*Cenchrus ciliaris*), and blue panic grass (*Panicum antidotale*).	(Nouman et al., 2012)
Ethanol MSE	2,4, 6, 8% (*v*/*v*)	Improved growth and nutrient contents in cancer bush (*Lessertia frutescens*).	(Buthelezi et al., 2023)

2.4.1 In Cereals

Moringa extracts exhibit concentration-dependent effects on key physiological parameters in cultivated cereal crops including seed germination, root and shoot length, and promote photosynthetic activity (Perveen et al., 2021). For example, exogenous application of MLE at the rate of 25, 12.5, 6.25, 3.13, and 1.56% improve seedling growth, soluble leaf proteins, and leaf relative water content in maize under drought stress (Pervez et al., 2017), while 25% (*w*/*v*) of Aq. MLE improved germination and seedling growth of the same crop (Zia et al., 2021. Furthermore, foliar application of Aq. MLE improved growth, grain, and biomass yield under field condition (Mvumi et al., 2013), while applying Aq. MLE two weeks after emergence and four weeks later increased plant height, fresh and dry shoot biomass, and yield of the same crop Biswas et al., 2016). Maize seed priming with Aq. MLE improved antioxidant activities (CAT and POD) by 22–56% that reduced hydrogen peroxide production. Additionally, a 3% (*v*/*v*) application of Aq. MLE resulted in hermetic effect in elevating salinity stress thereby improving germination, growth, photosynthesis, and antioxidant activities (Muneeba et al., 2024). The analysis of moringa extracts found numerous plant growth hormones including gibberellins (GA_4) and indole acetic acid (IAA) that have been reported to enhance growth in different cereal crops (Brockman et al., 2019). Moreover, GA_4 stimulates and facilitates the formation of hydrolytic enzymes such as amylase, which breaks down the reserved food substances held in seed endosperm into simple sugars to provide energy for germination and seedling growth (Zhao et al., 2022).

Wheat crop responded positively to the application of fresh Aq. MLE at the rate of 3% (*w*/*v*) and significantly improved seedling growth, productivity, and grain yield under drought stress conditions (Khan et al., 2017). It further increased the contents of

chlorophylls a and b when applied at tillering and heading stages (Nawaz et al., 2016). Although research has demonstrated a hormesis response to MLE (Perveen et al., 2021), spraying a 100% concentration of Aq. MLE improved growth parameters in sorghum, the results were attributed to the availability of zeatin, a natural substance used as a source of cytokinins along with other minerals and phytohormones that balance physiological and biochemical processes to enhance growth and yield of crops when applied exogenously (Maishanu et al., 2017).

Drought stress negatively affects physiological, biochemical, and yield parameters of various crops. It affects gas exchange attributes, antioxidant enzymes' activities, photosynthetic efficiency, yield, and yield components of rice (Khan et al., 2022). However, foliar application (3%) of Aq. MLE improved gas exchange traits, photosynthetic pigments, seedling establishment, growth, and yield of the same crop under normal and drought stress conditions (Khan et al., 2022). Biochemical improvements in SOD, CAT, and APX in rice provide evidence for the presence of antioxidants that protect the plant from oxidative damage (Hanafy, 2017). Additionally, MLEs function as exogenous growth enhancers due to the presence of zeatin, carotenoids, ascorbate, antioxidants, essential plant nutrients, and vitamins (Yasmeen et al., 2016; Khan et al., 2020). These improve endogenous hormonal balance during stress to improve overall plant growth, yield and yield components (Khan et al., 2020).

Similarly, MLE were effective in improving growth and productivity of cereal forages (millet, pearl millet, and Sudan grass) under salinity stress environment. A 1 mL^{-1} 10 mL of Aq. MLE contained the highest concentration of inorganic elements and growth hormones (cytokinin, auxin, gibberellins, and abscisic acid), these contributed to an increased fresh and dry forage yield by 17.67 and 4.87%, respectively (Abusuwar &

Abohassan, 2017). The analysis of MLE found rich contents of zeatin, a cytokinins which sustain the green colouring matter for photosynthetic activity that supports continued growth, accumulation of biomass, and higher grain yield under drought and salinity stress (Abusuwar & Abohassan, 2017). It is reported that the role of plant hormones in MPE is to regulate and maintain photosynthetic activity by protracting senescence and modify plant phenotypic response towards harsh environment. The presence of zeatin and its derivatives could explain the rapid germination and growth in different cereal species under abiotic stress.

2.4.2. In Leguminous Plants

Studies have demonstrated that low concentration of Aq. moringa extracts from young leaves diluted with water had stimulatory effects on seed germination and seedling vigour of leguminous crops including mung bean, cowpeas, snap beans, and chickpea (Elzaawely et al., 2017; Merwad, 2018; Phiri & Mbewe, 2010; Rady & Mohamed, 2015). MPE's antioxidants play essential roles in reducing or alleviating oxidative damage thereby enhancing plant growth and productivity under stress environments (Dias et al., 2021). A low concentration rate of 1:30 (*w*/*v*) at 25 mL per plant accelerated stem growth, increased the number of leaves, and branches in the range of 10–45% in cowpeas Maishanu et al., 2017). While a ratio of 1:20 (*w*/*v*) of MLE sprayed on 30 and 37 days after sowing speeded and supported an antioxidant system and tolerance under varied levels of salt stress thereby improving growth and yield in common/snap beans (Rady et al., 2015). Further, lower concentration rates of 1, 2, 3, and 4% significantly increased fresh pods yield, biological yield, protein content, and nutrient accumulation in pea plants; however, high protein content and photosynthetic activities were obtained at 4%

concentration (Merwad, 2018). The analysis of MLE found a higher concentration of GA_4 than other hormones.

Enhanced growth, yield, and yield components in legumes following the application of MLE has been linked to the availability of phytohormones and antioxidants (Elzaawely et al., 2017). It can be suggested that stimulatory effects of MLE on different legumes is due to the presence of natural zeatin, ascorbic acid, and quercetin, which enhances cell division, maintain membrane integrity, and modulate redox signalling pathways during optimal and suboptimal conditions. Antioxidants eliminate harmful free radicals and reduce oxidative stress that helps to protect plant cells from damage (Kumar et al., 2024). Phytohormones further synergistically upregulate photosynthesis-related genes and antioxidants enzyme activity including SOD, CAT, and APX which enhance chlorophyll biosynthesis, carbon assimilation, and stress tolerance in legumes thereby promoting plant productivity (Merwad, 2018). Protection allows plants to maintain their physiological functions and optimize nutrient uptake under salinity, thus improving photosynthetic efficiency, overall growth, vigour, and plant health (Latif & Mohamed, 2016; Merwad, 2018; Zia et al., 2021).

2.4.3. In Vegetable Crops

Although certain phytohormones possess similar effects in different plant species, research suggests that the specific response varies due to differences in plant physiological, hormonal receptors, interactions, environmental niche, hormone transport and distribution patterns, and genetic variation among plants (Sato et al., 2024). For example, spraying low concentration of MLE 1:10 (*w*/*v*) fortnightly (Aq. 25 mL per plant) improved growth parameters by 25% and increased yield in tomatoes and malabar spinach (Hoque et al., 2021). However, the effects on spinach were slightly lower than

those of tomatoes, providing further evidence that various crop species respond differently to allelochemicals. Furthermore, the application of Aq. MLE at the ratio of 1:30 (*w*/*v*) enhanced growth, fruit weight, and nutrient contents such as nitrogen, potassium, and phosphorus in egg plants (Hoque et al., 2020). It can be hypothesized that the presence of zeatin, GA_4, ABA, IAA and salicylic acid (SA) in MLE, each have distinct roles in regulating plant growth and development. Zeatin promotes cell division and lateral bud growth. Gibberellin, a plant hormone involved in stem elongation, seed germination, and flowering, ABA primarily regulates seed dormancy, stress responses by closing stomatal, and senescence, whereas IAA, an auxin, influences cell elongation, apical dominance, and root development. In turn, all these facilitate rapid growth, biomass accumulation, and crop yield (Elzaawely et al., 2017).

Application of 10% (*v*/*v*) concentration of Aq. MLE at a rate of 25 mL per plant positively influenced growth, photosynthetic pigments (anthocyanins), nutrient absorption, and reduced nitrate content by 12% in cabbage (Yaseen et al., 2023). Interestingly, applying a reduced concentration rate of 6% resulted in an even greater reduction in the nitrate content by 23%, contrasting previous findings that higher concentrations have detrimental effects on plant growth and development (Perveen et al., 2021). This signifies a differential response of plant species to bioactive compounds (Mushtaq et al., 2020). Similarly, foliar application of ethanolic MLE (20 g with 675 mL of 80% aq. ethanol) in the ratio of 1:10 (*v*/*v*) positively influenced vegetative growth and increased sweet pepper yield components by 1.68 kg per plant and 16.88 tons per hectare and a concentration of 4% significantly increased plant growth parameters and chemical composition of the same crop (Mehdawe et al., 2023; Hala et al., 2017).

A ratio of 1:20 (*w*/*v*) of Aq. MLE promoted the growth and development of cucumber fruits (fruit fresh and dry weights, and total fruit yield) and increased endogenous hormone levels (auxins, gibberellins, and cytokinins); and also promoted the activity of the antioxidant enzymes superoxide dismutase, catalase, and ascorbate peroxidase in cucumber leaves (Ahmed et al., 2020).

2.4.4. In Fruit Crops

Several studies have shown that moringa plant extracts positively influence fruit crops through the enhancement in fruit quality, increasing fruit shelf life, and promoting resistance to pests and diseases. For instance, applying Aq. MLE to the leaves at the rate of 3% (*w*/*v*) improved kinnow (mandarin) fruit size and weight, sweet, and increased the content of phenolic and ascorbic acids (Nasir et al., 2020) while application of a 6% (*w*/*v*) concentration of MLE enhanced fruit weight and anthocyanin levels in strawberries and enhanced firmness and colour traits in plums (Ismail et al., 2021). Foliar application of Aq. MLE at 3.5% (*v* /*v*) improved firmness, fruit diameter, and titratable acidity in grapevine fruits (Ali et al., 2020). The enhancements in fruit quality and its associated nutrient composition are due to the presence of essential nutrients in MLE that influence plant metabolism by augmenting the synthesis of vitamin C and other trace elements in leaves, thereby facilitating their transport to the fruit through the phloem (Nasir et al., 2020).

2.4.5. In Other Cultivated Plant Species

Foliar application of Aq. MLE at the concentration rate of 3% (*w*/*v*) enhanced the physiological and biochemical responses, quality attributes, as well as growth and yield components of quinoa. It further increased the activities of antioxidant enzymes including

SOD, CAT, and APX along with elevated levels of total phenolics and glycine betaine. The concentration of key mineral nutrients including K, Ca, and N was found to be highest in root and shoots in response (Rashid et al., 2021). Other studies have shown that the application of Aq. MLE and moringa root extracts (MRE) at 50% (*w*/*v*) significantly increased the yield and the yield components of common sunflower including the number of achenes per plant (Iqbal et al., 2020). Aq. MSE at the concentration rates of 2, 4, 6, and 8% increased growth characteristics, chlorophyll content, phenols, and flavonoids contents in cancer bush plants; however, 6 and 8% concentrations were highly effective. It further enhanced the concentration of micronutrients including zinc, copper, manganese, and sodium of the same plant.

Although research has demonstrated the hormesis phenomenon in plants (Jalal et al., 2021; Vargas-Hernandez et al., 2017), application of Aq. MLE at 100% (*v*/*v*) concentration significantly enhanced both fresh and dry biomass weight of the shoots, as well as the shoot and root lengths of purple nut sedge (Ali et al., 2015). Several grass species including barnyard grass, buffel-grass, and blue panic grass, were shown to benefit from seed priming with a 30% (*w*/*v*) MLE, which was proven to be efficient in enhancing seed germination and seedling vigour, crude protein, and phosphorus contents (Nouman et al., 2012). Further, maximum potassium, calcium, and magnesium contents of the same crop were found when the seeds were treated with moringa extracts. The effectiveness of MLE in promoting growth and development in various crops can be attributed to the presence of nutrients and plant growth regulation hormones, zinc, ascorbic acid, calcium, and potassium in moringa plant.

2.5 Moringa Plant Extracts as Plant Growth Inhibitors

The inhibitory effects of allelochemicals manifest in several ways including reduced seed germination, impaired seedling growth, nutrient uptake, suppression of photosynthetic activities by interfering with enzymatic activities and its metabolic pathways, resulting in overall decrease in productivity of infected plants (Amini et al., 2014; Kalisz et al., 2021). The effect entails biochemical interactions at different growth stages that affect the processes of germination, development and productivity of adjacent plants (Gurmani et al., 2021). Despite the MLE's significant concentration of phenolic acids, terpenes, and alkaloids, research has shown that the allelopathic efficacy of phenolic acids and their derivatives from plant roots are constrained by high biodegradability and strong adsorption to soil particles (Marchiosi et al., 2020). Hence, most studies on the allelopathic effects of moringa have concentrated more on leaf extracts than on root extracts and often conducted in controlled laboratory setups in which growth conditions mostly differ from natural environment. The negative allelopathic effects of MPE on leguminous, vegetable, fruit, and other crops are highlighted in Table 3.

Table 3. Inhibitory effects of moringa plant extracts on the growth and development of various crops.

Source	Concentration	Target Crop and Observed Effects	References
		Leguminous Crops	
Aq. MRE	10% (*v*/*v*)	Reduced growth parameters of mung beans (*Vigna radiata*).	(Hossain et al., 2012)
Aq. MLE	5–10% (*w*/*v*)	Inhibitory effects broad beans (*Vicia faba*) and chickpea (*Cicer arietinum*).	(Khan et al., 2017; Mangal et al., 2013);
Aq. MLE	1:10 (*w*/*v*)	Reduced germination and survival of cowpea (*Vigna unguiculata*) and groundnuts (*Arachis hypogaea*).	(Phiri & Mbewe, 2010)
		Vegetable, Fruit, and Other Crops	
Aq. MLE	0.45–0.90 $mg \cdot mL^{-1}$	Reduced germination and seedling growth of wild mustard (*Synapsis arvensis*).	(Tahir et al., 2018)
Aq. MPE	>10% (*v*/*v*)	Inhibited shoot and root length of curly/garden cress (*Lepidium sativum*).	(Perveen et al 2021)
Aq. MPE	100 (*v*/*v*)	Inhibited germination of okra (*Abelmoschus esculentus*).	(Waris et al., 2024)
Methanol MPE	0.03, 0.01, and 0.1 $g \cdot mL^{-1}$	Inhibited the growth of lettuce (*Lactuca sativa*) and curly cress (*Lepidium sativum*).	(Piyatida & Kato-Noguchi, 2010)
Aq. MLE	25 $mg \cdot mL^{-1}$	Reduce yield of eggplant (*Solanum melongena*) and sweet corn (*Zea mays*).	(Vélez-Gavilán, 2022)
Aq. MLE	2.5–10% (*w*/*v*)	Suppression of germination and growth of watermelon (*Citrullus lanatus*).	(Ahmed et al., 2019)
Ethanol MLE	20–25 $g \cdot mL^{-1}$	Inhibited germination and growth of tomatoes (*Solanum lycopersicum*).	(Vyas & Sharma, 2021)

MLE = moringa leaf extracts, MRE = moringa root extracts, Aq. = aqueous extracts, ethanol = ethanolic extracts, and methanol = methanolic extracts.

2.5.1. In Leguminous Plants

The effect of MPE has been described as hormesis (Perveen et al., 2021) i.e., higher concentrations exhibit growth inhibitory effect by reducing seed germination, early seedling growth, and chlorophyll degradation in several cultivated and non-cultivated plants (El-Rokiek et al., 2022). Although MLE have shown some positive allelopathic effects in various legume crops, MRE (Aq. water extracts) applied at the higher concentration rate of 10% (*v*/*v*) had detrimental effects on mung beans germination, shoot and root growth, resulting in total reduction in dry matter biomass, number of pods, and yield per plant by 40%, 41%, and 43%, respectively (Hossain et al., 2012). The differential effects observed between MLE and MRE in stimulating growth and development of mung beans demonstrate that various parts of the moringa plant may possess distinctive bioactive compounds that render differential physiological and biochemical functions in other plants.

Higher concentration rates of 5% and 10% (Aq. MLE *w*/*v*) had inhibitory effects on broad beans and chickpea, respectively. They reduced seed germination, seedling growth, biomass accumulation, and biochemical components by more than 50%, and recommended that all moringa plants should be rogued out before sowing broad beans and chickpea (Abou-Zeid & El-Darier, 2014; Khan et al., 2017; Mangal et al., 2013). The same authors reported the decreased efficiency in photosynthetic pigments and chlorophyll fluorescence spectra with greater effect on broad beans. Inhibition of growth in recipient plants is associated with oxidative burst caused by phenolic acid overaccumulation that leads to the disruption of cellular respiration and auxin transport system. Damage to cell membranes affects the entire metabolism and physiological process which affects intermembrane ion transport, accumulation and water imbalance,

causing malfunction of cellular activities and eventually inhibit plant growth (Kostina-Bednarz et al., 2023).

Aqueous MPE at the ratio of 1:10 (*w*/*v*) had an inhibitory effect on germination and survival of groundnuts and cowpeas (Phiri & Mbewe, 2010). It lowered the survival rate of cowpeas by 4% and groundnuts by 10%. It also reduced radicle length by 24% and 21% in cowpeas and groundnuts, respectively. Furthermore, the extracts reduced the development of seed hypocotyls by 4% in cowpeas and 66% in groundnuts. Research has shown that MPE contains benzyl isothiocyanate and ferulic acid which impede mitotic activity in apical meristem and disrupts rhizosphere microbial dynamics. This reduces the nodulation and nitrogen fixation capacity in legumes and eventually affects their growth and productivity (Alyssa & Tamara, 2015; Tahir et al., 2020).

2.5.2. In Vegetable, Fruit, and Other Crops

Allelochemicals affect distinct stages of plant growth and development in diverse ways. Some allelochemicals interfere with enzyme activities and other processes that occur during the initial stages of seed germination. For instance, (-)-catechin, a secondary chemical compound in moringa leaves, impeded enzymatic activity including amylase and protease, hindering the mobilization of nutrients essential for germination and seedling growth (Azam et al., 2020). Applying 0.90 $mg \cdot mL^{-1}$ of MLE during sowing reduced the number of seeds germinated and seedling growth of wild mustard than applying 0.45 $mg \cdot mL^{-1}$ (Tahir et al., 2018), indicating a concentration-dependent allelopathic activity.

Allelochemicals, such as caffeic acid, disrupt physiological functions and the balance of plant regulatory hormones involved in seed germination including ABA, GA_4, and auxins

(Xu et al., 2023). These allelochemicals enhance the effects of ABA, a hormone that suppresses germination, by interfering with the signaling pathways and biosynthesis of GA_4 (which promote seed germination) (Zhao et al., 2022).

Aqueous MPE (>10% *v*/*v*) inhibited shoot and root length of garden cress by 38% and 85%, respectively (Perveen et al., 2021). Metabolite analysis and profiling of MLE identified twelve compounds associated with allelopathic effect including p-coumaric acid, salicylic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, and gallic acids which have strong allelopathic effect, i.e., inhibit seed germination and seedling growth of various plant species (Alam et al., 2020; Dessalegn & and Rupasinghe, 2021; El-Rokiek et al., 2017). The bioactive compounds were further analysed in the leaves, seeds, and flowers of moringa, the results showed that MLE were rich in methyl 11,14, 17-eicosatrienoate (13.69%) and octadec-9-enoic acid (27.8%); flower extracts were rich in nonacosane (18.3%), methyl 12, 15-octadecadienoate (17.9%), and 9-octadecenoic acid methyl ester (36.9%); and root extracts were rich in (*E*)- 9-octadecenoic acid, methyl ester (36.94%) and octadec-9-enoic acid (16.66 %) (Tahir et al., 2018).

These chemical compounds enhance the permeability of the cellular membrane, resulting in damage to the protein membranes and their channels. This disrupts the transportation of nutrients to different parts of the plant, which in turn affects its metabolic activity (Cheng & Cheng, 2015). The damaged membranes hinder energy production pathways, decreasing growth and productivity in the majority of plants, specifically biomass accumulation (El-Rokiek et al., 2022). The allelochemical compounds and their respective quantities present in moringa could have exerted an inhibitory effect on the growth, yield, and yield components of garden cress.

Higher concentration of moringa leaf, seed, and flower extracts had a strong bioactive effect on various vegetable crops. For instance, 100% Aq. MLE caused maximum inhibition with (0%) germination, mean germination time, and shoot length of okra (Waris et al., 2024). Similarly, 20–25 $g \cdot mL^{-1}$ alcohol MLE was found to be toxic against germination and seedling growth of tomato plants (Vyas & Sharma, 2021). Some plants deploy advanced mechanisms to counteract harmful toxins or varied amounts of allelochemicals, while other plants are more susceptible to toxin concentrations emitted by donor plants in the soil. The observed germination and growth disorders in these crops can be attributed to allelochemical activity that induce physiological and biochemical malfunction, and hormonal imbalances in different plant species (Effah & Clavijo McCormick, 2024).

Methanol extracts of whole moringa plants at different concentrations, i.e., 0.03, 0.01, and 0.1 $g \cdot mL^{-1}$ had inhibitory effects on hypocotyl and root growth of curly cress and lettuce with concentrations more than 0.03 mL^{-1} exacerbated the effect to 100% (Piyatida & Kato-Noguchi, 2010). The presence of coumarin and cinnamic acids, coupled with other phenolic chemical compounds in moringa might have caused the inhibitory action. These organic acids compromise and hinder seeds from germinating (Marchiosi et al., 2020). They further cause oxidative stress in seeds and seedlings by making ROS, and the accumulation of ROS deters the function of proteins, lipids, and DNA in cells, thereby damaging and compromising seed viability, germination, and seedling growth (Wang et al., 2022).

Moringa has been described as extremely competitive with eggplant and sweet corn varieties with a reduction in yields by more than 50% (Vélez-Gavilán, 2022). A 25 $mg \cdot mL^{-1}$ concentration of Aq. MLE inhibited the growth of eggplant seedlings,

demonstrating the availability of compounds responsible for growth suppression (Vyas & Sharma, 2021). Suppression of seed germination, plumule, and radicle elongation were visible seven days after spraying Aq. MLE in watermelon at the concentration rate between 2.5 and 10% (*w*/*v*) (Ahmed et al., 2019). Harmful allelochemicals and light blocking are suggested to compromise and causes breakage in membrane which in turn slow down germination and seedling growth. Plant cell membranes play important roles in keeping the balance of water and nutrients and controlling the transport of molecules in plants (Gill et al., 2021). Allelochemicals break down cell membranes during initial stages of seedlings growth, causing leakage of cellular contents, this leads to the loss in cell integrity. This phenomenon compromises important physiological and biochemical processes such as osmotic regulation, ion transportation, and metabolic activities, which in turn affect seed germination, seedling growth, and the ability of plants to deal with stress factors in the environment (Cheng & Cheng, 2015).

2.6 Potential Allelopathic Effect of Moringa on Weeds

Weed management is one of the major concerns in sustainable crop production systems. Weeds cause significant losses in agriculture by competing with crops for essential resources including soil nutrients, light, water, and air. This has prompted farmers to use synthetic herbicides to control weed species. However, continued and heavy herbicide usage raises concerns over human health and the environment (Chandini et al., 2019). Hence, researchers and farmers have been exploring alternative environmentally friendly and effective solutions to control weeds including the use of plant extracts. Recent studies have demonstrated that some plant species possess potent allelochemicals that can be screened for potential and ecofriendly natural herbicides to control weeds (El-Rokiek et al., 2017; Kostina-Bednarz et al., 2023). Moringa has emerged as one of the plant species

with allelopathic potential due to its diverse of secondary metabolites including phenolic acids, flavonoids, glucosinolates, and isothiocyanates (Gill et al., 2021). Through MPE application, these have demonstrated significant bioactivity against broad range of weed species such as and lesser canary grass (*Phalaris minor*), jungle rice grass (*Echinochloa colona*), weed beet (*Beta vulgaris*), and wild poinsettia (*Euphorbia heterophylla*) (Gill et al., 2021; Oluwafemi, 2014).

Moringa leaf extracts have shown a strong herbicidal effect on some weed species. For example, applying MLE at lower concentration rates suppressed seedling growth of wild poinsettia, while increasing concentration from 5 g·100 mL^{-1} to 20 g.100 mL^{-1} inhibited seed germination of the same species (Oluwafemi, 2014). The results may indicate the availability of bioactive compounds with bio-herbicidal properties. Other studies have reported that a concentration of 5% (*v*/*v*) of Aq. MLE reduced the growth of both weed beet (broadleaf weed) and lesser canary grass, while at higher concentrations (>10%), it significantly reduced the growth of jungle rice grass (grassy weed) (Gill et al., 2021). Researchers found different quantities of polyphenols and flavonoids in leaf extracts, which might have played a major role in inhibiting seedling growth (Lopez-Rodriguez et al., 2020).

Moringa water extracts used in combination with carrot grass/weed (*Parthenium hystorophorus*) and hemp (*Cannabis sativa*) extracts (3% *v*/*v*) significantly suppressed weeds when compared with a distilled water control in a wheat-maize cropping system. This suggests that moringa extracts can be used synergistically with other plant extracts for effective weed control in agriculture. This treatment reduced leaf number, leaf length, shoot length, chlorophyll content, and photosynthetic rate in all tested weeds and the effect was linked to phenolic compounds (Lopez-Rodriguez et al., 2020). Regarding the

potential mode of action, exogenous phenolic compounds can penetrate cell membranes and accumulate within the cell disrupting enzymatic activity, hormone signalling, antioxidant systems, respiration and photosynthetic activity among other vital plant functions (Hossain et al., 2012; Kumar et al., 2024).

2.7 Conclusion and Future Prospectives

Moringa plant extracts possess biochemical compounds that can positively and negatively influence the growth and development of various crops and weed species. Based on the findings of this review, the stimulatory properties of moringa allelochemicals, such as enhanced stress tolerance and growth promotion, can be utilised or applied in the development of biofertilizers or biostimulants. Conversely, moringa's allelochemical inhibitory effects on seed germination and seedling growth can be harnessed for the development of bioherbicides for sustainable weed management. Despite moringa allelochemicals possessing dual allelopathic roles, there is a lack of research exploring the underlying physiological and molecular mechanisms. Therefore, future studies should focus on how biochemical pathways and gene expression are influenced by moringa extracts to better understand their mode of action and optimize their application in sustainable agriculture production.

Several gaps in the literature were identified. For instance, there is little information on the effect of moringa allelochemicals on temperate pasture species like perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*). Also, effects on native, endemic, ornamental, managed tress i.e., pines, and naturalised plants that are not cultivated are poorly explored. Additionally, most studies on allelopathic impacts of moringa were found to involve leaf extracts with fewer studies exploring extracts from other parts of the plant such as bark, flowers, or roots.

Investigating the allelopathic effects of root extracts and exudates can be of particular interest given that roots are potential carriers of numerous bioactive compounds (Mangal et al 2013), and these compounds can be released into the rhizosphere influencing the growth and development of nearby species. More studies to characterise root-specific metabolites and laboratory and field trials to investigate their effects on other crops, native species and weeds, and the mechanisms behind the observed direct impacts are recommended.

The indirect impacts of moringa extracts are poorly documented, and future research should also concentrate on how moringa allelochemicals may affect other plants indirectly through changes in soil properties, plant-insect, and plant-microbe interactions. Additional research could also explore how environmental stress factors (such as drought or extreme temperatures) influence allelochemical production and activity in moringa, given that such factors are known to impact the production and release of secondary metabolites and the ecological interactions these mediate (Bhuker et al., 2023; Clavijo-McCormick et al., 2023).

This review has summarised key findings and identified avenues for future research; however, as the body of the literature develops, controlled trials to explore moringa's allelopathic potential conducted before its introduction into a new ecosystem or using its extracts on other plant species as biofertilizers or biopesticides are recommended and encouraged.

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We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Blair Moses Kamanga
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In which chapter is the manuscript/published work? 3

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

B.M.K. and A.C.M developed the main idea for the manuscript, conducted literature search, and wrote the original draft. D.L.C., A.C.M., and C.M., as doctoral supervisors of B.M.K., provided significant input and editing. All authors read and agreed to the published version of the manuscript.

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Chapter 3

Cold stress responses and adaptation mechanisms in *Moringa oleifera* Lam.: A metabolite-centred review

Moringa plants in a plant growth chamber at 4°C. Photo credits: ***Blair Moses Kamanga***

This chapter reviews the adaptation mechanisms of moringa and explores the coordinated roles of primary and secondary metabolites in response to cold stress in plants. It also summarises the breeding programs for cold tolerant cultivars of moringa and identifying research gaps for future research. The chapter is presented in the style of the journal *Plants* with minor structural modifications. The chapter was published to the journal *Plants* as:

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Abstract

Moringa oleifera Lam. (moringa) is a desirable crop for intensive cultivation due to its multiple use in human and animal nutrition, medicine, and ecological applications. Its resilience and adaptability to various environmental conditions make it an attractive option for farmers seeking alternative cash crops that can thrive in challenging agricultural environments. While its resilience is well documented in tropical and subtropical climates, limited information exists on its growth dynamics and adaptation mechanisms to prolonged cold stress, which constrains its expansion and cultivation in temperate regions. This review synthesises current knowledge on cold stress adaptation mechanisms and coordinated functional roles of primary and secondary metabolites in response to cold stress in plants. Although considerable progress has been made in understanding morphological adjustments in moringa plants, limited attention has been given to elucidating the physiological, metabolic, and genetic regulatory mechanisms underlying the adaptive responses to cold stress. Moreover, despite the potential roles of primary and secondary metabolites in coordinating protective functions against cold stress in plants, specific metabolites and their functional roles against cold stress remain insufficiently characterised in moringa. While genetic improvement and selective breeding have improved key agronomic traits, including growth rate, biomass yield, and nutritive value, progress in breeding for enhanced cold stress tolerance remains insufficiently explored. Future studies should focus on integrative metabolite profiling, identification, and selection of cold-tolerant provenances to aid in the development of cold-tolerant gene pools to broaden the cultivation range to temperate regions.

Keywords: Antioxidative defence, cold stress, growth dynamics, hormonal regulation, metabolites, morphological traits, physiological adaptation

3.1 Introduction

Low temperature is a critical abiotic stress factor that compromises plant growth and limits their geographical distribution (Devireddy et al., 2021; Hatfield & Prueger, 2015). For plants that grow well in tropical and sub-tropical environments, low-temperature stress encompasses both chilling (0–15°C) and freezing stresses (<0°C), each of which elicits distinct physiological and biochemical responses (Theocharis et al., 2012). Exposure to low temperature induces morphological, physiological, molecular, biochemical, and hormonal alterations that disrupt normal plant function, growth and productivity (Muhl et al., 2011; Ntsangani, 2018; Soares et al., 2023). Cold stress injury not only causes a reduction in crop productivity and quality in cultivated crops but also affects morphogenesis and may induce cold acclimation (Gismondi et al., 2023; Soualiou et al., 2022). Cold acclimation refers to an increased tolerance to freezing that develops through repeated exposure to low, non-freezing temperatures and involves coordinated physiological, morphological, biochemical, and cellular reconfigurations (Ritonga et al., 2020; Hasanuzzaman et al., 2013; Gismondi et al., 2023).

While plants from tropical and subtropical environments are typically sensitive to cold stress and lack cold acclimation capacity, temperate species are tolerant to seasonal climatic fluctuations and easily adapt to cold stress (Ritonga & Chen, 2020; Hasanuzzaman et al., 2013; Yavas et a., 2023). Cold adaptation mechanisms can be broadly classified as either avoidance (i.e., transpiration cooling, stomatal closure, leaf orientation, and early maturation) or tolerance (i.e., altered transcripts, free radical scavengers, and the production of osmo-protectants) (Shanmugavel et al., 2021).

Moringa oleifera Lam. (moringa) which belongs to Moringaceae family, is one of the most cultivated and versatile tropical plants that is adapted to a wide range of

environments (Palada et al., 2019). Moringa is highly valued for its nutritional and medicinal uses for humans, and its leaves, flowers, seeds, and roots are utilised in agriculture for livestock feed and in natural resource management (Bancessi et al., 2020). Its resilience and adaptability to various environmental conditions make it an attractive option for farmers seeking alternative cash crops in different agricultural systems including areas outside its natural range (Kamanga et al., 2025).

The species' worldwide distribution and adaptation to various agroecological zones could be attributed to a wide range of genetic variation and adaptation mechanisms among cultivated and wild provenances (Mutiso et al., 2022; Ondieki et al., 2023). Moringa also produces a wide variety of secondary metabolites with biological activity linked to competitive advantage, stress responses, and defence, allowing it to thrive in new environments (Kamanga et al., 2025). However, the sensitivity of moringa to cold stress poses a major natural barrier for its natural expansion and introduction into temperate regions (CABI, 2022). Hence, this review explores the growth dynamics and adaptative mechanisms of moringa in response to cold stress by integrating research findings from recent studies. It further proposes avenues for future research that can be integrated into the development of cold-tolerant cultivars suitable for temperate environments.

3.2 Literature rationale and review methodology

Although considerable progress has been made in characterising the morphological adjustments of moringa to low temperatures (Muhl et al., 2011; Soares et al., 2023), less effort has been directed toward the mechanisms underlying metabolic, physiological, biochemical and genetic regulation aspects. This knowledge gap constrains a holistic understanding of integrative and coordinated strategies that enable moringa to perceive, respond to, and tolerate cold stress. Therefore, multidisciplinary studies to bridge

morphological, physiological, and biochemical regulations are essential to address the following research questions: what are the growth dynamics of moringa and its adaptation mechanisms to cold stress? What information is needed for effective breeding programs for cool-tolerant cultivars? Does moringa possess coordinated primary and secondary metabolites that are responsible for cold stress adaptation?

To address the above questions, a systematic review was conducted to synthesise recent advances in growth dynamics, adaptative mechanisms, and breeding initiatives associated with moringa. As a complementary line of inquiry, the coordinated roles of primary and secondary metabolites in the plant cold stress response were examined to clarify how metabolically driven adjustments may contribute to the broad ecological distribution of this species. The comprehensive search for relevant scientific literature on moringa across various scientific databases, e.g., Google Scholar, Web of Science, PubMed, and Scopus, was conducted using a combination of keywords, including 'moringa' AND 'adaptation mechanisms', 'moringa' AND 'growth dynamics', 'moringa' AND 'breeding', 'moringa' AND 'genetics', to obtain in-depth information on the current knowledge of various adaptation mechanisms and growth dynamics. Article suitability was determined by evaluating methods aimed at screening titles and abstracts, which have been described as an efficient approach for identifying relevant literature on a specific topic of study (Affengruber et al., 2024).

3.3 Adaptations and potential distributions of moringa in temperate regions

Conflicting reports exist regarding the growth dynamics and climate suitability of moringa, particularly in relation to temperature and precipitation gradients. Earlier studies characterised moringa as adapted to arid and semiarid tropical environments (CABI,

2022), with optimal growth observed between mean temperatures of 12 °C and 40 °C (Roloff et al., 2009). However, emerging evidence indicates a broader thermal tolerance than previously reported. Recent studies have demonstrated that moringa can withstand light frost to −3 °C, whereas some provenances exhibit a freezing tolerance threshold (LT_{50}) of approximately −2.8 °C (Soares et al., 2023).

Conversely, moringa is sensitive to severe or prolonged freezing, with significant tissue injury reported at temperatures below -5°C (NRC, 2006). Supporting evidence indicates that moringa may survive transient frosts between -1°C and -3°C during cooler months but can also endure high thermal extremes of up to 48°C under arid conditions (Bancessi et al., 2020). These contrasting findings emphasise the substantial degrees of ecotypic variation and physiological plasticity within the species. Nevertheless, despite its broad adaptive range, moringa achieves optimal growth, photosynthetic performance, and biomass accumulation with a moderate thermal range of 25–35°C (Palada et al., 2019). However, laboratory experiments have demonstrated the importance of exposure duration; for example, a 10/5 °C Day–night temperature regime sustained for 8 days resulted in unrecoverable injury after a 4-day recovery period (Soares et al., 2023).

Previous studies provide a quantitative threshold for freezing injury but should be interpreted cautiously since tolerance varies with growth stage and experimental conditions (Kandilli et al., 2025). For example, seedlings are more frost-sensitive whereas established plants may tolerate short temperature declines due to greater structural protection and carbohydrate reserves (Richetti et al., 2025). Cellular damage in moringa under freezing conditions could be due to the extracellular ice formation and cellular dehydration, which disrupt membrane integrity and metabolic activity as shown for other tropical species such as oil palm species (*Elaeis guineensis* Jacq.) (Song et al., 2025),

nevertheless, recent studies suggest that moringa shows little or no capacity for cold acclimation (Soares et al., 2023).

In New Zealand, studies on climate suitability have identified the top of the North Island, i.e., Northland, and the surrounding microclimates as suitable for the introduction and cultivation of moringa (Kamanga et al., 2025. These locations are characterised by a mean annual temperature of approximately 25 °C, with annual precipitation ranging from 2000 mm to 2700 mm (NIWA, 2023). These conditions align closely with the optimal thermal and hydric requirements previously reported for moringa establishment and cultivation, where temperatures ≥ 25 °C and rainfall exceeding 1000 mm promote vigorous growth and sustained physiological performance (Palada et al., 2019; Haile and Ayansa, 2022). This climatic congruence between these North Island zones and moringa's native or naturalised habitats demonstrates the species' potential adaptability to subtropical environments.

3.4 Adaptation mechanisms of moringa in cold stress environments

As sessile organisms unable to escape adverse conditions, plants depend on a suite of integrated adaptation mechanisms to withstand harsh environmental conditions including cold stress. These mechanisms encompass coordinated physiological, morphological, anatomical, molecular, biochemical, hormonal, and genetic responses that enhance plant tolerance to low temperatures (Figure 3) (Chelli-Chaabouni, 2014; Jalal et al., 2021; Raza et al., 2023; Aftab et al., 2025; Satyakam et al., 2022; Takahashi et al ., 2021; Tian et al., 2016; Wany et al., 2018). Physiologically, plants mitigate freeze-induced cellular damage through antifreeze proteins, osmotic adjustment through compatible solute accumulation, and structural modifications such as thicker cuticles, altered leaf morphology, and insulated root systems (Muhl, 2011; Roychowdhury et al., 2025; Zahra et al., 2021). At

molecular and biochemical level, cold stress triggers the synthesis of cryoprotective metabolites, activation of antioxidant defences, remodelling membrane lipid composition to maintain fluidity, and induction of stress-responsive proteins including dehydrins and late embryogenesis abundant (LEA) proteins (Raza et al., 2023; Roychowdhury et al., 2025; Hundertmark and Hincha, 2008; Hussain et al., 2018).

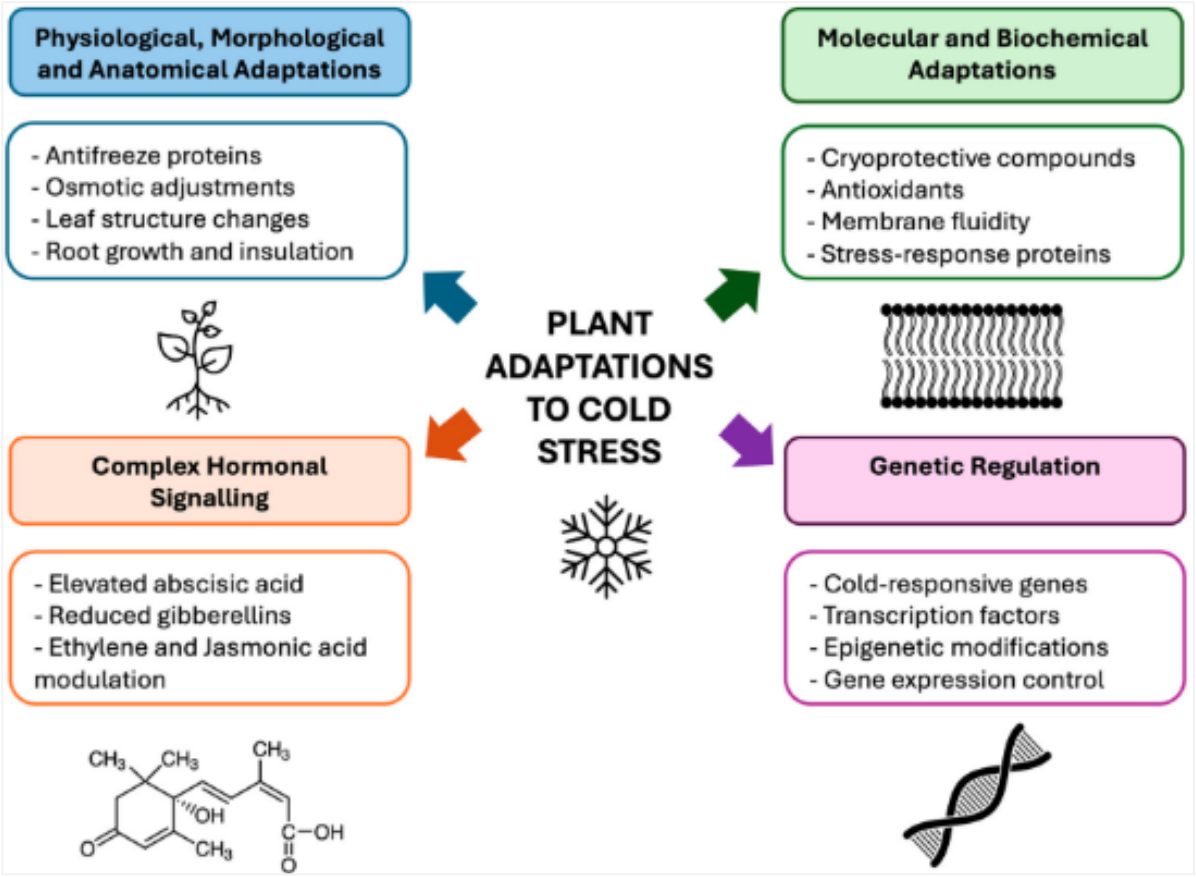

Figure 3. Integrated mechanisms underlying cold adaptation in plants. The diagram illustrates the multi-layered responses plants employ to perceive and withstand low-temperature stress. Physiological, morphological, and anatomical changes including antifreeze protein accumulation, osmotic adjustment through compatible solutes, alterations in leaf structure, and root system insulation help to maintain cellular stability during freezing conditions. Molecular and biochemical adaptations involve the production of cryoprotective metabolites, activation of antioxidant systems, adjustments in membrane lipid composition to preserve fluidity, and induction of stress-responsive proteins such as dehydrins. Cold-induced shifts in hormonal balance, i.e., elevated abscisic acid (ABA), reduced

gibberellins (GA), and modulatory roles of ethylene and jasmonic acid (JA), coordinate growth restraint with activation of defense pathways. These processes converge under genetic regulation which is mediated by calcium-dependent signalling cascades, transcription factors, cold-responsive (COR) gene expression, and epigenetic modification that improves transcriptional responses to cold stress.

These processes are strongly integrated with shifts in hormonal homeostasis, where increased abscisic acid (ABA) promotes cold-responsive gene expression and stomatal regulation, reduced gibberellin (GA) levels suppress growth to conserve energy, and ethylene and jasmonic acid (JA) modulate cross-talk among stress pathways (Hu, 2024). Ultimately, cold acclimation is governed by complex genetic regulation involving calcium-mediated signalling cascades, activation of C-repeat/dehydration-responsive element binding factors (CBF/DREB), expression of cold-responsive (COR) genes, and the epigenetic modifications that adjust chromatin structure to improve stress-responsive transcriptional processes (Gismondi et al., 2023; Raza et al., 2023; Diao et al., 2020; Guo et al., 2018; Kong et al 2024; Ma et al., 2022).

3.4.1 Physiological, morphological, and anatomical mechanisms

Plants possess various morphological adaptation mechanisms in response to cold stress through phenotypic plasticity, such as reduced leaf area, thicker cuticles, and increased root-to-shoot ratios (Gratani, 2014). These mechanisms minimise energy loss, mitigate freezing damage, and maintain productivity. Stomatal modification is an anatomical defence mechanism against cold stress aimed at promoting water use efficiency and stress tolerance (Kamanga et al., 2018; Soltys-Kalina et al., 2016). In moringa, the number of stomata per unit leaf area was significantly greater at lower temperatures (10/20 °C; day/night temperatures) than at 20/30 °C; although the stomata were larger at higher

temperatures. The lower stomatal density at 20/30 °C resulted in a reduction in leaf conductance (Muhl et al., 2011). The decline in leaf conductance limits the diffusion of CO2 into the leaf mesophyll for photosynthesis, reduces the catalytic activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and consequently compromises plant growth and productivity (Zhang et al., 2014).

Leaves of moringa plants grown under a low-temperature regime of 10/20 °C and a 10 °C decrease in day and night temperature presented an average increase in leaf thickness of 43.1%, i.e., from 0.14 mm to 0.24 mm (Muhl et al., 2011). Plants grown at 10/20 °C also presented more spongy mesophyll tissue and longer palisade cells than did the leaves of plants grown at 20/30 °C (Muhl et al., 2011). Plants modify their leaf structure in response to cold stress to stimulate tolerance. They exhibit specific morphological and anatomical adaptations, such as increased palisade thickness, increased tightness of the tissue structure, and an increased palisade spongy mesophyll tissue ratio, to minimise the effect of chilling stress (Wu et al., 2020; Zeng et al., 2020). Similar trends have been observed in other plants, e.g., Mastic tree (*Pistacia lentiscus* L.) (Stefi et al., 2022), and Shearer's Phoebe (*Phoebe sheareri* Hemsl.) (Shi et al., 2023). Modifications in leaf anatomy across plant species provide evidence of how plants adapt to and thrive in temperate regions.

The thickening of leaves observed in moringa serves as a protective mechanism to minimise water loss and maintain cellular integrity under cold conditions. For example, exposing moringa plants to 15/10 °C for 96 h increased leaf thickness by 17.3%, with elongated and swollen spongy mesophyll cells (Ntsangani, 2018), while the increase in palisade thickness suggests that plants allocate more resources to photosynthetic tissue in

response to chilling stress. Plants usually increase the efficiency of photosynthesis by minimising the damaging effects of photoinhibition at lower temperatures (Yadav, 2010).

Cold stress compromises the integrity of the thylakoid membrane in tropical plant species, which is the primary site of electron transport and photochemical reactions. This results in reduced membrane fluidity, impaired photosynthetic efficiency, and increased electrolyte leakage due to oxidative stress (Bhattacharya, 2022). Moreover, cold-stressed plants exhibit a reduction in the contents of chlorophyll a and b, which are key pigments essential for light absorption and energy conversion during photosynthesis. This reduction is indicative of broader disruption of photosynthetic function and overall physiological impairment (Kita et al., 2024; Ritonga & Chen, 2020). In moringa plants, cold stress at 15/10 °C and 10/5 °C decreased the maximum quantum yield of photosystem II (Fv/Fm) by 28.7% and 73.7%, respectively, with full recovery after one day of exposure under both temperature regimes (Soares et al., 2023). Nevertheless, prolonged exposure of the plants for four days exacerbated the effect, with reductions of 32.2% and 87.7% at 15/10 °C and 10/5 °C, respectively, without recovery. The reduction in Fv/Fm at 10/5 °C may indicate the failure of moringa provenances to adapt to or defend against cold stress over long exposure periods.

3.4.2 Molecular and biochemical adaptation mechanisms in moringa

Cold stress influences the production and accumulation of primary and secondary metabolites to counteract the effects of cold stress, which disrupts biochemical and hormonal processes in plants (Yadav, 2010). Research has shown that plants respond differently to cold stress on the basis of species type and stress-dependent effects. For example, exposing moringa plants to 15/10 °C for 48 and 96 h resulted in increases in phenolic contents of 21.6% and 26.5%, respectively (Ntsangani, 2018). Similarly, in

grapes (*Vitis vinifera* L.), more tolerant cultivars were characterised by higher contents of phenolic compounds and better radical-scavenging capacity (Król et al., 2015). This type of response is indicative of an adaptation strategy, as phenolic compounds modulate the activity and expression of key antioxidant enzymes to increase the cellular redox balance under cold stress (Hayat et al., 2010). Nevertheless, specific metabolites responsible for cold acclimation and tolerance in moringa have yet to be characterised.

The accumulation of non-structural carbohydrates, phenolics, and proteins in plants has been previously reported as a response mechanism to cold stress. For example, in Jatropha (*Jatropha curcas* L.) which belongs to family Euphorbiaceae, the accumulation of soluble sugars, including starch, maltose, sucrose, glucose, galactinol, and raffinose, was observed at 12 °C (6–48 h) (Haibo Wang et al., 2018). These sugars function not only as osmoprotectants and energy reserves but also as signalling molecules that regulate stress-responsive gene expression to increase membrane stability and mitigate oxidative damage (Sengupta et al., 2015). Cryoprotective sugar alcohols, including sorbitol, ribitol, and inositol, are known to accumulate under cold stress conditions; these polyols protect cellular structures through maintaining membrane fluidity, scavenging ROS, and reducing the risk of freeze-induced injury in plant leaves (Tak et al., 2023).

Contrasting patterns have been observed regarding proline and carbohydrate accumulation under elevated temperature conditions (Mohammed et al., 2021), with earlier findings indicating an increase in phenolic contents with decreasing temperature (Ntsangani, 2018). Several studies have shown that exposure to abiotic stresses such as cold induces the accumulation of polyamines such as spermidine, spermine, and putrescine in rapeseed (*Brassica napus* L). (Jankovska-Bortkevič et al., 2020). However, there is insufficient information on the changes in polyamines in moringa plants that

survive light frost. In general, the total soluble sugar, free amino acid, proline, phenolic, and flavonoid contents of leaves increase when moringa plants are exposed to abiotic stress conditions as survival mechanisms (Mohammed et al., 2021).

3.4.3 Complex hormonal balance as an adaptation mechanism

Most plants, including moringa, contain numerous phytohormones, such as abscisic acid (ABA), auxins, cytokinins, and gibberellins (Hemasundar Alavilli et al., 2022; Bharath et al., 2021; Raza et al., 2023). As a key stress-responsive phytohormone in plants, ABA coordinates complex regulatory networks that enable plants to perceive and adapt to cold-induced cellular dehydration and oxidative stress (Kane & McAdam, 2023). During cold stress, ABA functions as a regulatory hormone to increase cold tolerance through the regulation of stress-responsive genes, the accumulation of protective metabolites, and increased membrane integrity (Bharath et al., 2021; He et al., 2023; Kane & McAdam, 2023).

Elevated ABA levels activate the expression of cold-responsive (COR) and late embryogenesis abundant (LEA) genes to produce antifreeze proteins and osmoprotectants that stabilise cellular structures and cold-induced dehydration (Hundertmark & Hincha, 2008; Mansoor et al., 2019). For example, studies in common beech (*Fagus sylvatica* L.) (Fagaceae) have shown that ABA mediates stomatal closure to reduce cuticular transpirational water loss and stimulates antioxidant defense systems that mitigate oxidative damage (Kane & McAdam, 2023). However, the level of ABA in moringa provenances that survive brief periods of chilling and light frost stress down to −2 °C (Soares et al., 2023) has not been investigated to establish its adaptive course. Therefore, further studies are needed to establish the role of ABA and other phytohormones in moringa in relation to cold tolerance.

Plant hormones such as ABA, auxins, cytokinins, and ethylene play important roles in regulating the expression of NAC transcription factors [No Apical Meristem (NAM); Arabidopsis transcription activation factor (ATAF1/2); and cup-shaped cotyledon (CUC2)] (Diao et al., 2020). These transcription factors directly or indirectly regulate plant gene expression in response to developmental and environmental stress (Diao et al., 2020). The presence of ABA in moringa (Arif et al., 2023) could be associated with NAC transcription factors, which enable the plant to tolerate various environmental conditions, including cold-induced stress. Both ABA and NAC transcription factors are widespread in plants (Cao et al., 2017), suggesting that these may play a role in moringa responses to cold stress.

3.4.4 Genetic regulation as an adaptation mechanism

Plants synthesise and produce large numbers of microRNAs (miRNAs) known to control a variety of plant functions in response to biotic and abiotic stressors (Islam et al., 2023). These noncoding RNAs regulate the expression of genes in eukaryotic cells (21–24 nucleotides), are transcribed as primordial miRNAs, and are precursors of RNAs by RNA polymerase-II inside the nucleus (O'Brien et al., 2018). Research also suggests that plants subjected to different environmental stress factors, including cold, synthesise stress-responsive proteins to increase their survival under adverse conditions (Gismondi et al., 2023; Diao et al., 2020). The induction of miR171d and miR2118a in moringa plants exposed to cold stress in dark conditions suggests a genetic component of the cold stress adaptation mechanism since these miRNAs are known to regulate plant gene expression in response to various abiotic stimuli (Gismondi et al., 2023; Lu et al., 2020; Yuan et al., 2016).

Furthermore, the elevated expression levels of specific miRNAs in moringa leaves and callus tissues exposed to cold stress suggest that the activation of post-transcriptional regulatory pathways enhances stress tolerance (Pirrò et al., 2019). The ability of moringa to thrive and adapt across diverse climatic environments demonstrates its underlying genetic variability, which may account for the differential adaptive mechanisms observed among the provenances and their associated effects on relative growth rates under varying environmental conditions (Mridha, 2015; Muhammad et al., 2016).

Most plants survive and reproduce under adverse temperatures because NAC factors are among the adaptation mechanisms for survival. They play essential roles in various stress responses, including low temperature, drought, and pathogen attack (Liu et al., 2023). These gene families form a complex regulatory network that modulates the expression of stress-responsive genes, including those involved in detoxification, osmotic regulation, and antioxidant defense, to optimise plants' chances of survival under stress conditions (Liu et al., 2023). For example, gene expression (NAC) was evident in *Madhuca longifolia* (L.) J.F.Macbr plants (Want et al., 2024) and alfalfa (*Medicago sativa* L.) (Ye et al., 2024) under cold stress.

Meta -analyses have demonstrated that overexpression of NAC transcription factors, i.e., PbeNAC1 (*Pyrus betulifolia* NAC1) and SlNAC (*Solanum lycopersicum* NAC), enhances cold tolerance by activating cold-responsive regulatory pathways that lead to improved cell membrane stability and increased scavenging of ROS under low-temperature stress (Chen et al., 2025; Figueroa and Gómez, 2022). The ability of certain moringa provenances to survive cold stress provides evidence of the existence of genotypes with desirable traits that could be enhanced through breeding and utilised for cultivation in temperate regions.

3.5 Coordinated functional roles of metabolites in response to cold stress in plants

Plants native to arid and semiarid tropical environments are susceptible to cold stress (chilling at 0–15 °C or freezing at <0 °C). Cold stress causes membrane rigidification in plant cells, which has been described as a primary biophysical alteration that triggers downstream signalling cascades (Devireddy et al., 2021). Initially, there is a transition phase that compromises membrane fluidity and affects embedded proteins; this disrupts enzymes and leads to photoinhibition, membrane damage, and reduced photosynthetic capacity in most plant species (Rácz et al., 2023). At the molecular level, chilling stress interferes with gene expression and translation by promoting the formation of RNA secondary structures (Raza et al., 2023).

When freezing stress is more severe, it can cause lethal and physical damage to plant organs and tissues. Freezing injury begins with the nucleation of extracellular ice, which induces cellular dehydration and mechanical injury, resulting in plant death (Ding et al., 2019). Metabolic shifts in response to environmental stressors represent strategic physiological, biochemical, and hormonal reconstitutions aimed at increasing the survival rate and productivity. As sessile organisms, plants deploy various adaptation mechanisms associated with complex interactions between primary and secondary metabolism. (Mushtaq et al., 2020). Figure 4 illustrates the coordinated roles of primary and secondary metabolite-driven pathways culminating in enhanced cold tolerance in plants. These processes could be linked and contribute to the adaptive responses of moringa plants to environmental stressors including low-temperature stress by supporting cellular protection and physiological stability.

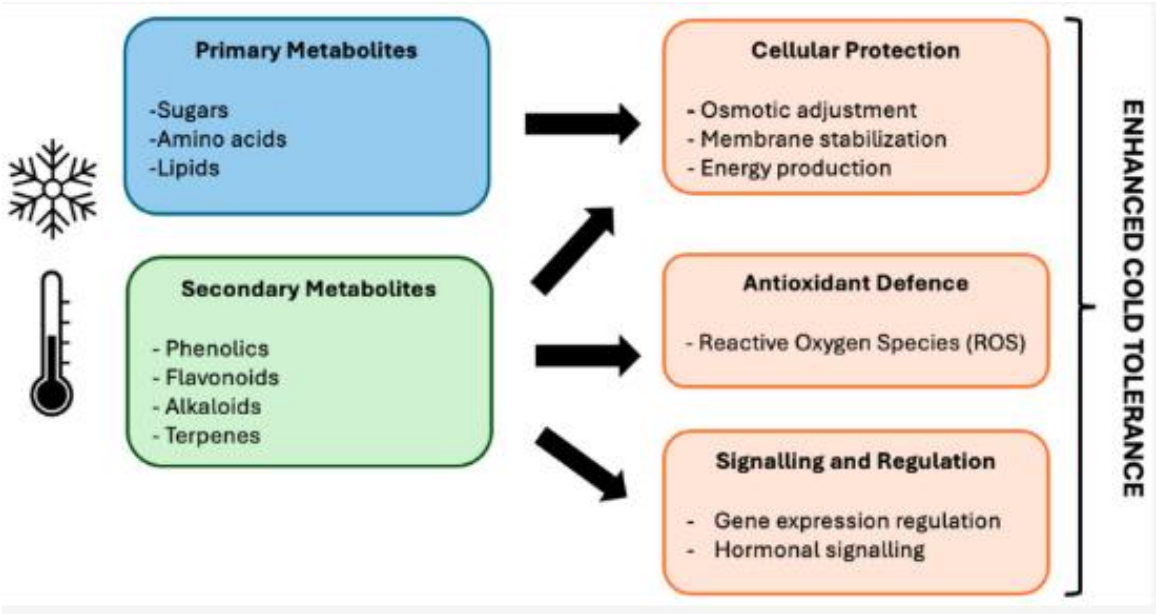

Figure 4. Schematic illustration of coordinated roles of primary and secondary metabolites in plant cold stress responses. Primary metabolites support osmotic adjustment, membrane stabilisation, and energy balance, while secondary metabolites contribute to antioxidant defence and signalling functions to enhance cold tolerance in most plant species.

3.5.1 Coordinated functional roles of primary metabolites in the response to cold stress

The accumulation of soluble sugars is one of the adaptation mechanisms that plants use to survive cold stress (Ritonga & Chen, 2020). Low temperatures induce rapid accumulation of soluble carbohydrates, including glucose, sucrose, fructose, and maltose, which are all essential primary metabolites (Salam et al., 2023). They perform multiple functions as compatible osmolytes, cryoprotectants, scavengers of ROS, and signalling molecules (Hajihashemi et al., 2018; He et al., 2023). These sugars not only function as osmolytes but also stabilise membranes and proteins to protect cellular structures during freeze–thaw cycles. They provide the energy required for various metabolic functions,

such as respiration, photosynthesis, and the transportation of molecules, under extremely cold conditions (Bhattacharya, 2022).

The accumulation of soluble carbohydrates such as disaccharides, e.g., trehalose and sucrose; oligosaccharides such as raffinose and stachyose; and polymers of fructose molecules, has been linked to increased freezing tolerance in various temperate species such as Jatropha seedlings (Wang et al., 2018). These carbohydrates, together with their corresponding metabolic enzymes, play essential roles as compatible osmolytes and scavengers, enabling plants to maximise their productivity under cold stress (Hajihashemi et al., 2018; Kita et al., 2024).

Cold stress delays plant growth due to cell death or cellular injury; this decreases membrane integrity, causes malfunctions in antioxidant defences and enzyme activities, and modulates hormonal signalling responses (Raza et al., 2023; Zahra et al., 2021). This results in the low production of energy required for plant growth and development. However, primary metabolites such as proline and γ-aminobutyric acid (GABA) accumulate during cold stress conditions to help with ROS detoxification, redox buffering, and osmotic adjustment (Ghosh et al., 2022; Singh et al., 2024). Moreover, GABA accumulates to provide an alternative pathway for energy production and the modulation of intercellular pH and signalling (Yu et al., 2023).

In other plants, cold stress has been shown to induce the accumulation of GABA and proline. For instance, in *Medicago ruthenica* (L.) Trautv. (Li et al., 2022) and tea plants (*Camellia sinensis* L) (Wang et al., 2023), these metabolites contribute to osmotic regulation, stabilisation of cellular structures, and mitigation of oxidative damage (Singh et al., 2023; Wang et al., 2023). Although such responses have not been extensively characterised in moringa, similar metabolic adjustments may contribute to its

physiological response to cold stress and play a significant role in maintaining cellular homeostasis under adverse environmental conditions. These findings further suggest that moringa plants maintain cellular homeostasis during cold stress due to increased synthesis of primary metabolites, indicating a conserved energy strategy for adaptation.

3.5.2 Coordinated functional roles of secondary metabolites in response to cold stress

While primary metabolites are involved in plant growth activities, secondary metabolites are synthesised as defense and plant communication mechanisms under various stressors (Clavijo-McCormick et al., 2023). Secondary metabolites such as flavonoids, alkaloids, and terpenoids are regulated under cold stress, function as antioxidants and play regulatory roles in adaptation mechanisms (He et al., 2023; Sana et al., 2025). This type of adaptation mechanism involves the synthesis and production of complex chemical compounds that contribute to the structural function of various metabolic processes for plant survival and productivity.

The cold-induced accumulation of phenolic acids (gallic, caffeic, protocatechuic, and chlorogenic acids) in plant cells decreases the freezing point, maintains water potential, and protects the cells from bursting (Tank et al., 2021). The deposition of phenolic compounds into the cell wall matrix through increased lignification due to cold stress has been thoroughly reviewed (Naikoo et al., 2019). In moringa, cold stress may stimulate the accumulation of phenolic compounds and increased lignification within the cell wall matrix, which can contribute to structural reinforcement and protection against cellular damage. The subsequent thickening of the cell wall strengthens its mechanical properties to mitigate freeze-induced dehydration and cellular distortion. This structural reinforcement is a critical component of the overall freezing tolerance mechanism of

plants. Additionally, flavonoids such as quercetin, kaempferol, and anthocyanins mitigate photoinhibition by filtering excess light and harmful ROS to protect photosystem integrity through cold-induced stomatal closure under altered light conditions (Wang et al., 2024).

The dynamic adjustment of terpenoid metabolism and accumulation in plants is due to its role in cold stress tolerance through various protective mechanisms, such as membrane stabilisation, antioxidant activity, and hormonal signalling (Kong et al., 2024). However, the mechanisms behind this accumulation still lack a systematic explanation. Hence, understanding cold response mechanisms in plants remains complex and ambiguous. Nonetheless, carotenoids, monoterpenoids, and sesquiterpenes function as antioxidants and cell membrane stabilisers in most plants, whereas some terpenoids play crucial signalling roles during cold acclimation (Li et al., 2025). Terpenoids further play a vital role in lipid metabolism and accumulation. For example, the exposure of walnut (Juglans regia L.) plants to cold stress increased the concentration of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Phytol) to mitigate the effects of cold injuries (Terletskaya et al., 2024).

Glucosinolates (GSLs) are characteristic secondary metabolites of moringa that have traditionally been associated with defence against herbivores and pathogens. However, accumulating evidence indicates that glucosinolates also contribute to plant responses to abiotic stresses, including low-temperature stress, through the regulation of redox homeostasis, antioxidant defence, and stress-signalling pathways (Chowdhury, 2022; Sana et al., 2025). Cold stress has been shown to alter glucosinolate biosynthesis and metabolism, resulting in the accumulation of specific glucosinolate compounds that may protect cellular components against oxidative damage and support physiological acclimation. Therefore, characterising glucosinolate responses under chilling conditions

provides valuable insight into the metabolic mechanisms underpinning cold tolerance in moringa, while also identifying metabolites that may serve as potential biomarkers for selecting cold-tolerant germplasm.

Research has shown that plants increase the synthesis and production of GSLs in response to cold stress as an adaptation mechanism (Chowdhury, 2022; Sana et al., 2025). GSLs are a class of specialised metabolites found abundantly in the brassica family, where they contribute significantly to cold stress tolerance by modulating key physiological processes and interacting with other defence pathways (Ljubej et al., 2021). Cold stress alters the balance of GSL types, which in turn affects the overall concentration and expression of genes involved in their synthesis (Tang et al., 2023). Under normal conditions, GSLs tend to be inactive, but they are hydrolysed upon tissue damage due to cold stress caused by the enzyme myrosinase (Ljubej et al., 2021). The hydrolysis of GSLs results in the production of chemical compounds with antioxidant properties that help scavenge the ROS generated during cold stress. However, the mechanisms underlying the accumulation of GSLs in moringa plants in response to cold stress have not been thoroughly studied to understand their specific roles in adaptation mechanisms.

There is a clear interaction between primary and secondary metabolism in adaptation mechanisms to cold stress. For example, carbohydrates are produced and accumulate during primary metabolism and serve as precursors for the synthesis of secondary metabolites to prevent freezing stress (Biswas et al., 2021). While sugar-derived precursors are fed into the phenylpropanoid and terpenoid pathways, amino acid metabolism coincides with polyamine and alkaloid biosynthesis to counteract the effects of cold stress (Kawade et al., 2023; Singh et al., 2024). This phenomenon not only helps plants during stress acclimation but also helps them optimise growth and defense

mechanisms under suboptimal temperatures. The relationship between primary and secondary metabolites in connection with cold stress indicates high metabolic coordination in response to environmental stressors. However, this type of coordination has yet to be characterised in moringa, especially for provenances that have shown brief tolerance to cold.

3.6 Research progress on genetic and breeding programs for cold tolerance in moringa

The genetic patterns and morphological traits of moringa have been studied to select provenances with desirable agricultural traits (Shahzad et al., 2013). Since 1976, the Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR) has been conducting extensive exploration and germplasm collection of selected vegetable crops, including moringa, for improvement of agronomic traits (Bhatt et al., 2022). Global efforts to collect, select, conserve, and exchange moringa germplasm have been a driving force for the identification of elite provenances for breeding programs of new cultivars with yield-related traits (Hemasundar Alavilli et al., 2022).

Additionally, Palada (2017) reported that the World Vegetable Centre has distributed moringa germplasm to research institutions, non-governmental organisations, and private companies in more than 15 countries to promote moringa production. These programs have transformed and contributed to the genetic improvement of major quantitative and qualitative traits of moringa (Balakumbahan & Boopathi, 2021). However, current breeding programs have focused more on yield and its associated quality parameters.

The current genetic diversity assessment of moringa has provided an opportunity to develop various cultivars, including two novel cultivars with high-yielding traits (PKM-1 & PKM-2), for commercial cultivation (Hemasundar Alavilli et al., 2022).

Although moringa is regarded as a “miracle tree” with numerous benefits, the plant remains neglected in breeding for cold tolerance (Saraswathi et al., 2023). Targeted improvement of this trait is essential for supporting species expansion, resilience, and reliable production in temperate environments.

Induced mutagenesis via a low dose of ethyl methane sulfonate (EMS) produced enhanced leaf mutant lines with potential germplasm and functional genomic resources for improving leaf architecture, nutrient yield, and adaptive traits to stressors (Prasanth et al., 2020). While new cultivars have been developed, molecular breeding efforts in moringa remain limited compared with those in other major crops (Boopathi, 2025). For example, advancements in molecular marker development for germplasm characterisation and the application of DNA barcoding in moringa remain minimal (Boopathi et al., 2021; Lakshmidevamma et al., 2021). However, the availability of genomic resources in moringa, such as SSR markers, genome sequence data, and other biotechnological tools (Shyamli et al., 2021; Tian et al., 2015), provides a foundation for breeding new cultivars with desirable traits suitable for cultivation in temperate regions.

3.7 The current knowledge gap and potential research perspective of moringa

Despite moringa exhibiting resilience across a range of abiotic stresses, the mechanistic basis behind this adaptability remains insufficiently characterised. Emerging evidence shows that in most plants; adaptive capacity is mediated through complex reconfigurations of primary and secondary metabolic pathways. These processes enable dynamic adjustments in osmotic balance, energy allocation, and redox homeostasis for plant survival in stressful environments (Aazami et al., 2021; Diao et al., 2020; Jahed et al., 2023; Mansoor et al., 2019; Ritonga & Chen, 2020). While most of these mechanisms

have been extensively studied in other plants through metabolomics analyses, coordinated regulation between metabolic networks and their integration with transcriptional, hormonal, and signalling cascades in moringa remain insufficiently studied. This information, alongside other resources, is critical to inform successful modern cold-breeding programmes in moringa.

Metabolomics (i.e., the systematic study of the unique chemical fingerprints left behind by plant metabolic processes under specific conditions) offers a powerful framework for advancing biochemical research aimed at breeding cold-tolerant crop varieties (Arbona et al., 2013; Lixia Zhou et al., 2025). By profiling metabolites such as soluble sugars, proline, polyamines, and antioxidants under chilling and freezing conditions, metabolomic analyses allow the identification of biochemical markers that consistently distinguish tolerant from sensitive genotypes and thus can be used as robust selection tools in breeding programmes (Bilbrey et al., 2021). Beyond marker discovery, metabolomics also provides mechanistic insights by mapping coordinated changes in primary and secondary metabolic pathways during cold stress, thereby linking observed physiological traits, such as membrane stabilization, reactive oxygen species (ROS) detoxification, and improved osmotic adjustment, to their underlying biochemical networks and regulatory circuits (Roychowdhury et al., 2025).

Comparative metabolite profiling further enables early discrimination between cold-tolerant and cold-sensitive genotypes at seedling or early vegetative stages, significantly accelerating selection compared with reliance on later field-based winter survival data. When integrated with genetic data through QTL mapping, GWAS, or metabolite-QTL analyses, metabolomics strengthens candidate gene discovery by establishing direct links between metabolite signatures and genomic regions controlling cold-tolerance traits (Bilbrey et al., 2021; Tang et al., 2025), thereby enhancing the precision of marker-

assisted and genomic selection strategies. Finally, metabolomic studies that track metabolic reprogramming during cold acclimation, de-acclimation, and re-acclimation provide crucial insights into genotype resilience under increasingly variable winter temperature regimes, informing breeding decisions for climate-adapted cultivars capable of maintaining performance under future environmental fluctuations (Bilbrey et al., 2021; Tang et al., 2025).

In figure 5, we provide a schematic summary of potential mechanisms of cold stress adaptation in moringa, the resources needed to develop effective cold-breeding programmes and how metabolomics can contribute to these efforts.

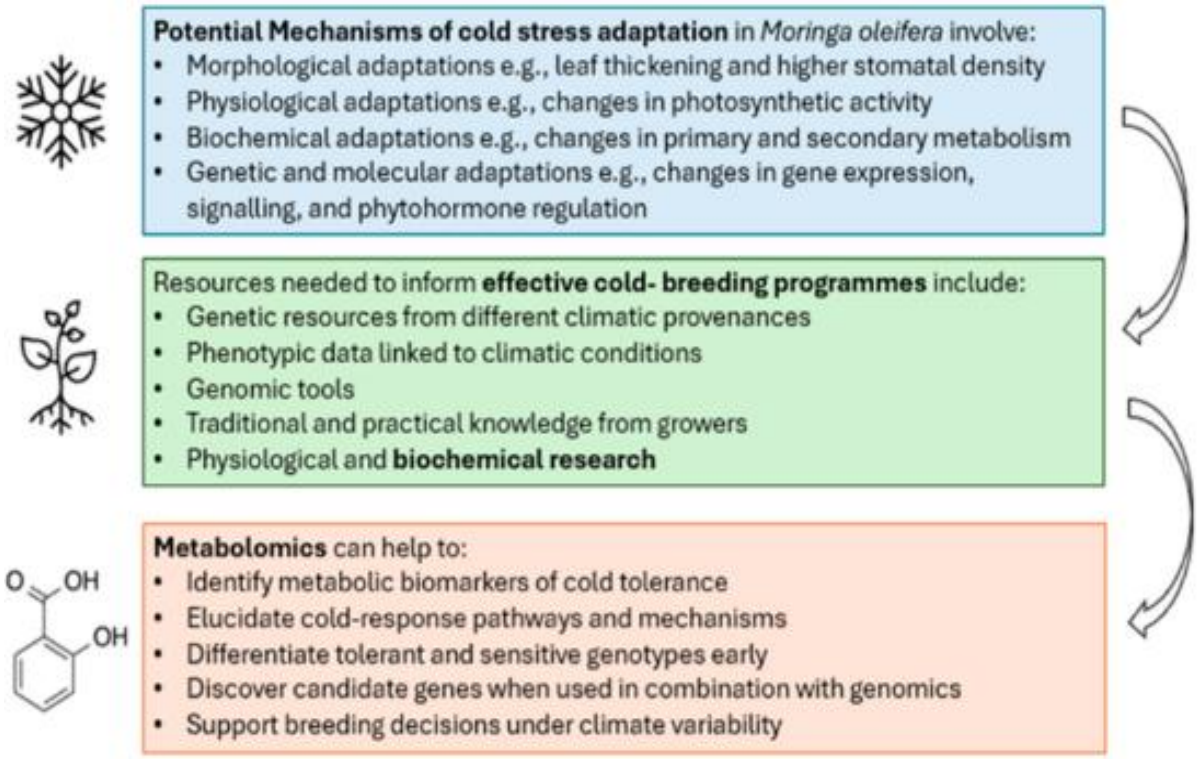

Figure 5. Considerations for breeding of cold tolerant varieties of moringa, and the role of metabolomics in contributing to biochemical research.

Future studies should prioritise integrative approaches that combine metabolomic profiling with genomic, transcriptomic and epigenomic assessments to identify metabolites, genes and regulatory networks associated with cold tolerance in moringa. Additionally, biochemical and molecular approaches linking carbon and nitrogen

metabolism with the biosynthesis of secondary metabolites such as phenolics, glucosinolates, and flavonoids are strongly encouraged. Moreover, most studies have been confined to foliar tissues, neglecting the metabolic versality of other plant organs. Roots comprise a significant portion of plant biomass and are essential to water and nutrient absorption, as well as to ecological interactions, making their study a fundamental piece of the puzzle. Strategic provenance selection from cold environments coupled with omics-driven breeding programs is vital for new cultivars suitable for temperate regions.

Table 1 highlights some of the current knowledge, research gaps, and future prospects that can help in understanding multiomics integration and functional validation to unravel the metabolic networks governing stress resistance in moringa. A comprehensive understanding of these processes not only defines or clarifies the adaptive plasticity of a species but also provides a biochemical framework for enhancing stress tolerance through metabolic engineering and sustainable crop management.

Table 4. Research gaps and future directions for the coordinated regulation of primary and secondary metabolites in moringa under abiotic conditions

Aspect	Current understanding	Knowledge gap	Future research direction
Metabolomic coordination under stress	Both primary (sugars, amino acids, organic acids) and secondary metabolites (e.g., phenolics, flavonoids, glucosinolate) contribute to stress mitigation in plants (Shiade et al., 2024; Tak et al., 2023)	The integrative coordination between primary and secondary metabolic networks under specific abiotic stress conditions (e.g., cold) remains poorly defined in moringa.	Use integrative metabolomic-transcriptomic approach to define network-level coordination and cross-regulation of metabolic pathways under cold stress.
Regulatory/signalling mechanisms	Transcription factors such as MYB, bZIP, and WRKY families are involved in metabolic regulation in model species (Kajla et al., 2023; Rabeh et al., 2025).	The regulatory hierarchy linking carbon and nitrogen metabolism to secondary metabolite biosynthesis and stress signalling is not yet established in moringa.	Identify and characterise key transcriptional regulators and hormonal cues e.g., ABA, jasmonic and salicylic acids that govern metabolic reconstitution during cold stress adaptation.
Stress-specific metabolite dynamics	Individual stresses modulate the accumulation of compatible solutes and antioxidants such as proline and phenolics (Kajla et al., 2023; Naikoo et al., 2019).	Comparative profiling of metabolite changes under combined or sequential stresses is lacking, this limits the understanding of metabolite plasticity.	Conduct time and multi stress metabolomic analyses to identify synergistic or antagonistic interactions between metabolic responses.
Tissue-specific metabolite responses	Most existing studies emphasise moringa leaf metabolites under stress (Kane & McAdam, 2023; Y. Kong et al., 2024; Ntsangani, 2018).	The metabolic functions of roots and stems, particularly their role in synthesis and systemic signalling-remain underexplored.	Implement tissue-specific metabolite mapping and transcriptomic profiling to reveal organ-dependent metabolic adjustments under stress environment.
Functional validation of metabolites	Correlations between metabolite accumulation and stress tolerance have been reported to be involved in metabolic regulation in model species (Kajla et al., 2023; Rabeh et al., 2025).	Functional validation of key metabolites or genes through biochemical assays or genetic manipulation remain undefined in moringa.	Utilise functional genomics, enzyme assays, and heterologous expression systems to validate the roles of candidate metabolites in stress tolerance.
Ecological and allelopathic integration	Several secondary metabolites in moringa exhibit allelopathic and ecological relevance (El-Rokiek et al., 2022).	The ecological significance of stress-induced metabolites in mediating interspecific interactions or rhizosphere ecology remains poorly understood.	Examine how stress-driven metabolic shifts influence allelopathic potential and rhizosphere interactions to link physiology with ecological function.

3.8 Conclusions

Moringa is a promising multipurpose crop with high nutritional, medicinal, and ecological value. While the plant is resilient under tropical and subtropical climates, its cultivation in temperate climates is constrained by its sensitivity to low temperatures. There is limited information on moringa growth dynamics under cold stress. However, the available evidence indicates that moringa deploys a suite of morphological adjustments, physiological acclimatisation, biochemical modulation, and genetic regulation as cold adaptation mechanisms. The coordinated functional roles of primary and secondary metabolites play a central role in stress mitigation in plants; however, their precise roles and regulatory pathways in moringa remain insufficiently understood. The establishment of a joint network among research institutions working on moringa, and engagement with current moringa growers and traditional knowledge owners, would generate and provide important information vital for the development of cold-tolerant germplasms and production systems in temperate regions. Metabolomics and its integration with other -omics and information sources promises to be a helpful tool in advancing plant breeding to develop cold-tolerant varieties.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Blair Moses Kamanga
Name and title of main supervisor:	Dr Donita L. Cartmill

In which chapter is the manuscript/published work? 4

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

C.M., A.C.M., and B.M.K Conceived the research idea; A.B. and A.M. Provided information regarding the growth of Moringa in its native range; B.M.K. and S.H. Curated the information, performed climate modelling, and implemented risk assessment tools; A.C.M. and B.M.K. Prepared the submitted draft; All authors reviewed the manuscript and agreed to its submission.

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Chapter 4

Combining Climate Models and Risk Assessment Tools to Evaluate the Invasive Potential of Intentional Plant Introductions: A Case Study of *Moringa oleifera* Lam. in New Zealand

Moringa plants grown for 4 weeks in a plant growth chamber. Photo credits ***Blair Moses Kamanga***

This chapter investigates the climate suitability and risk invasive potential of intentional introduction of moringa in New Zealand using weed risk assessment tools and climate adaptation models. This chapter is presented in the style of the journal *Discover Plants*. This chapter was published in the journal as:

Kamanga B.M., McGill C., Halloy S., Bhuker A., Malik A., and Clavijo McCormick A. Combining climate models and risk assessment tools to evaluate the invasive potential of intentional plant introductions: A case study of *Moringa oleifera* in New Zealand *Discover Plants* 2, 195 (2025).

Abstract

An increasing number of alien plants are escaping cultivation and becoming invasive worldwide, with devastating ecological consequences. As a result, it is crucial to better assess the risks associated with intentional plant introductions before widespread cultivation, even when these plants offer potential economic benefits. In this study, the use of climate suitability and weed risk assessment (WRA) tools to evaluate the invasiveness risk of *Moringa oleifera* Lam. (moringa) in New Zealand is explored. Moringa is valued globally for its nutritional content and potential health benefits, making it an attractive candidate for cultivation outside its native range. Although not yet widely grown in New Zealand, the rising demand for moringa food products could lead to increased cultivation and distribution. Therefore, it is essential to ensure that this crop's expansion is both environmentally and socially responsible, preventing it from becoming invasive and threatening native species. Climate suitability analysis identified Northland and surrounding areas as potential sites for moringa growth under current climate. The Composite Match Index, which measures the climatic similarity between current global distribution sites and potential locations in New Zealand, showed an increase from 0.52 in 2030 to 0.55 in 2070 under medium climate change scenarios, suggesting that climate change will facilitate the plant's expansion. The invasive risk assessment model classified moringa as a low-risk species for invasiveness in New Zealand, with a low uncertainty score for its impact potential (0.04) and higher uncertainty regarding its spread potential (0.2). However, further research on the plant's growth dynamics and interactions with other species is necessary to inform responsible decision-making regarding moringa's cultivation and management in New Zealand.

Keywords: Adaptability, climate match, invasive potential, uncertainty index, risk analysis

4.1 Introduction

The number of alien plants escaping cultivation and becoming invasive worldwide is on the rise. While the introduction of non-native plants has historically been a cornerstone of agriculture, the rapid increase in human mobility, global trade, and climate change in recent decades have significantly influenced the patterns and frequency of alien plant introductions. These factors not only facilitate the entry and spread of alien plants to new regions but also create favorable conditions for their establishment leading them to become unwanted weeds, with devastating ecological consequences (van Kleunen, Essl, Pergl, & Brundu, 2018). A more cautious approach to intentional plant introductions - one that assesses their potential to spread, establish, and negatively impact native species - could help prevent and mitigate future risks, informing decision-making. Although weed risk assessment tools, including climate change models, are commonly used to evaluate weeds of global concern, they also show great potential for assessing plants considered for introduction in horticulture and agriculture (Pheloung et al., 1999). In this study, an approach that combines climate change models and weed risk assessment tools to evaluate the weed/invasive potential of moringa, a plant of growing interest for commercial cultivation in New Zealand, is proposed.

Moringa (*Moringa oleifera* Lam.) is one of the most intentionally introduced plant species across diverse agroecological zones worldwide, attributed to its adaptability to varying climatic conditions (Bancessi et al., 2020; Vélez-Gavilán, 2017). It is distributed in most parts of the world, predominantly in the tropical and sub-tropical regions (Anwar et al., 2007). Although moringa is indigenous to western and sub-Himalayan tracts including India and Pakistan, it has been successfully introduced and widely cultivated in several other countries in Asia, Africa, America, and Oceania (Arif et al., 2023; Asati et al.,

2021), where it is used as a feed for livestock, human consumption, and medicinal and industrial purposes (Amad & Zentek, 2023; Horn et al., 2022). However, the species' sensitivity to cold temperatures and frost, limits its introduction and distribution in temperate regions (Godino et al., 2017). Nevertheless, climate change may alter this pattern due to shifts in temperature and an increase in weather conditions may create opportunities for the adaptation and expansion of moringa in new regions (Bania et al., 2023).

Moringa has garnered significant attention for its exceptional nutritional and medicinal properties, but its potential as a multipurpose crop extends far beyond these traditional uses. As global agricultural systems face increasing challenges from climate change, soil degradation, and resource scarcity, moringa present a compelling case for sustainable land use management and agroecosystem resilience (Amaglo et al., 2017; Palada et al., 2019). While moringa has gained popularity for its multipurpose uses, concerns have been raised about the potential of becoming an invasive alien species to the environment (Mashamaite et al., 2024). For example, based on growth characteristics such as rapid growth, adaptability to different environments, and potential for naturalization outside its native habitats, the species has been documented as invasive/weed or environmental escape in some countries including China, Philippines, British Indian Ocean Territory, Cuba, Solomon Islands, and Palau (Vélez-Gavilán, 2017), Cuba (Oviedo-Prieto et al., 2012), the state of Florida in the USA (UF-IFAS, 2021), and South Africa (Mashamaite et al., 2024). Nevertheless, the plant's impacts on native species and natural habitats remain poorly documented.

There are tools available to assess the risk and prevent the introduction of potential invasive species, these are often used for known weeds that are at risk of spreading

worldwide but less so on plants introduced for commercial cultivation, these include, but are not limited to, Weed Risk Assessment (WRA) questionnaires and climate suitability assessments (climate modelling). WRA tools are used to evaluate the possibility of introduced plants becoming invasive and cause detrimental impact on the environment, economy, and disruption of bio-ecosystem (Broadbent et al., 2018). WRA encompasses climatic conditions, species biology, entry potential, establishment likelihood, spreading capacity, and ecological impact (Pheloung et al., 1999). It has proven to be effective in various countries by successfully screening of aquatic plants in Switzerland (Oertli et al., 2018), New Zealand and the Great Lakes region of North America (Champion & Clayton, 2009; Gantz et al., 2015), and Malaysia (Saba et al., 2021); island ecosystems such as Hawaii and Pacific Islands (Daehler et al., 2004); and a variety of terrestrial ecosystems from Mediterranean regions (Andreu & Vilà, 2010), to Western African rainforests sites (Dawson et al., 2009).

The distribution of both cultivated and invasive species is driven by environmental and climatic factors including temperature, precipitation, soil conditions, and ecological interactions. Climate change is expected to impact the establishment, spread, and distribution of plant species over time (Guan et al., 2020; Haeuser et al., 2017); as well as their chemical behaviour and ecological interactions (Clavijo-McCormick et al., 2023). Temperature and precipitation have been singled out as key determinants in the establishment of crops and invasive species (Bell et al., 2021; Early et al., 2016). Consequently, climate suitability assessments serve as essential tools for informed decision-making regarding crop species selection and site suitability while also offering insights into how climate change may shape the landscape, altering the distribution of both cultivated and invasive plants (Kwesiga et al., 2003). Thus, understanding the

connection between plant species and climate suitability is crucial for predicting potential invasions (Epanchin-Niell, 2017; Valentin et al., 2018).

In New Zealand, moringa is only available as a household ornamental plant and not yet widely cultivated (Amaglo et al., 2017; Vélez-Gavilán, 2017). However, there is an increasing demand for its products supplied by Fiji and India for food and medicinal purposes (Hemasundar et al., 2022; Joshi et al., 2016). In January 2024, a formal application was lodged to Food Standards Australia New Zealand (FSANZ) to allow the use of moringa leaf, pods, and seed oil as a food in Australia and New Zealand (Newton, 2024). This amendment and the potential business opportunities it creates for moringa producers, is leading to a growing interest in the local cultivation of the species (AgriFutures-Australia, 2024). While the cultivation of a newly introduced crop can offer commercial and competitive advantages, the risks of unwanted outcomes such as invasiveness and negative impacts on native species and ecosystems must be carefully considered. Therefore, this study was conducted to assess potential risks associated with the introduction and cultivation of moringa in New Zealand, and to inform decision-making by growers and other stakeholders.

4.2 Materials and methods

4.2.1 Suitability and climate match analysis

Initially, suitability characterisation maps were generated for individual climatic factors i.e., temperature and precipitation. The approach involves generating suitability characterisation for temperature and precipitation individually and then combining them through a minimum argument. The minimum argument approach was used because it considers that both temperature and precipitation are critical factors for determining the

suitability of an area for agricultural production (Peter et al., 2020). To determine climate suitability sites for moringa in New Zealand under current climate, a fuzzy overlay analysis using the spatial analysis tool in Arc Map 10.8 of GIS software (https://www.esri.com/en-us/arcgis/products/arcgis) was used to identify suitable sites for moringa establishment in New Zealand. This method was chosen due to its ability to analyse data that have no discrete polygons or classified boundaries to determine the likelihood of whether the site is suitable or unsuitable. To conduct this analysis, the worldwide distribution of moringa species was determined using online data sets (11, 450 occurrences) sourced from Global Biodiversity Information Facility (GBIF.Org, 2023).

Temperature and rainfall parameters were the main climatic criteria considered when identifying suitable sites for moringa establishment and distribution in New Zealand. The average annual temperature and rainfall raster TIF files of New Zealand (1970 – 2000) were downloaded from Landcare Research website, New Zealand (LrisPORTAL, 2023). The TIF files were reclassified using Arc Map 10.8 into temperature and precipitation suitability classes in reference to how they influence the growth of moringa plants. Following moringa climate suitability classes in Haile and Ayansa (2022), suitability was classified into four classes: 1) most suitable class (mean annual temperature 25-30°C, rainfall >1000 mm) the optimum conditions where moringa thrives exceptionally well, 2) suitable class (temperature 18-25°C, rainfall 700-1000 mm), conditions that are favourable for growth but may not optimal; 3) moderately suitable (temperature 10-18°C, rainfall 340-700 mm), comprises of conditions where the plant can grow but growth may be limited as compared to suitable class; and 4) not suitable class (temperature <10°C, rainfall <340 mm), conditions that cannot support plant growth. Thereafter, the reclassified tiff files of temperature and rainfall were subjected to the fuzzy overlay analysis. Out of the many fuzzy overlay analysis types available, the ‘and’ type was used

for the analysis because of its ability to identify suitable sites that meet all the assigned criteria according to plant requirements.

Climate matching (similarities) between New Zealand and the rest of the world for moringa was calculated using the Better Border Biosecurity (B3) climate match app, designed in New Zealand for biosecurity purposes, which was imbedded with Match Climates Regional algorithm of Hearne Software's CLIMEX-DYMEX package at the default settings during its development following procedures in Phillips et al. (2022) and Roigé and Phillips (2021). The B3 app was chosen because it was specifically designed for the New Zealand context using species occurrence (presence only, 11450 occurrences) records from various sources to provide information about the climate similarities of the uploaded locations. Briefly, the app uses historical climate data 1970-2000 "1985" released January 2020 as current scenarios. Future climate scenarios include projected 20-year averages from the HadGEM3-GC31-LL Global Circulation Model for 2021-2040 "2030", 2041-2060 "2050", and 2061-2080 "2070" under shared socioeconomic pathways (SSP) 126 "low emission", 245 "medium emissions", and 585 "high emissions" (Phillips et al., 2022). The app's algorithm calculates a composite match index (CMI) for each non-New Zealand location which ranges from zero (no match) to one (perfect match). Niche overlaps were determined based on CMI using standard variables of maximum and minimum monthly temperature and rainfall based on species' global occurrence and distribution in GBIF. Climatic similarities between NZ and the world were calculated and visualized using 2.5-minute resolution data for New Zealand and 5-minute data for the rest of the world.

Species distribution models such as MaxEnt and CLIMEX are widely used to predict the potential geographical distribution of species using occurrence records and environmental

variables. However, these approaches are primarily designed to estimate the realised ecological niche of naturally occurring populations and rely on extensive, high-quality occurrence datasets (Song et al., 2026). In contrast, the objective of this study was to evaluate the climatic suitability of New Zealand for the cultivation of moringa, rather than predict its natural distribution. Henceforth, climatic suitability was assessed using the B3 climate app, which implements a climate-matching framework based on physiologically relevant climatic thresholds. This approach was considered appropriate because it directly evaluates whether regional climatic conditions satisfy the environmental requirements for crop establishment and survival, independent of potential biases associated with cultivated occurrence records. Furthermore, the B3 framework was specifically developed to support biosecurity and agricultural decision-making in New Zealand.

The climate niche envelope approach according to the procedures in Roigé and Phillips (2021) were used to demonstrate global climate change and shifts for possible moringa cultivation. Briefly, locations for climate niche comparisons were chosen from the extremes of moringa native and non-native distributions. This provides an 'envelope' of the potential areas where moringa can grow successfully. Sites where moringa is native were identified in India and Pakistan to provide a range from the hotter southern distributions (Tamil Nadu) to the somewhat cooler northern distributions in the Himalaya foothills (Haripur); at the same time providing a range from more continental (Madurai) to coastal (Puducherry); and including cultivated and disturbed forest. Non-native GBIF records (excluding indoor and greenhouse plants) were included to provide information on potential climate niche and growth or invasiveness i.e., San Bernardino (California), Auckland (New Zealand), Brisbane (Australia), and Pretoria (South Africa). The climate niche approach was used to visualize the climate niche overlaps in maps and graphs. Of

all the types of ellipses available, the inclusive ellipses were used due to their ability to surround all the points in a group of sites available.

The description and prediction of phenological events such as the potential crop establishment, development stage, and phenology, a growing degree-days (GDD) phenomenon was used. A temperature derived index that determines the amount of heat available for the growth of plants including moringa (Mokgehle et al., 2022). GDD were calculated by taking integral warmth above a base temperature 8℃ (T_b) and below an upper limit maximum temperature for crop growth of 48℃ (Tcutoff) (Ibraimo et al., 2016; Trigo et al., 2020), following the equation in (Mokgehle et al., 2022):

$$GDD = \int (T(t) - T_b)\, dt$$

Where integration is over the period with Tcutoff > $\boldsymbol{T}(t)$ > $\boldsymbol{T}_b$.

4.2.2 Invasive risk assessment model and analysis

The weed risk assessment tool developed by the Plant Protection-Quarantine-Weed Risk Assessment (PPQ-WRA) from the United States Department of Agriculture (USDA) (PPQ, 2022), a modified version of WRA developed by the Australian government was used with modifications (Appendix 1) in relation to New Zealand climatic conditions following procedures in Pheloung et al. (1999). A questionnaire-style of weed risk assessment that includes forty-nine (49) questions (Pheloung et al., 1999) was used to assess the probability of moringa becoming a weed or an invasive species to the environment in New Zealand. The tool uses an additive approach, with a set of scores ranging from -3 to 5 but mostly from -1 to 1, depending on the answer to questions which range from distribution and climate requirements to cultivation and invasion history to biological traits of the plant species (Mughal et al., 1999; USDA, 2019). The

questionnaire answers were coded to convert to a number with high values corresponding to qualities that make the species more likely to become invasive, and the final score is the sum of the values for each question. Thresholds are assigned to define categories where species would be 'accepted' for values below 0, would need further evaluation for insufficient information, between 0 and 6 and would be rejected outright for values above 6 (Pheloung et al., 1999). However, thresholds can be adjusted according to the acceptable level of risk and risk aversion within the model.

The PPQ-WRA tool was coded with uncertainty risk analysis model and a logistic regression equation model for probability analysis of species becoming major, minor, or non-invader (Caton et al., 2018). The question weights are calibrated based on chi-square tests of independence to optimize the model accuracy with predictive questions having greater weights during development of the tool. The tool model assesses the risk of species becoming invasive or non-invasive based on assigned points and probabilistic logistic regression (Koop et al., 2012). The approach involves assigning numerical values (points) to distinct factors or characteristics associated with a species and these points are then used to calculate the risk scores. These risk scores serve as inputs to a logistic regression equation, which in turn helps predict the probability of the species falling into one of the specified categories of major, minor, and non-invader plant. The PPQ-WRA tool assesses the probabilities obtained through this process and compares them to the predetermined threshold to assign an overall risk rating as high risk, low risk or evaluate further (Caton et al., 2018; Koop et al., 2012).

In PPQ-WRA tool, the uncertainty ratings (index) for establishment/spreading, impact, and entry potential are generated based on a combination of weighted scores, uncertainty ratings, and the maximum uncertainty values for each question in the assessment. High

uncertainty indices are applied in situations where assessors encounter weak or conflicted evidence. Although, the evidence may not be conclusive, it is deemed sufficient to lean towards one response as being more likely than the alternatives and only those that surpass 50% are chosen (Caton et al., 2018). To the contrary, when insufficient information is found, the tool generates the response as "unknown" and maximum uncertainty is automatically assigned in such situations within the WRA tool (Caton et al., 2018). The risk score for the species (solid black symbol) is plotted relative to the risk scores of the species used to develop and validate the PPQ-WRA (Koop et al., 2012). The results from the uncertainty analysis are plotted around the risk score for the species in question. The smallest, black box contains 95% of the simulated risk scores while largest contains 99% (Fig. 4). The black vertical and horizontal lines in the middle of the boxes represent the medians of the simulated risk scores of N=5000 (PPQ, 2022).

To effectively use the tool, a systematic literature review from peer reviewed journals, online reports, and expert-based knowledge of the plant was conducted to assess moringa's environmental and climatic requirements in places of origin, naturalized, and where it has been introduced and distributed. A comprehensive search for relevant scientific literature on invasiveness and climate suitability of moringa was conducted across various scientific databases including Springer, PubMed, and Scopus. The search utilized keywords "moringa" in combination with each one of the following key concepts and phrases "invasiveness" and "climate suitability," to capture the breadth of current research of the species, only English-language articles were analyzed further. Literature search was focused on studies published over the last decade between 2014 and 2024, and article suitability was determined by reviewing titles and abstracts which have been described as efficient approach in identifying relevant research publications (Tullu, 2019). The taxon's climatic requirement was determined based on global occurrence

locations and the comparisons on soil requirements were not conducted because moringa tolerates a wide range of soil types and thrives in infertile soils with little or without fertilization (Alshoaibi, 2021; Bancessi et al., 2020). The potential entry risks were not evaluated because moringa is already present in New Zealand as an ornamental plant, although it is not widely cultivated (Amaglo et al., 2017).

4.3 Results

4.3.1 Climate match and suitability analysis of moringa in New Zealand

The product of the fuzzy overlay analysis produced three suitability classes for moringa species i.e., suitable, moderately suitable, and not-suitable (Figure 6a). The most suitable class (rainfall >1000 mm and temperature 25-30°C) was not found because none of the reclassified tiff parameters (temperature and precipitation) met the criteria for climate suitability classification in New Zealand. The analysis for suitability and establishment found North Island, particularly areas surrounding Northland, as a potential site for moringa germination, establishment, and potential cultivation in New Zealand.

Based on the minimum argument approach using temperature and rainfall, the most suitable area in the North Island has a combination of high temperature and low precipitations, while unsuitable areas (mostly in the South Island) have an opposing pattern with high precipitation and low temperatures (Figure. 6b and 6c). However, other areas with similar precipitation and temperature conditions are found in the North Island (e.g., Bay of Plenty, Gisborne, and some of Waikato), making them potential for expansion under current climate scenarios.

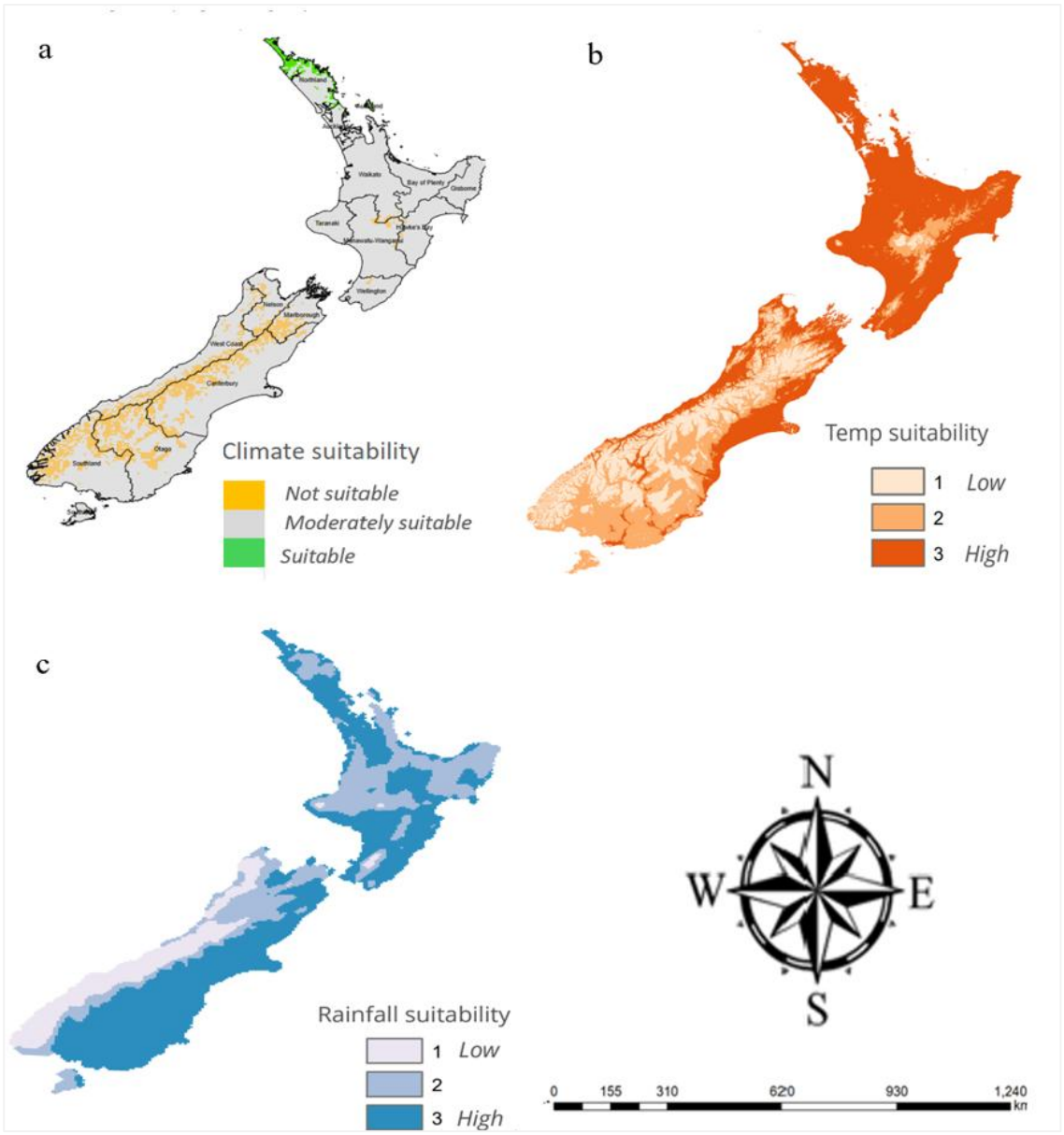

Figure 6. (6a) Overall potential suitability for establishment and distribution of moringa in New Zealand, areas shaded green are climatically suitable for the taxon based on fuzzy overlay analysis of Arc Map 10.8, (6b) temperature and (6c) precipitation suitability analysis for moringa growth and cultivation in New Zealand based on a minimum argument approach.

The results from running GBIF occurrences onto the B3 climate app provided additional information on the climatic similarities between New Zealand and other regions where moringa is cultivated. The frequency of CMI cells overlapping with unique occurrences,

where multiple occurrences within a single CMI cell were counted only once, was 967 after adjusting for spatial correlation. In New Zealand, the average CMI is on average 0.52, with approximately 15% of the CMI cells (over 150 cells) having a CMI >=0.7, the threshold considered suitable for species establishment and growth (Figure 7).

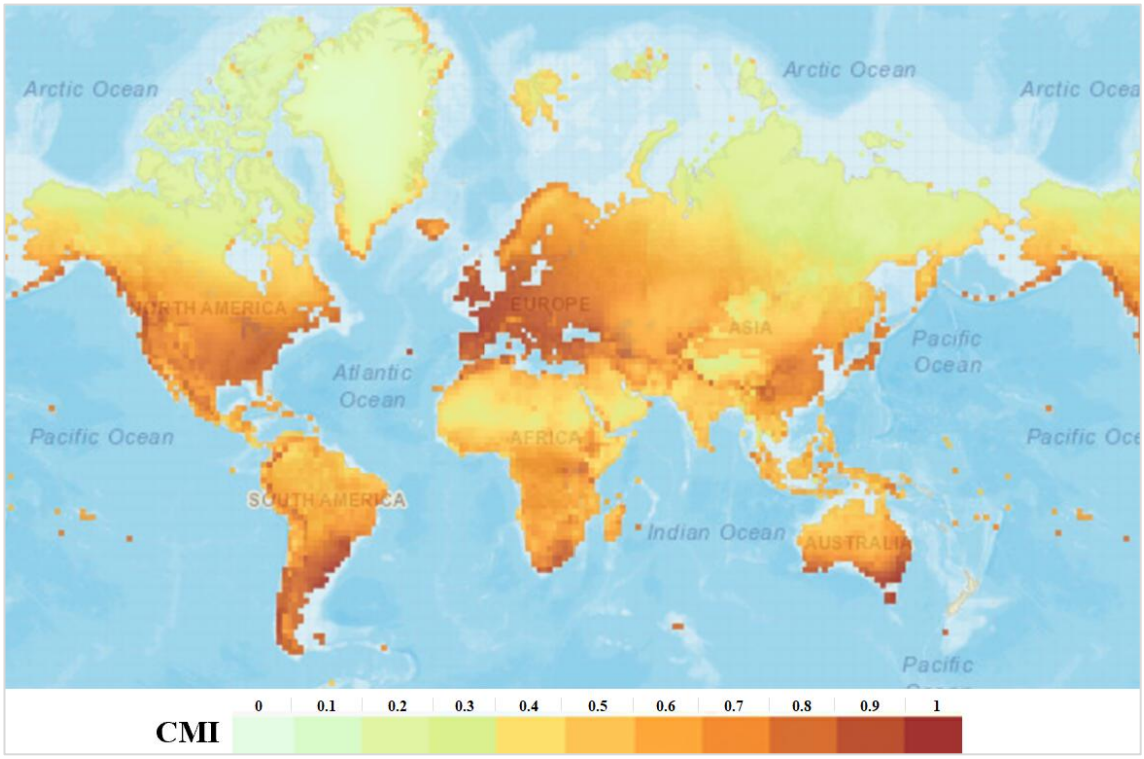

Figure 7. Climate matching index between all places in New Zealand (2.5-minute resolution) and the rest of the world where moringa is reported to occur (5-minute resolution) derived from the 3B climate matching app. The colour gradient indicates CMI values for each non-New Zealand location, which ranges from zero (no match) to one (perfect match) with CMIs >= 0.7 interpreted as or indicating that two climates are sufficiently similar for species to thrive in both locations.

Together these two measures of distribution indicate a somewhat narrow restriction to sub-tropical conditions in New Zealand mainly in North Island. Climate match analyses for 2030-, 2050-, and 2070-year scenarios found that the average CMI for moringa

worldwide distribution sites versus New Zealand rose from 0.52 for 2030 to 0.55 for 2070 under medium scenarios. In the same comparison, the proportion of CMI cells with values >=0.7 which is considered enough to establish, rose from 11.2 to 15.9%, demonstrating potential impacts of climate change on plant distribution.

Climate niche overlap analyses between New Zealand and known sites of moringa's current occurrences and distribution show that while native distribution sites closer to the equator (Madurai, Puducherry) are entirely outside New Zealand's present climatic conditions, however, another native distribution site, Haripur, does show considerable overlap with New Zealand climate niches (Figure 8a), cooler areas where moringa has been introduced have a high overlap with New Zealand climate (Figure 8b), suggesting that moringa is able to grow and adapt to cooler climates with respect to GDD.

For example, it was found that San Bernardino has 3180, Pretoria 3474, and Brisbane 4000 GDD as compared with Auckland having 2239. Additionally, sites further north in the Himalayan foothills (Haripur and Pathankot) have cooler winters with frosts and considerable overlap with New Zealand including cooler regions of New Zealand (Figure 8b) but have much warmer summers, leading to higher degree days than Auckland i.e., Haripur are close to 3000. Hence, moringa may thrive and grow in Auckland with relatively lower GDD due to cooler summers (~24°C max) leading to slower growth and potentially a longer maturation period.

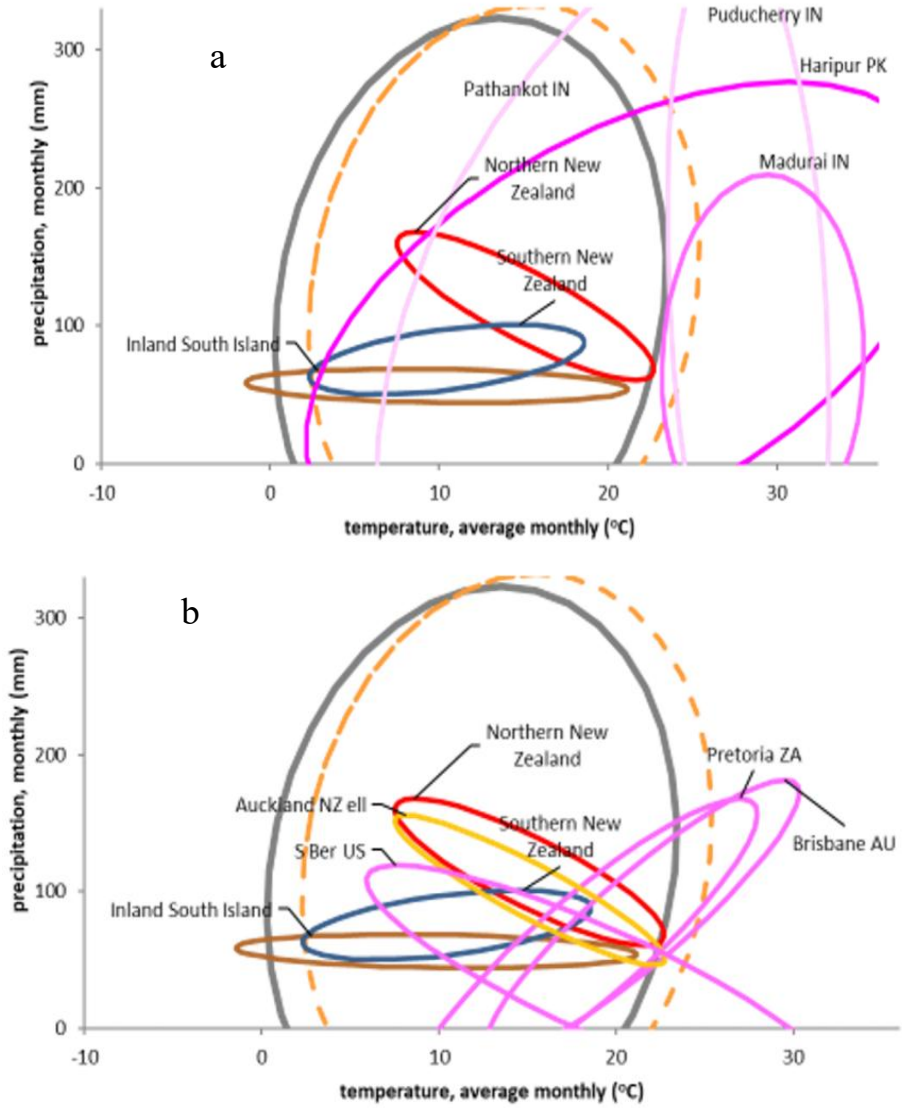

Figure 8. (a) Climate niche overlap between New Zealand (blue, brown, and red ellipses)and moringa native occurrences in pink ellipses and (b) Climate niche overlap between New Zealand (blue, brown, and red ellipses) and coolest sites where moringa is recorded (pink ellipses and Auckland – in yellow). Mean New Zealand climate is represented by the large grey ellipse, moving to the orange dashed ellipse with a climate change of +2°C mean annual temperature and +10 mm monthly precipitation.

4.3.2 Weed Risk Assessment

Several different results are presented from different models within the PPQ- WRA tool for risk and uncertainty index scores (Table 5) and model probabilities for moringa

becoming invasive in New Zealand (Table 6). The uncertainty index for establishment/spread potential (0.21) was higher due to limited information on soil seed bank formation and the period of seed storability while uncertainty index for impact potential (0.04) was lower due to multiple literature and reports describing moringa as a beneficial plant. Information on the resources consulted for the risk assessment is available in Table S1. Model analysis found no evidence to suggest that moringa would become a major invader under the current New Zealand climatic conditions. The species had risk scores ranging from -4 to 2, having an overall total risk score of one (1) with the model classifying moringa as a 'low risk' for invasiveness in New Zealand.

Table 5. The risk scores and uncertainty risk analysis of moringa on establishment/spread and impact potential to the environment in New Zealand based on the PPQ-WRA model.

No.	Parameter	Total risk score	Uncertainty Index
1	Establishment/Spread potential	1	0.21
2	Impact Potential	1	0.04
3	Entry potential	-	-
4	Secondary screening		Not applicable

The model's risk scores on establishment/spread and impact potential were used to predict the probabilities of invasiveness and assess the overall risk of moringa in New Zealand. The risk score and result of the uncertainty analysis (Figure 9), illustrates the major findings of the model. The probability of moringa becoming a major invasive species was found to be 3.6%, a value considered too low to cause severe environmental damage. Hence, under current climate scenario, moringa may be classified as a non-invader in New Zealand.

Table 6. Model probability analysis of species becoming major, minor, or non-invader based on risk scores of establishment/spreading and impact potential of WRA tool of moringa becoming invasive in New Zealand.

No.	Taxon invasiveness	Model probabilities
1	P (Major-invader)	0.036
2	P (Minor-Invader)	0.514
3	P (non-invader)	0.450
4	Model Risk Rating	Low risk

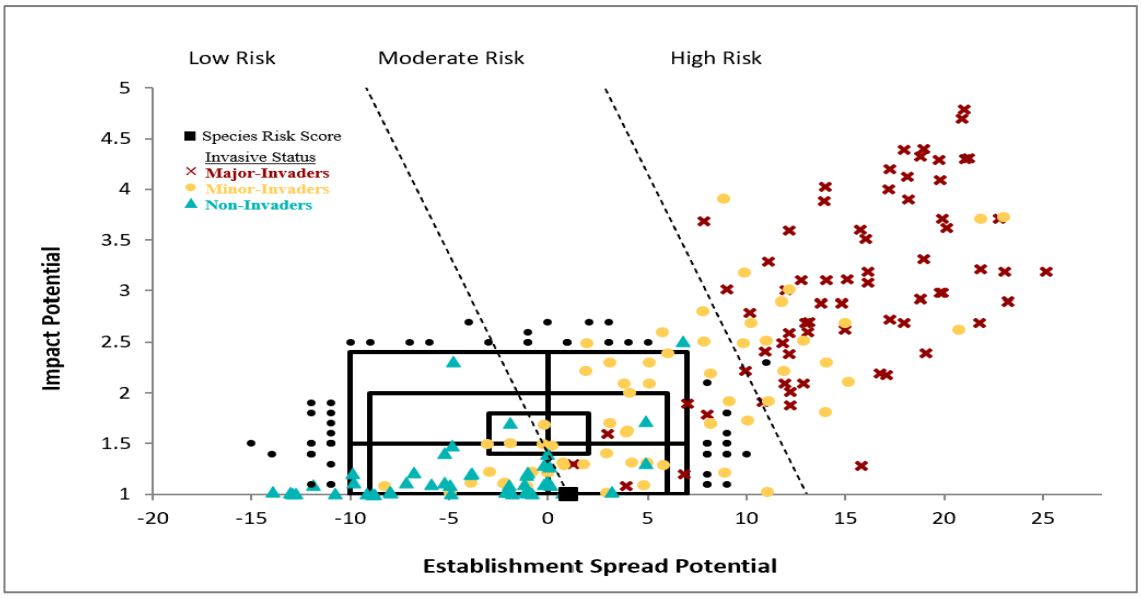

Figure 9. Risk and uncertainty results for *Moringa oleifera* species in New Zealand. The average risk score for this species (solid black square) is plotted relative to the risk scores of other introduced plants used to develop and validate the model (Koop et al., 2012). The smallest box contains 50% of the outcomes, the second box contains 95%, and the largest contains 99%. The remaining 1% of the outcomes are shown as outliers outside of the boxes represented by small black dots. The black vertical and horizontal lines in the middle of the boxes represent the medians of the simulated risk scores (N=5000) of uncertainty analysis (Caton et al., 2018) with an overall 'low-risk' result.

The plant was further rated as a minor invader with a score (probability) of 51.4%, suggesting that while moringa has the potential to cause some environmental concerns, it is not classified as a major invasive species or currently causing environmental problems. Furthermore, the probability of moringa being classified as a non-invader was 45.0%, indicating relatively low potential to become a significant invasive species in New Zealand.

4.4 Discussion

4.4.1 Climate match and suitability analysis of moringa in New Zealand

According to the model analysis, moringa is more suitable for growth and cultivation in most parts of the North Island compared with the South Island. Based on existing knowledge from the fuzzy overlay analysis and the B3 climate app, the climatic suitability for growth of moringa in New Zealand is expected to be limited to subtropical regions, primarily in Northland and the surrounding areas of the North Island.

Low temperature is a major limiting factor for the potential distribution and growth of moringa in temperate regions (Abdoun et al., 2023; Godino et al., 2017). Moringa thrives in warm to semi-arid tropical conditions, with optimum temperature range of 25-35℃ (Soares et al., 2023), which are rarely found in New Zealand (NIWA, 2023). However, a minimum growth rate (within a suitable range) occurs at temperatures between 18-25℃ and annual precipitation of 700-1000 mm (Haile & Ayansa, 2022). These conditions are mainly found in the North Island and may become more prevalent in other regions in New Zealand in the future under climate change scenarios with proportion of 951 cells with CMIs ≥ 0.7 raising from 11.2% to 15.9% under 2070 medium scenarios (Figure 7). However, moringa's ability to withstand droughts, light frosts, and poor soils (James &

Zikankuba, 2017), enhances its potential to thrive in New Zealand under current climate scenarios.

While New Zealand's temperate climate differs from moringa's native tropical range, the plant is cultivated in other temperate regions such as San Bernardino, Brisbane, and Pretoria (Figure 8b). The ability of plants to tolerate frost for many tropical and subtropical species depends on how 8plants grow and harden over a warm summer period i.e., the accumulation of sufficient Growing Degree Days (GDD). For moringa, GDD values above 10°C are well over 6000 in southern India, dropping to 3000 in northern India and Pakistan in the Himalayan regions where moringa is suggested to have originated. The coolest sites where moringa is cultivated or naturally occurs range from 3000 to 4000 GDD, with many sites in the North Island falling within this range. This provides an opportunity for moringa growth, as some provenances within moringa species can tolerate brief exposure to cooler temperatures ranging from -1°C to -3°C (Godino et al., 2013; Soares et al., 2023). The current prediction based on the proportion of CMI cells with values ≥ 0.7 rising from 11.5 to 15.9% suggests that in the future, most locations in New Zealand could become suitable or most suitable for moringa growth and cultivation.

Biogeographic suitability maps provide valuable insights into how a species may adapt to new niches based on the environmental factors in its current distribution (Peter et al., 2020). They contribute to sustainable and resilient cropping systems by aligning crop choices with the environmental conditions of a specific region and facilitating successful crop introduction (Mahaut et al., 2021). Climate niche overlap results, based on CMI values for temperature and precipitation indicate a relatively higher future overlap in moringa's climate suitability between New Zealand and other regions (Figure 7). Several

climatic factors contribute to this overlap including the projected climate change scenarios of a +2℃ increase in mean annual temperature and a +10 mm rise in monthly precipitation. These trends are expected to continue, leading to mean annual temperature of 25-35℃ and precipitation levels of 300-1500 mm, conditions that have been described as optimal for moringa growth (Alavilli et al., 2022; Palada et al., 2019).

As temperature and precipitation patterns shift, suitable areas for moringa cultivation may expand under future climate change scenarios. By 2050 and 2070, highly suitable areas are projected to increase substantially (Bania et al., 2023), concurring with our findings. However, potential distribution of species refers not only to the climatic conditions where it could survive but also to the availability of suitable habitats that provide the necessary conditions for growth. Factors such as competition, pests, diseases, predators, and habitat characteristics influence species establishment (Roigé & Phillips, 2021). Vegetation composition and other ecological elements will determine moringa's suitability within a given biogeographical zone (Harrison et al., 2020; Paz-Kagan et al., 2021). The potential interactions between moringa and New Zealand native species remain unknown and require further investigation.

4.4.2 Invasive Risk Assessment

No evidence for moringa becoming a major invader under New Zealand climatic conditions was found. The current score (-4 to 2) for moringa is lower compared with the range of 13 to 26 for well-known invaders like gorse (*Ulex europaeus*) but higher than the -5 to -3 for non-invaders such as wheat (*Triticum aestivum*). However, the score is closely comparable to subclover (*Trifolium subterraneum*) with its scores ranging from 0 to 6 (GISP, 2024; PIER, 2017), which implies a requirement for 'further evaluation'. While moringa is often acknowledged for adaptation and versatility, contrary views and

some studies including Randal's compendium of weed (2017) have reported instances of potential weediness and environmental escape associated with the species in different countries mainly in tropics (Mashamaite et al., 2024; Randall, 2017). This underscores the species' potential to exhibit invasive behaviour under certain conditions mainly in tropical and subtropical regions, highlighting the need for careful management and monitoring systems in non-native environments to prevent unintended ecological impacts.

The current results align with findings from Hawaii, where moringa received an overall score of 1 after secondary screening, classifying the species as 'low risk' for invasiveness (PIER, 2017). Henceforth, moringa is actively promoted for cultivation in Hawaii, reinforcing its low potential for invasiveness. In contrast, our results differ from a previous risk assessment in Trinidad and Tobago, which assigned moringa an overall risk score of 0 (Evaluate further), classifying it as naturalised but not invasive (Bridgemohan & Bridgemohan, 2014). These differences may be attributed to contrasting climatic conditions, as Trinidad and Tobago has a tropical climate characterized by consistently high temperatures and distinct wet and dry seasons. (TTMS, 2023). These conditions provide optimum conditions for moringa growth and development (Santos et al., 2015). In contrast, New Zealand experiences a temperate maritime climate with mild temperatures and high rainfall (NIWA, 2023) which could reduce moringa's rapid growth and development, limiting its potential of becoming invasive.

The impact potential risk element assesses a species' capacity for direct and indirect impacts on production, natural, and anthropogenic systems based on existing evidence of invasiveness (Caton et al., 2018). The overall impact potential score (1.0), together with the low uncertainty index (0.04), indicates that current evidence and available literature suggest moringa poses a negligible risk of becoming invasive or causing significant

adverse impacts on agricultural systems or natural habitats. However, knowledge gaps remain regarding certain aspects of its ecology, particularly its potential to affect native ecosystems under different environmental conditions.

The very low uncertainty for this risk element is due to the abundance of literature referring to species' positive impact on agricultural production as the plant has been extensively used in agriculture with numerous nutritional and production benefits (Ahmed et al., 2020; Taiwo et al., 2022), and natural resource management (Amad & Zentek, 2023; Arora et al., 2023), but there may be some bias and over reporting of positive outcomes. The present analysis supports previous reports that moringa has no documented history of causing adverse impacts on agricultural systems, anthropogenic environments, or natural ecosystems (Csurhes & Navie, 2016; Mashamaite et al., 2023).

The establishment/spread potential risk evaluates the likelihood of a species to escaping, naturalising, and spreading in a vulnerable area by considering various factors related to its biological behaviour in an environment. Its risk scores range from -25 to 32 points, with higher values indicating a higher likelihood (Caton et al., 2018). In comparison, the current risk assessment values (-4 to 2) are relatively low for moringa to escape or spread. The lack of information on the formation of soil seed and seed storability period of moringa is suggested to have influenced the model's outcome. However, under favourable conditions, Moringa oleifera produces abundant seeds that are dispersed primarily through intentional human activities, with wind and water playing only minor roles. (Csurhes & Navie, 2016; Makkar & Becker, 1997; PIER, 2017). The ability of moringa to spread and naturalise is highly dependent on environmental conditions, highlighting the importance of localised risk assessment.

The plant was further classified as a minor invader with 51.4% rating, indicating that it has the potential to cause minor environmental impacts. This suggests that while moringa is not a major invasive species with widespread disruptive impacts, it possess certain growth traits that may enable the taxon to spread and establish beyond its native or cultivated areas (Abdoul-Salam et al., 2021; Asati et al., 2021). Further research in needed to assess its ecological impacts when grown alongside native species and other introduced plants such as *Pinus radiata*. Additionally, the model classified moringa as a non-invader species with a 45.0% probability, categorizing it as a low-risk plant. A higher probability score of minor-invader does not necessarily mean moringa is currently causing problems in different ecosystems, it does indicate that moringa possess some biological traits such as rapid growth and prolific seed production (Makkar & Becker, 1997; Shaltout et al., 2017) that raise concerns about its potential to become invasive under certain environmental conditions (Renteria et al., 2021).

Although some countries have classified moringa as a potential invasive species, mainly in tropical and subtropical regions (Mashamaite et al., 2024; UF-IFAS, 2021), the current analysis was conducted in a temperate region with cooler climatic conditions which may reduce the risk of invasiveness and environmental escape. Invasive risk assessments are predictive tools based on the information available at the time of evaluation. While they provide a good indication on whether a species is likely to become invasive, they cannot predict the behaviour of a species under all circumstances. The tool not only provides valuable information about the species' potential suitability and invasiveness risk but highlights gaps in the knowledge of potentially invasive plants that require further exploration. Caution must always be exercised when introducing any species that is known to naturalise or be invasive elsewhere. Additionally, regular monitoring and assessment of the impacts of introduced plant species are key components

of effective species management. The approach allows for early detection of potential invaders and enables timely intervention to prevent the negative impacts on ecosystem (Epanchin-Niell, 2017; Mandrak & Cudmore, 2015). Robust risk assessment approaches are needed before considering the introduction and cultivation of plant species into new ranges.

4.5 Conclusion and recommendations

The introduction of non-native species, such as moringa, into new environments is increasingly becoming a concern due to the rising global trends in human mobility, trade, and climate change. While moringa cultivation may present an opportunity for industrial crop development in New Zealand, assessing the risk of its potential to become invasive is critical. Although the present assessment indicates a low risk of moringa becoming invasive under present climate conditions, this could change as other areas of New Zealand become suitable for its growth due to climate change. This assessment also highlights key knowledge gaps, particularly regarding traits such as seed bank formation, which may limit assessment accuracy. Furthermore, for cultivated species, negative ecological impacts may be underreported, potentially biasing risk assessments. Therefore, further research and periodic risk assessments are essential to elucidate the growth dynamics of moringa, its interactions with native and introduced species, and its potential to cause environmental harm.

The rapid spread of alien plants and their increasing role as invasive species highlights the importance of assessing the ecological, social, and economic risks posed by new introductions. A more cautious approach to intentional introductions, incorporating the use of climate change models and weed risk assessment tools, can help prevent future negative impacts. This approach not only informs better decision-making but can also

support the development of management practices that can mitigate the risks. As New Zealand explores the commercial cultivation of moringa, continued monitoring and further studies will be essential to ensure that the species does not unintentionally contribute to the decline of native species and ecosystems.

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4.8 Appendix 1: Supplementary information to chapter 4

Appendix1.1 All the information/evidence and associated references related to the evaluation of the potential invasive risk of moringa using WRA-PPQ tool including the answers, uncertainty rating, and scores for each question used to evaluate the establishment-Spread (ES), and Impact potential (Imp) of the taxon

No. Question ID	Answer-Uncertainty	Score	Notes and associated references
ES-1 What is the taxon's spread establishment status outside its native range?	e - high	2	Moringa is naturalised in some countries in Asia, Africa, and Middle East Vélez-Gavilán, 2017)
ES-2 (Is the species highly domesticated)	y - high	-3	Spreading and cultivated in semi-arid, tropical, and sub-tropical regions of several countries (Horn et al., 2022; Joshi et al., 2016; Spandana et al., 2016).
ES-3 (Significant weedy congeners)	n - negl	0	No species of Moringa has been reported to have weedy congeners (Mashamaite et al., 2023).
ES-4 (Shade tolerant at some stage of its life cycle)	n - low	0	Not shade tolerant (Palada, 2021; PFAF, 2021).
ES-5 (Plant a vine or scrambling plant, or forms tightly appressed basal rosettes)	n - low	0	Moringa has been described as shrub or small tree (Boopathi & Abubakar, 2021; Vélez-Gavilán, 2017).
ES-6 (Forms dense thickets, patches, or populations)	y - mod	2	The plant appears to spread slowly and forms dense thickets around parent trees (Csurhes & Navie, 2016).
ES-7 Is the taxon an aquatic plant?	n - negl	0	Terrestrial plant and grows near the bans of the river (Vélez-Gavilán, 2022).
ES-8 Is the taxon a grass (Poaceae)?	n - negl	0	Deciduous tree or shrub (Isitua et al., 2015; Kumar et al., 2019).

ES-9 (Nitrogen-fixing woody plant)	n - mod	0	Moringa does not fix nitrogen (Olson, 2017).
ES-10 (Does it produce viable seeds or spores)	y - high	1	Moringa do not have an innate dormancy, produces viable seeds (Candelaria-Martínez et al., 2019; Csurhes & Navie, 2016)
ES-11 (Self-compatible or apomictic)	y - low	1	Moringa has a mixed mating system, however, cross-pollination dominates. (Alhassan et al., 2022; Krieg et al., 2017).
ES-12 (Requires specialist pollinators)	y - high	-1	Moringa require many insects and sunbirds (Nectaria spp.) or other insects for a successful pollination (Alhassan et al., 2022; Popoola et al., 2016; Ramos et al., 2010)
ES-13 (What is the taxon's minimum generation time?)	a - mod	2	The plant blooms during the first 6 months to one year of planting (Alhassan et al., 2022; Takase et al., 2022).
ES-14 (Is the taxon a prolific seed producer)	y - high	1	The plant produce numerous viable seeds (Parvin et al., 2023; Ruiz et al., 2021; Shaltout et al., 2017; Zhigila et al., 2015)
ES-15 (Propagules likely to be dispersed unintentionally by people)	n - negl	-1	Moringa seeds are dispersed intentionally by human beings. (Csurhes & Navie, 2016).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	n - low	-1	No evidence was found on propagules likely to be disperse in trade as contaminants due to the nature of seed.
ES-17 (Number of natural dispersal vectors)	2	-4	Moringa seeds can be dispersed by wind, water, and human beings (Csurhes & Navie, 2016; Edwards et al., 2000; Roloff et al., 2009)
ES-17a (Wind dispersal)	y - low	N/A	The seeds are strongly winged, this feature enables the seeds to be

			dispersed over a short distance from the parent tree (Csurhes & Navie, 2016; Edwards et al., 2000)
ES-17b (Water dispersal)	y - low	0	The seeds are dispersed by water (Csurhes & Navie, 2016; Roloff et al., 2009).
ES-17c (Bird dispersal)	n - negl	N/A	Birds feed on leaves but no evidence of seed dispersal was found (Francis et al., 2019).
ES-17d (Animal external dispersal)	n - negl	N/A	There is no evidence of seed dispersal by animals externally, seeds can be dispersed through water, wind, and human beings (Csurhes & Navie, 2016; Roloff et al., 2009).
ES-17e (Animal internal dispersal)	n - negl	N/A	Seed-eating animals probably eat seeds, but no evidence has been reported to support this claim (Csurhes & Navie, 2016; Parrota, 2021).
ES-18 (Evidence that a persistent (>1yr) propagule bank (soil seed bank) is formed)	n - mod	-1	No information on the formation of soil seed bank (Gomaa & Picó, 2011)
ES-19 (Tolerates/benefits from mutilation, cultivation, or fire)	y - high	1	High tolerant and benefits from mutilation, cultivation, or fire (duToit et al., 2020; Soares et al., 2023).
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - negl	0	Moringa has been found to be sensitive to herbicides (Shulner et al., 2021).
ES-21 [Number of whole, cold hardiness zones suitable for its survival: (a) 1-3; (b) 4-9; (c) 10-13]	a - mod	-1	Moringa tolerates a wide range of climates and soils (A Bancessi et al., 2020; Godino et al., 2013; James & Zikankuba, 2017; Palada et al., 2019).

ES-22 [Number of broad climate types suitable for its survival: (a) 1-2; (b) 3; (c) 4-12]	c - mod	2	Tolerate a wide range of climates and soils (A Bancessi et al., 2020; James & Zikankuba, 2017; Palada et al., 2019).
ES-23 [Number of precipitation bands suitable for its survival: (a) 1-4; (b) 5-7; (c) 8-11]	b - mod	1	Moringa tolerates a range of precipitation with an average annual rainfall of 250-1500 mm (Mashamaite et al., 2022; Palada et al., 2019).
Imp-G1 (Allelopathic)	? - mod	-	Due to conflicting evidence, the taxon was rated as "?" (Bulgari et al., 2019; El-Mageed et al., 2017; Khan et al., 2017; S Perveen et al., 2021; Sinha, 2012; Soliman et al., 2017; Tahir et al., 2018).
Imp-G2 (Is a taxon parasitic, and does is it have host in the WRA area?)	n - negl	0	Moringa is not in a parasitic family (USDA, 2021).
Imp-N1 (Does a taxon changes ecosystem processes and parameters that affect other species)	? - mod	0	Due to conflicting evidence and inadequate information to justify its invasiveness or negative impact on native plants and natural habitats a taxon was rated "?" (Csurhes & Navie, 2016; Vélez-Gavilán, 2017).
Imp-N2 (Does the taxon changes habitat structure)	n - low	0	Lacks information to justify its invasiveness (Csurhes & Navie, 2016; Mashamaite et al., 2023; Olson, 2017).
Imp-N3 (Changes species diversity)	n - low	0	No history of invasiveness and lacks information to justify its invasiveness (Csurhes & Navie, 2016; Mashamaite et al., 2022).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species?)	n - low	0	No history of invasiveness and lacks information to justify its invasiveness (Csurhes & Navie, 2016; Olson, 2017).

Imp-N5 (Is it likely to affect any NZ or globally outstanding ecoregions?)	n - low	0	There's inadequate information to justify its invasiveness or its negative impact on native plants and natural habitats (Csurhes & Navie, 2016).
Imp-N6 [What does the taxon's weed status in natural systems?	a - low	0	No records of invading natural flora (Csurhes & Navie, 2016; Olson, 2017).
Imp-A1 (Negatively impacts individual property, human safety, or public infrastructure)	n - negl	0	No literature has reported the negative impact on individual property, human safety, and public infrastructure but rather individuals grow few trees around their homestead as live fence (Amaglo et al., 2017).
Imp-A2 (Changes or limits recreational use of an area)	n - negl	0	No records of invading natural flora (Csurhes & Navie, 2016; Olson, 2017)
Imp-A3 (Affects desirable and ornamental plants, and vegetation)	n - negl	0	No evidence of invading natural flora (Csurhes & Navie, 2016; Olson, 2017)
Imp-A4 [What does the taxon's weed status in anthropogenic systems?	a - low	0	No evidence of invading natural flora (Csurhes & Navie, 2016; Olson, 2017).
Imp-P1 (Does the taxon reduces crop/product yield)	? - low	0	Due to conflicting evidence, the taxon was rated "?" However, it improves crop yield and quality (Mvumi et al., 2013; Ngcobo & Bertling, 2021; Pervez et al., 2017).
Imp-P2 (Lowers commodity value)	n - low	0	No literature evidence was found
Imp-P3 (Is it likely to impact trade?)	n - low	0	It does not negatively affect trade but promotes international trade through moringa health products (Reynolds & Robinson, 2022).

Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - negl	0	Moringa is tolerant to drought and not a heavy feeder in terms of water and nutrient requirements (Abdoun et al., 2023; A Bancessi et al., 2020).
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - low	0	To the contrary, Moringa is used as feed for livestock (Abdoun et al., 2023; Amad & Zentek, 2023).
Imp-P6 [What does the taxon's weed status in production systems? (a) Taxon not a weed; (b) Taxon a weed but no evidence of control; (c) Taxon a weed and evidence of control efforts]	a - negl	0	There is no information of the species invading agricultural fields or displacing native flora (Olson, 2017)
Ent-1 (Plant already here)	y - mod	1	The plant is present in New Zealand under homestead cultivation (Amaglo et al., 2017; Vélez-Gavilán, 2017).
Ent-2 (Plant proposed for entry, or entry is imminent)	--	N/A	No documentation, however, Moringa products and seed is available on the market and cultivated around the homestead (Amaglo et al., 2017).
Ent-3 Human value & cultivation / trade status:	--	N/A	Commercially cultivated or other evidence of trade or resale (Outani et al., 2023; Reynolds & Robinson, 2022).

Statement of Contribution to Chapter 5

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GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

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Chapter 5

Root-derived allelochemicals from *Moringa oleifera* regulate germination and early seedling growth in pasture, native, and weed species

Moringa roots from pot experiments. Photo credits: ***Blair Moses Kamanga***

This chapter evaluated the allelopathic potential of moringa root extracts/exudates on perennial ryegrass, white clover, fathen, crabgrass, and mānuka. It further conducted untargeted metabolomics analysis to identify bioactive compounds responsible for allelopathy. The chapter is presented in the style of the journal *Plant Signalling & Behaviour*. This this chapter was published as:

Kamanga, B.M., Barrett, P., Cartmill, D.L., McGill, C., and Clavijo McCormick, A. Root-derived allelochemicals from *Moringa oleifera* regulate germination and early growth in pasture, native, and weed species. *Plant Signalling & Behaviour, 2026*.

Abstract

Moringa oleifera Lam. (moringa) is not currently commercially cultivated in New Zealand, but there is growing interest in its integration into farming systems. However, there are questions regarding its ecological impacts. One area of interest is its allelopathic traits, which can affect native and other introduced plant species. Hence, this study assessed the allelopathic effects of moringa root exudates on common pasture, weed, and native species and conducted untargeted metabolomics to identify potential bioactive compounds associated with allelopathic activity. Root exudates from two moringa provenances were assessed across concentration gradients (1–100%) in the laboratory and under semi controlled natural conditions (plant–plant interactions) via germination and seedling growth bioassays. Statistical analyses revealed a dose-dependent and species-specific response to moringa root allelochemicals, with white clover, a key pasture species in New Zealand, being the most adversely affected. LC–MS/MS analysis revealed several bioactive compounds with potential allelopathic activities dominated by phenylpropanoids and benzenoids, with relatively small contributions from organoheterocyclics, oxygenated compounds, and organic acids. Therefore, the utilisation of moringa within white clover pastural systems may not be advised. Isolating and testing individual compounds will be essential for determining how moringa may influence plant growth and population dynamics in pastoral and native ecosystems.

Keywords: Allelochemicals, target species, inhibition, metabolomics, species interaction, stimulatory effects, provenances

5.1 Introduction

Plant roots constitute a significant portion of plant biomass and perform essential functions, such as anchorage, and the acquisition, uptake and storage of soil-based resources (mainly water and ions). However, roots are also involved in the production, storage and release of primary and secondary metabolites (Canarini et al., 2019; Jackson et al., 2007). Primary metabolites are essential for plant growth and reproduction and have regulatory properties, whereas secondary metabolites play vital roles in plant–plant interactions and ecosystem dynamics (Fletcher et al., 2020; Vives-Peris et al., 2020). They are released into the soil through root exudation, leaching, volatilisation, and decomposition of root tissues, which impacts nearby plants and soil biota (Bulgari et al., 2019). The chemical compounds that elicit a response in another plant or organism are collectively known as "allelochemicals" and may have positive or negative impacts on the growth, reproduction or behaviour of the receiver organisms (Mushtaq et al., 2020; Kumar eta al., 2024; Mansoori et al., 2020).

Root allelochemicals represent a diverse group of bioactive compounds with the potential to improve crop growth through their positive effects on physiological activities, nutrient uptake, and plant stress tolerance mechanisms (Bachheti et al., 2020; Kamanga et al., 2025; Hierro et al., 2021). Conversely, certain root allelochemicals have inhibitory effects on the germination and growth of neighbouring plants (El-Rokiek et al., 2022; Perveen et al., 2021). While the inhibitory traits of certain root compounds could be beneficial for weed suppression in an agricultural system, they could also pose serious challenges in managed and natural ecosystems by inhibiting other agricultural and important native species and further disrupting the ecological balance (Hickman et al., 2021; Perveen et al., 2021). This complex nature of allelochemicals emphasises the need for a nuanced

understanding and application of these traits in agricultural and ecological contexts. Adding to this complexity, receiving plants are known to react differently to allelochemicals released by species they coevolved with versus not, and their concentration determines the overall effect of a given chemical compound or blend (Effah & Clavijo McCormick, 2024).

In response to climate change and resource availability pressures, producers are increasingly exploring the establishment of resilient, multi-purpose perennial species such as *Moringa oleifera* L. (moringa) within cropping and pasture systems. This approach aims to diversify production (e.g., animal feed, human consumption, biofuel), while enhancing structural complexity and biodiversity (Swanepoel et al., 2021; Palada et al., 2019; Abdoul-Salam et al., 2021; Abdoun et al., 2023). Moringa is native to the Indian subcontinent; however, it has been cultivated worldwide across a range of climates, predominantly in tropical and subtropical regions (Singh et al., 2020). While moringa is neither native to nor commercially cultivated in New Zealand, there is growing interest in its integration into local farming systems (AgriFutures, 2024). However, there are questions regarding its potential ecological impact, especially any allelopathic traits that could impact established native and introduced pastoral species (Kamanga et al., 2025; Clavijo-McCormick et al., 2023; Effah et al., 2019).

New Zealand pastoral systems are dominated by high value forage species such as perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.), which support pasture productivity, persistence, and biological nitrogen fixation (Sutharsan et al., 2025; Widdup and Barrett, 2011). Since these species form the foundation of temperate pasture ecosystems, any allelochemical interference arising from the introduction of other species such as moringa requires careful assessment. Studies have

demonstrated that legumes are more sensitive to allelopathic compounds (Gomaa et al., 2015), whereas ryegrasses provide a robust indicator of early competitive responses within a mixed sward (Widdup et al., 2011). Therefore, these commonly sown species were selected for growth assays to evaluate their susceptibility to moringa root-derived allelochemicals and to determine the potential compatibility of moringa within temperate pastoral systems.

Research has shown that different parts of a plant, including flowers, fruits, seeds, and roots, produce unique bioactive compounds with specific compositions and properties (Bhalla et al., 2021; Preece & Peñuelas, 2020). While moringa leaf extracts (MLEs) have received much attention for their bioactive compounds, including phenolic acids, flavonoids, tannins, and glucosinolates) that are known to suppress seed germination and early seedling growth, (Kamanga et al., 2025; El-Rokiek et al., 2022), moringa root extracts (MREs) remain insufficiently studied (Kamanga et al., 2025) despite roots being important sources of secondary metabolites (Loyola-Vargas & Miranda-Ham, 1995). Since the concentration of these metabolites varies with genotype and in response to the environment where the plant is grown, two moringa provenances were used to capture potential differences in allelopathic potency. Provenances from hotter or drier regions typically accumulate higher levels of secondary metabolites, whereas those from more moderate climates often contain lower or more balanced concentrations (Qaderi et al., 2023; Pant et al., 2021; Ahmed et al., 2019). Comparing these provenances could therefore provide insights on how moringa allelochemicals may vary in response to different environments.

his study was conducted to assess the chemical composition of MREs from moringa plants from two provenances in India and the allelopathic effects of different

concentrations of MREs on the germination and seedling growth of New Zealand forages, including perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Weed species assessed, included fathen (Chenopodium album L.) and crabgrass (*Digitaria sanguinalis* L. Scop.), and a native species i.e., mānuka (*Leptospermum scoparium* J.R.Forst. et G.Forst.) was also tested. Furthermore, pot-based plant–plant interference assay (seeds grown together with moringa plants on the same soil) were conducted. We hypothesised that root-derived allelochemicals from moringa modulate germination and growth trajectories of pasture, native, and weed species in species-specific and concentration-dependent ways. By identifying bioactive compounds in moringa roots and evaluating their allelopathic effects, we aim to advance our understanding of the ecological interactions that underpin the sustainable integration of moringa into New Zealand agricultural systems.

5.2 Materials and methods

5.2.1 Experimental site and plant materials

Plants were grown under greenhouse conditions between June and November 2024 at the Plant Growth Unit (PGU), Massey University (−40.37807°S, 175.61362°E), New Zealand. Temperatures were maintained between 24–27°C (±3°C), relative humidity between 65−80%, and plants were exposed to natural light. Seeds of two moringa provenances (PKM-1) were used for the cultivation of donor plants, i.e., provenance_t from Tamil Nadu (South India) and provenance_g from the villages of Deoria District, Uttar Pradesh, (North India). The target species were mānuka seeds collected from the Pasture and Crop Research Unit (PCRU), Massey University (2024), ‘Nui’ perennial ryegrass (ryegrass) and ‘Kotuku’ white clover seeds (2024) were sourced from the

AsureQuality Seed Laboratory (New Zealand) and weed species seeds (fathen and crabgrass) were collected in Christchurch, New Zealand, in 2022. Allelopathic effects were assessed using two complementary approaches: root extract assays on steel blue germination paper and pot-based plant–plant interference assays.

5.2.2 Root sample preparation for LC–MS, metabolite data processing and analysis

Moringa root tissues (100 g) were collected from 120-day-old plants, snap-frozen in liquid nitrogen, freeze-dried, and ground (≈50–150 µm) before metabolite extraction. Metabolomic analysis followed the procedure of Barrett et al. (2024), with minor modifications. Briefly, root samples were collected between the months of July and November 2024 from moringa plants raised in a glasshouse at 25±3 °C. Approximately 200 mg of root tissues were collected and folded in aluminium foil, snap-frozen in liquid nitrogen before being transferred to −80 °C for storage. All samples were freeze dried then stored for 2 weeks at −20 °C prior to grinding to approximately 50–150 µm particle size before extraction. At least 50 ± 2.0 mg of ground sample were weighed into 2 mL Eppendorf microcentrifuge tubes, each with a 2.5 mm glass bead and extracted with 800 µL of pre-chilled chloroform CHCl3: MeOH (1:1 v/v). Samples were homogenised for 5 mins at 25 Hz sec−1 using a Retsch MM400 Mill/Tissue Lyser, then stored for 1 hr at −20 °C.

Then, 400 ml of Ultra High-Performance Liquid Chromatography (UHPLC) grade H2O was added to each tube, similarly homogenised for 5 mins, and centrifuged (Sigma 1–14K microcentrifuge) at 4 °C and 11000 RPM for 15 mins to create a biphasic layer. For each sample, 250 µl of the upper layer was pipetted into a 2 mL microcentrifuge tube for reverse phase C18 analysis and a second 250 µl aliquot taken for hydrophilic interaction

liquid chromatography (HILIC) analysis. A final 250 µl aliquot from each treatment was added to a 150 ml plastic tube, ultimately pooling every sample of each treatment into a homogenous mix, then sub-aliquots (250 µl) of this mix were transferred into microcentrifuge tubes to use as quality controls (QC's). All tubes were then dried down under a continuous flow of N2 (3.5 L min−1) and at 40 °C for 55 min using a BT Lab Systems sample concentrator, then immediately stored at −80 °C until reconstitution.

Reconstitution solvents which included an internal standard (MSK-QC-KIT), (Cambridge Isotope Laboratories Inc.) at a concentration of 10 µl ml−1 were prepared for C18 analysis in ACN: H2O (1:9, v/v) and for HILIC in ACN: H2O (1:1, v/v). Immediately prior to UHPLC-MS (Mass Spectrometry) analysis, all samples plus 7 quality controls (QCs) were reconstituted by adding 250 µl of solvent. Samples were vortexed until dissolved and transferred by pipette into a 250 µl glass insert in a clear autosampler vial, capped, kept dark and chilled until loading. The sequence was five vials of reconstitution solvent only (blanks),1 amino acid standard (A9906; Sigma-Aldrich, Auckland, New Zealand), two QC's then the samples with a QC every 9th slot to finish with a final amino acid. Analyses were conducted using a Thermo LC–MS system (Thermo Fisher Scientific, Waltham, MA, USA) consisting of a Dionex UltiMate 3000 UHPLC system coupled with a high-resolution Q Exactive Focus Quadrupole-Orbitrap mass spectrometer utilising heated electrospray ionisation to run in both positive and negative modes.

Thermo-derived raw files from C18 and HILIC (positive/negative) modes were converted to mzXLM (MSConvert) (Adusumilli et al., 2017) processed in MZmine (Du et al., 2020) for peak detection and alignment and further refined in XCMS Online "xcmsonline.scripps.edu" for exploratory analysis. Mode-specific parameters were

optimised for m/z deviation, peak width, noise, and signal-to-noise threshold to maximise feature detection. Background variability was reduced by removing chromatogram features with $p > 0.05$ or elevated in blanks (QC vs blank t-test). The resulting data matrices i.e., m/z, retention time, ion intensity were imported into MetaboAnalyst 6.0Citation 38 for quality control, removal of variables with high percent relative standard deviation (RSD) > 30% and auto-scaling. Gaussian distribution of variables was confirmed, ensuring comparability of feature intensities prior to statistical analysis.

A principal component analysis (PCA) was conducted to explore chemical composition and metabolite differences between two moringa provenances. Pathway enrichment analysis was conducted in MetaboAnalyst 6.0 for identified chemical classes and metabolite sets disproportionately represented among the detected allelochemicals relative to KEGG and HMDB background libraries. Overrepresentation was quantified using enrichment ratios and assessed for significance via Fisher's exact test with false discovery rate (FDR) correction ($p < 0.05$), enabling detection of dominant biosynthetic categories with potential ecological roles in allelopathic interactions.

5.2.3 Allelopathic screening using root extracts method on steel blue germination paper

To investigate the allelopathic effects of MRE, a three factorial experiment was established using a completely randomised design (CRD). The factors included (i) two moringa provenances: provenance-t and provenance-g; (ii) six extract concentrations, i.e., 0% (control, distilled water), 1%, 10%, 20%, 50%, and 100% (w/v); and (iii) five target species, including mānuka, ryegrass, white clover, fathen, and crabgrass, and five biological replications were used for each treatment. These concentrations were selected based on results from preliminary experiments carried out prior to the main study. These

preliminary tests were used to identify dilution levels that generated detectable allelopathic responses without causing complete germination failure, ensuring that the final concentration range captured both sub-inhibitory and inhibitory effects.

Aqueous root extracts were prepared by homogenising fresh roots collected from 120-day-old moringa plants grown under glasshouse conditions at temperatures ranging from 24–27 °C (±3 °C), while light and relative humidity were not controlled. The roots were soaked in double distilled water for 24 hours, followed by double filtration to obtain the working concentrations (w/v). To prepare the extract dilutions, a stock solution of MREs was first produced by homogenising a known mass of fresh root tissue in a defined volume of distilled water i.e., 1:10 w/v followed by filtration to obtain a liquid extract. This stock was treated as 100% extract, representing the undiluted filtrate. All subsequent concentrations (0−100%) were prepared by diluting the stock extract with distilled water.

For each treatment, the top-of-paper method (ISTA, 2025) was used, where 10 mL of each extract concentration was applied uniformly to Steel Blue Germination Paper (Anchor Paper Company, St Paul, MN), which was placed in plastic containers (15 × 12 × 10 cm, PS Limited, Auckland, New Zealand). Fifty seeds of each target species were placed on germination paper in a container and sealed with a lid before being placed into a controlled growth unit (Conviron Ltd., Canada). The germination conditions were set at 20/25 °C for white clover and mānuka and 20/30 °C (day/night temperatures) for perennial ryegrass, fathen, and crabgrass. Germination was scored as radicle emergence (RE) (2 mm) every 4 h for ryegrass and white clover. The final germination count (normal seedlings) was determined seven days after sowing (ISTA, 2025). For mānuka, fathen, and crabgrass, RE was observed every 24 hours, and the final count was performed 21 d after sowing. These observation periods were selected based on the results from

preliminary experiments carried out prior to the main study. Germination (%) was calculated on the basis of the final count of normal seedlings (seedlings without germination defects) as a fraction of the total number of seeds planted multiplied by 100. The mean germination time (MGT) was calculated following the formula of Ellis and Roberts of Ellis and Roberts (1981):

$$\mathrm{MGT} = \frac{\sum(\mathrm{ni} \cdot \mathrm{ti})}{\sum \mathrm{ni}}$$

where n_i = the number of seeds that germinated on day t_i, ti = the number of days from the beginning of the experiment, and ∑ni = the total number of seeds that germinated at the final count.

5.2.4 Pot based plant–plant interference assay for screening moringa root allelochemicals

Besides the use of MREs, the allelopathic potential of moringa root allelochemicals were assessed through the pot-based assay following the procedures of Wu et al. (2001), with minor modifications. Briefly, a single moringa plant and fifty seeds of each target species were co-cultivated in 12.5 cm diameter pots with seeds sown in a circular pattern around the moringa plant. Each pot was filled with 1500 g of sterilised sand and compost mixed at a ratio of 1:2 (v/v) to serve as the growth medium. The target species were introduced approximately 120 d after moringa was established to ensure full root distribution within the pots. A bioassay ring (10 cm inner diameter, 2 cm height) was gently pressed onto the pot surface, leaving the moringa litter layer undisturbed. Within each ring, fifty surface-sown seeds of the target species (one species per pot per time) were evenly spaced, lightly covered (≈2 mm) with the same potting substrate, and sieved through a 2 mm mesh to

standardise seed–soil contact. Pots without moringa plants were included as positive control treatments.

The target species were mist irrigated daily with deionised water, and no fertiliser was applied during the experiment. The germination percentage (radicle ≥ 2 mm) was recorded 7 d after sowing (DAS) for white clover and ryegrass (ISTA, 2025), 21 DAS for fathen and crabgrass, and 28 DAS for mānuka (DAS for each species were selected from preliminary experiments). At 10 DAS, five randomly selected white clover and ryegrass seedlings per ring were gently excavated, and their root and shoot lengths (cm) were measured using digital callipers; at 22 DAS, the same measurements were taken for crabgrass and fathen; and at 29 DAS for mānuka. The remaining white clover and ryegrass seedlings were harvested at 12 DAS, 22 DAS for crabgrass and fathen, and 30 DAS for mānuka, oven dried at 70 °C for 72 hours and weighed separately to determine shoot and root biomass (g).

5.2.5 Statistical analyses for response species

To determine the concentration effects of the MRE, data were subjected to general linear models (GLMs) to account for potential non-normal distributions in the response variables using R-statistical package ver. 4.4.2 (R-CoreTeam, 2024). Fixed effects included variety, concentration, species, and their interactions; whereas replicates were treated as random effects to account for variability across experimental runs. The significance of fixed effects was tested using Type III fixed effects, which evaluate the significance of each factor after accounting for all other fixed effects and their interactions in the model, and treatment effects were evaluated relative to the control treatment (distilled water). When the interaction was significant, the concentration effect was probed by comparing the concentration effect within each species using Tukey–Kramer-

adjusted least-square means (LSMeans) ($p < 0.05$), and estimates are presented as LSMeans ± SEs.

For the pot-based plant–plant interference experiments, statistical analysis was performed using one-way ANOVA to test for significant differences among the treatments, and mean separation was conducted with Tukey's HSD test ($p < 0.05$).

The allelopathic potential of the aqueous root extracts and root exudates was determined in terms of germination, root and shoot length, and root and shoot biomass. The degree of inhibition or stimulation was determined by comparing the growth responses of the seedlings of the target species to those of the control plants via the allelopathic response index (ARI) via the following equation according to Wang et al. (2022):

$$RI = 1\text{-}C/T\ (T \geq C),\ RI = 1\text{-}T/C\ (T < C)$$

where T represents the data from the treatment variables, C represents the data of the control group, $RI < 0$ represents an inhibitory effect and $RI > 0$ represents a stimulating effect. A heatmap was used to determine the extent of the allelopathic effect, i.e., deep red (RI) ≤ -0.50 indicates strong inhibition, whereas deep blue (RI) ≥ 0.25 indicates a potential stimulatory effect on the target species.

5.3 Results

5.3.1 Metabolite profiling and allelochemical identification in moringa provenances

Principal component analysis (PCA) revealed considerable overlap in the metabolite profile of the moringa provenances (g and t) without clear clustering and/or significant separation along the principal component axes (Figure 10). The 95% confidence ellipses

for both provenances converged showing high similarity in metabolite composition. Consistently, permutational multivariate analysis of variance (PERMANOVA) confirmed that the differences in metabolite profiles between the two moringa provenances were not statistically significant ($p > 0.05$).

A total of 3167 molecular features (metabolites) were detected in the MREs across the four ionisation modes of C18 and HILIC (positive and negative), of which 52 were successfully annotated based on MS/MS fragmentation and spectral-library matching, while the remaining 3115 remained unidentified. Annotated metabolites were considered to exhibit allelopathic interactions when MREs showed a significant, concentration-dependent inhibitory effect on germination or early seedling growth, demonstrated consistently across both moringa provenances and relative to the control treatment (distilled water). These compounds were dominated by phenylpropanoids and benzenoids, with smaller contributions from organic acids, oxygenated compounds, and organoheterocyclics. Among the 52 metabolites, 36 were annotated with level three identification confidence (putatively characterised on the basis of spectral similarity; see Appendix 2.1), whereas 16 metabolites achieved level two confidence confirmed by spectral library matching (Table 7).

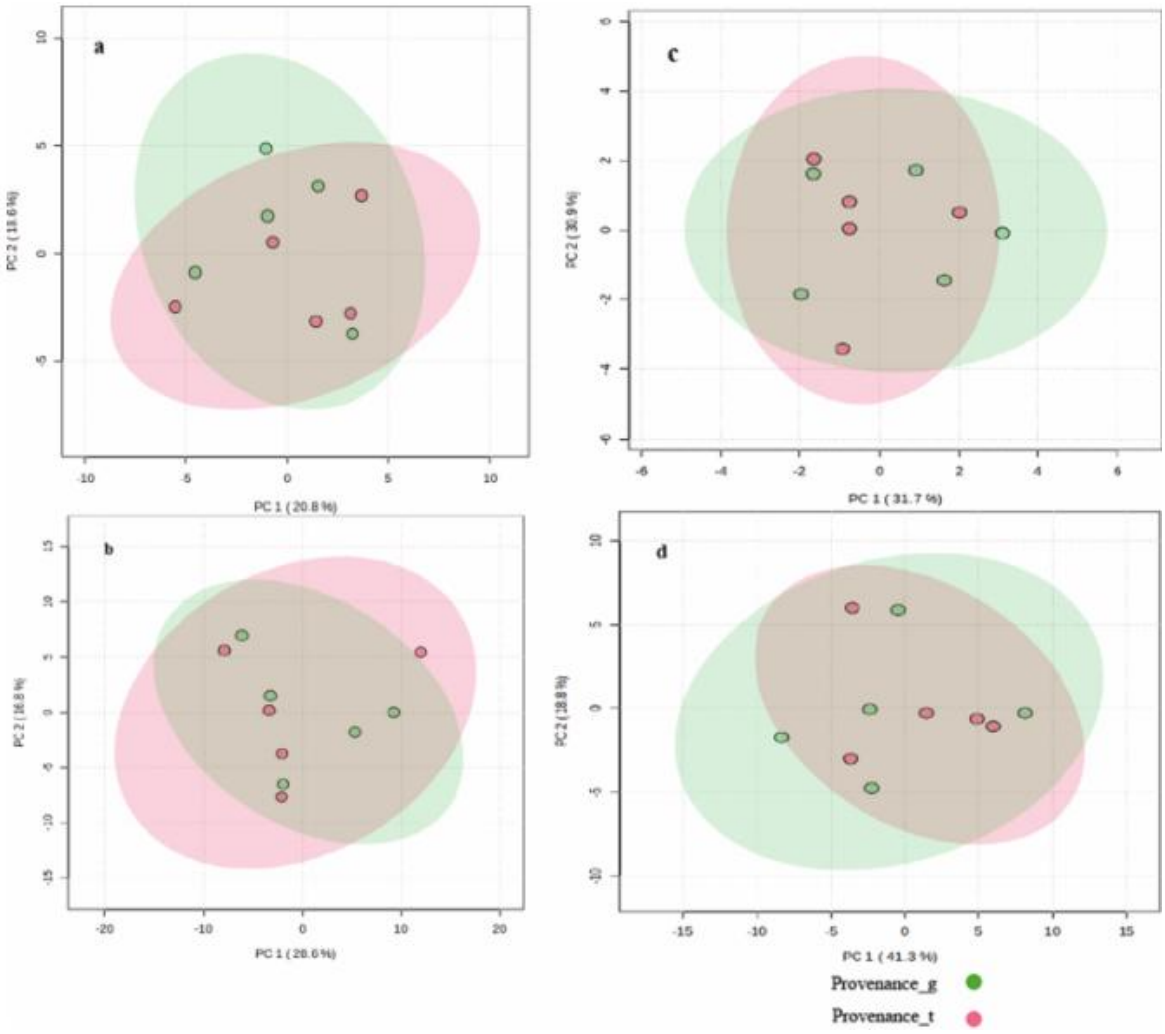


Figure 10. Principal component analysis (PCA) score plots comparing the metabolite profiles of two moringa provenances under different ionisation modes: a) C18 positive ($F = 0.5973$, $R^2 = 0.069$, $p = 0.5410$), b) C18 negative ($F = 0.05762$, $R^2 = 0.0720$, $p = 0.9470$), c) HILIC positive ($F = 0.8129$, $R^2 = 0.0922$; $p = 0.4570$), and d) HILIC negative ($F = 0.2646$, $R2 = 0.0327$, $p = 0.5745$). The 95% confidence ellipses show partial overlap between the provenances in all ionisation modes, which indicates a high degree of similarity in their metabolomic profiles. The minor variation observed may reflect environmental influences from their original provenances. PERMANOVA (999 permutations) confirmed that the differences between groups were not statistically significant ($p > 0.05$).

Table 7. Identified allelopathic metabolites from moringa root extract with level 2 confidence based on LC-MS/MS spectral library matching.

Class/Sub/sup class	Compound Name	KEGG	Stream	m/z	rt.	Fragments
Cinnamic acids	M-trans-cinnamic acid	-	C18 +	163.0751	8.30	132.0507, 103.0530
Hydroxycinnamic acids	Caffeic acid	C01481	C18 +	181.0492	7.79	163.0373
Flavonoids	Naringenin	C00509	C18 +	273.0752	8.89	123.0421, 119.0455
Coumarins	4-Hydroxycoumarin	C20414	C18 +	163.0387	7.45	65.0367
Flavonoids	Isoquercetin	C05623	C18 +	465.1021	9.17	303.0433, 304.0470
Flavonoids	Epicatechin	C09728	C18 +	291.0857	8.31	139.0362
Coumarins	Umbelliferon	C09315	C18 +	163.0387	7.45	65.0077
Phenylpropanoids	Coumarin	C05851	C18 +	147.0438	8.30	103.0519
Methoxyphenols	Homovanillic acid	C05582	C18 +	183.0649	11.49	153.0541, 107.0486
Flavonoids	Naringin	C09789	C18+	581.1855	9.13	563.1536
Flavonoids	(-)-Epicatechin	C09727	C18-	289.0711	8.28	245.0879
Alpha-Amino acids	L-Phenylalanine	C00079	Hilic pos	166.086	14.21	121.084
N-acyl-alpha amino acids	Indole-3-acetyl-L-tryptophan	-	C18 +	362.1496	8.25	344.1028
Alpha-Amino acids	L-Tryptophan	C00078	Hilic pos	205.0968	14.65	188.0687
Dihydrochalcones	Aspalathin	-	Hilic neg	451.1236	7.47	332.0806
Carboxylic acids	Glutaric acid	C00489	Hilic neg	131.0339	15.36	113.0272

The identification of the compounds followed the level 2 criteria of the metabolomics standards initiative (MSI), m/z = mass–charge ratio, rt. = retention time.

5.3.2 Enrichment analysis of identified allelochemicals in moringa provenances

The enrichment of bioactive chemical compounds was evaluated by quantifying feature abundance relative to the control (blanks) treatments, which served as the baseline for determining enriched metabolites. Pathway enrichment analysis of the moringa root allelochemicals revealed high isoflavonoids biosynthesis (0.97); significant enrichment for phenylalanine, tyrosine, and tryptophan biosynthesis (0.79), and a strong contribution from flavone/flavonol and broader flavonoid biosynthesis (0.69). Other significantly enriched pathways included glucosinolate, flavonoid, and tyrosine metabolism and CoA

biosynthesis (Figure 11). These pathways are driven by hydroxycinnamic acids (caffeic, ferulic, and 4-methoxycinnamic acids), flavonoids and glycosides (quercetin, kaempferol, and luteolin), coumarins (scopoletin and umbelliferone), and indolic derivatives (DOPA and indole-3-carboxylic acid).

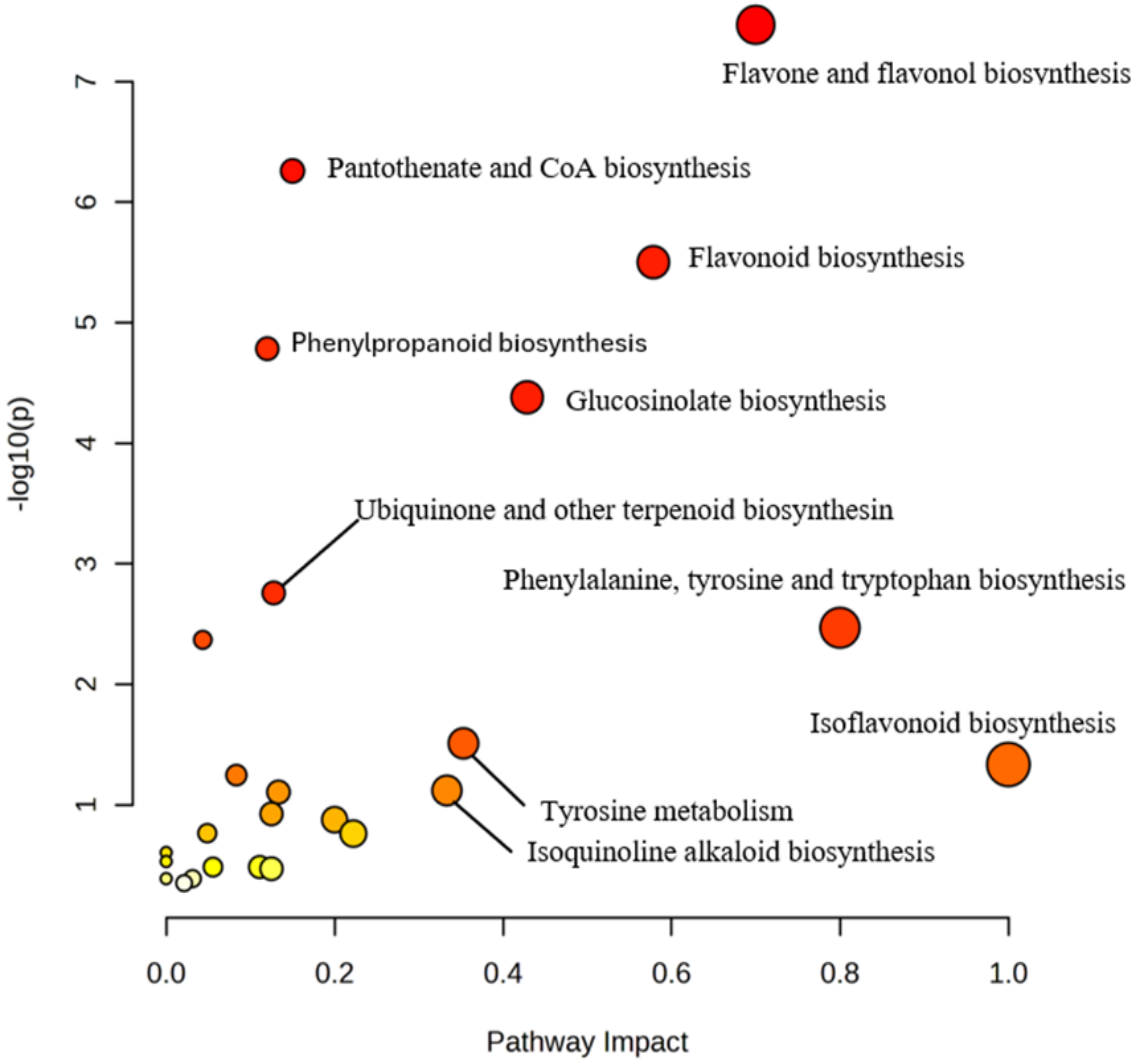


Figure 11. Pathway enrichment analysis of moringa root metabolites revealed significant flavonoid/ isoflavonoids biosynthesis; phenylalanine, tyrosine, and tryptophan biosynthesis; and flavone and flavonol biosynthesis, driven by phenolic acids, flavonoids, and coumarins.

5.3.3 Effects of moringa root extracts on the germination and growth of target plant species

Type III fixed effects ANOVA revealed significant interspecific variation in the responses of target species to moringa allelopathic treatments, which indicated that each species exhibited a distinctive sensitivity profile to the applied extract concentrations. The allelochemical concentration, target species, and their combination significantly influenced seed germination, mean germination time (MGT), shoot and root length, and shoot and root biomass ($p < 0.05$). However, the effects of moringa provenance alone or in combination with other factors were not significant (Table 8).

The combined concentration effect analyses indicated that germination and seedling growth traits varied significantly among the target species in response to increasing extract concentrations (Figure 12a-f). Although germination decreased with increasing extract concentration, the magnitude of inhibition was strongly species-specific and concentration dependent. Perennial ryegrass maintained high germination percentages associated with reduced MGT across all the extract concentration gradients.

In contrast, white clover, crabgrass, and fathen were sensitive to allelochemicals, with germination declining continuously from the 10% concentration, falling below 50% at the full-strength extract (100%) and subsequently increasing the MGT. Mānuka displayed an intermediate response with increased MGT at a 100% concentration. The germination and seedling growth traits at relatively low extract concentrations (1–10%) did not significantly differ from those in the control treatment for most target species. However, the inhibitory effects became increasingly visible at higher concentrations (20–100%) in more sensitive species, such as white clover.

Table 8. Type III tests of the fixed effects of mean germination time (MGT), germination, root length, shoot length, and root and shoot dry biomass were performed, with the fixed factors of species, provenance, concentration, and their interaction.

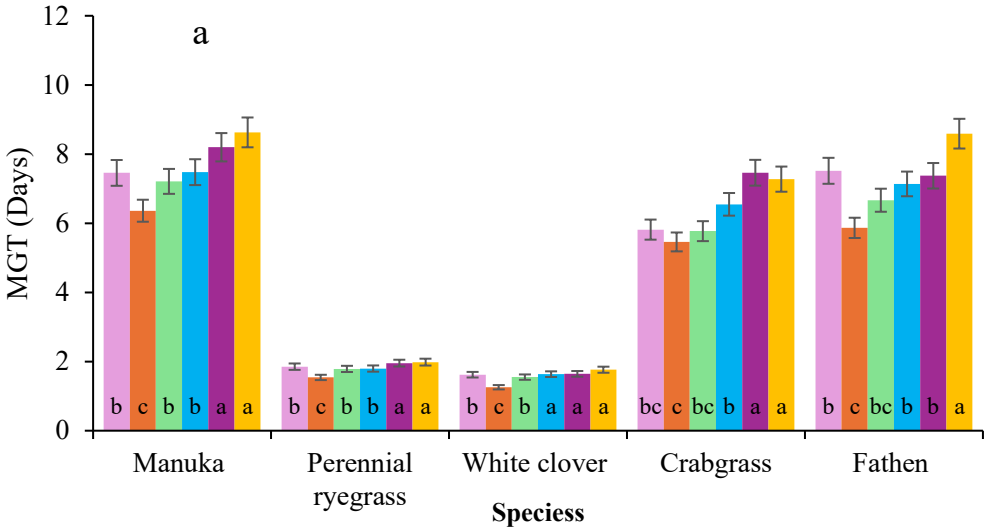
Effect	**MGT**		**Germination**		**Root length**		**Shoot length**		**Root biomass**		**Shoot biomass**	
	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value
S	6808.03	< .001	614.67	< .001	29434.2	< .001	31276.6	< .001	6613.56	< .001	31300.7	<.001
P	0.01	0.9324	0.51	0.4348	0.1421	0.9918	0.21	0.9054	0.18	0.6696	0.18	0.6722
C	191.73	< .001	237.35	< .001	863.56	< .001	168.42	< .001	238.56	< .001	276.94	<.001
S x P	0.21	0.9309	0.82	0.6149	0.5413	0.7041	0.25	0.9054	0.65	0.6293	0.06	0.9937
S x C	23.69	< .001	47.93	< .001	117.56	< .001	68.94	< .001	46.63	< .001	163.93	<.001
P x C	0.90	0.4820	0.34	0.8428	0.47	0.7962	0.14	0.9831	0.52	0.7616	0.06	0.9973
S x P x C	1.43	0.1271	1.32	0.1639	0.45	0.9801	0.15	1.0000	0.36	0.9951	0.09	1.000

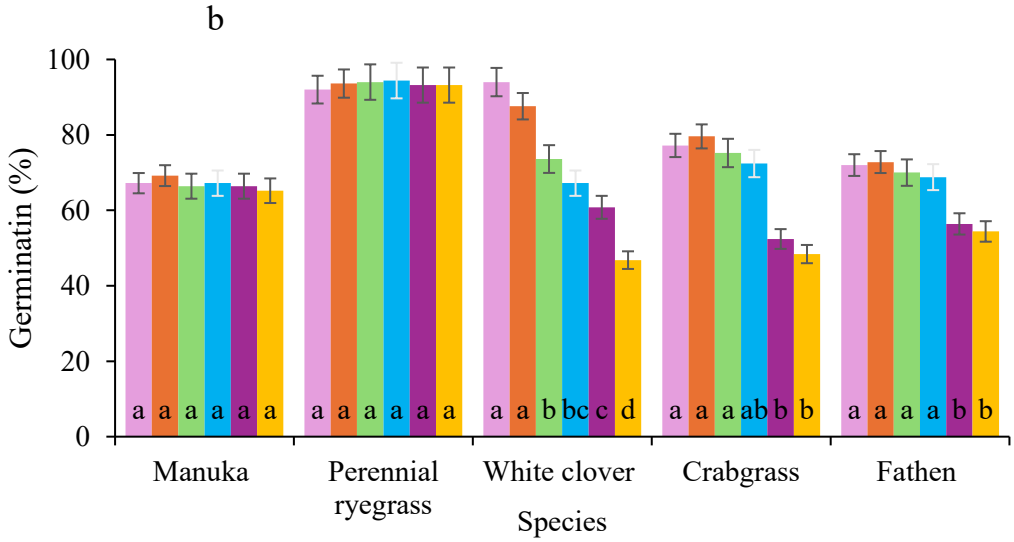
S = species, P = provenances, C = concentration, S × P = species × provenance, S × C = species × concentration, P × C = provenance × concentration, and P × S × C = provenance × species × concentration interactions. Differences were considered significant at $P < 0.05$.

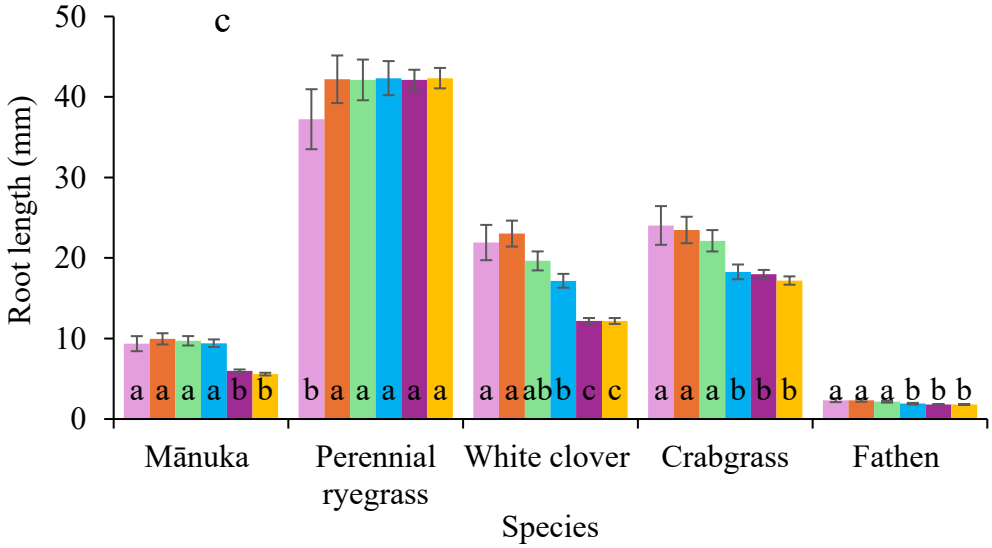
Target species: perennial ryegrass, white clover, fathen, crabgrass, and mānuka.

Moringa provenances: provenance_t from Tamil Nadu and provenance_g from the villages of Deoria district, Uttar Pradesh, India.

Concentration gradients: 0, 1%, 10 20, 50, and 100% (w/v).


a
MGT (Days)
0
2
4
6
8
10
12
b c b b a a
b c b b a a
b c b a a a
bc c bc b a a
b c bc b b a
Manuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Speciess


b
Germinatin (%)
0
20
40
60
80
100
a a a a a a
a a a a a a
a a b bc c d
a a a ab b b
a a a a b b
Manuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Species


c
Root length (mm)
0
10
20
30
40
50
a a a a b b
b a a a a a
a a ab b c c
a a a b b b
a a a b b b
Mānuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Species

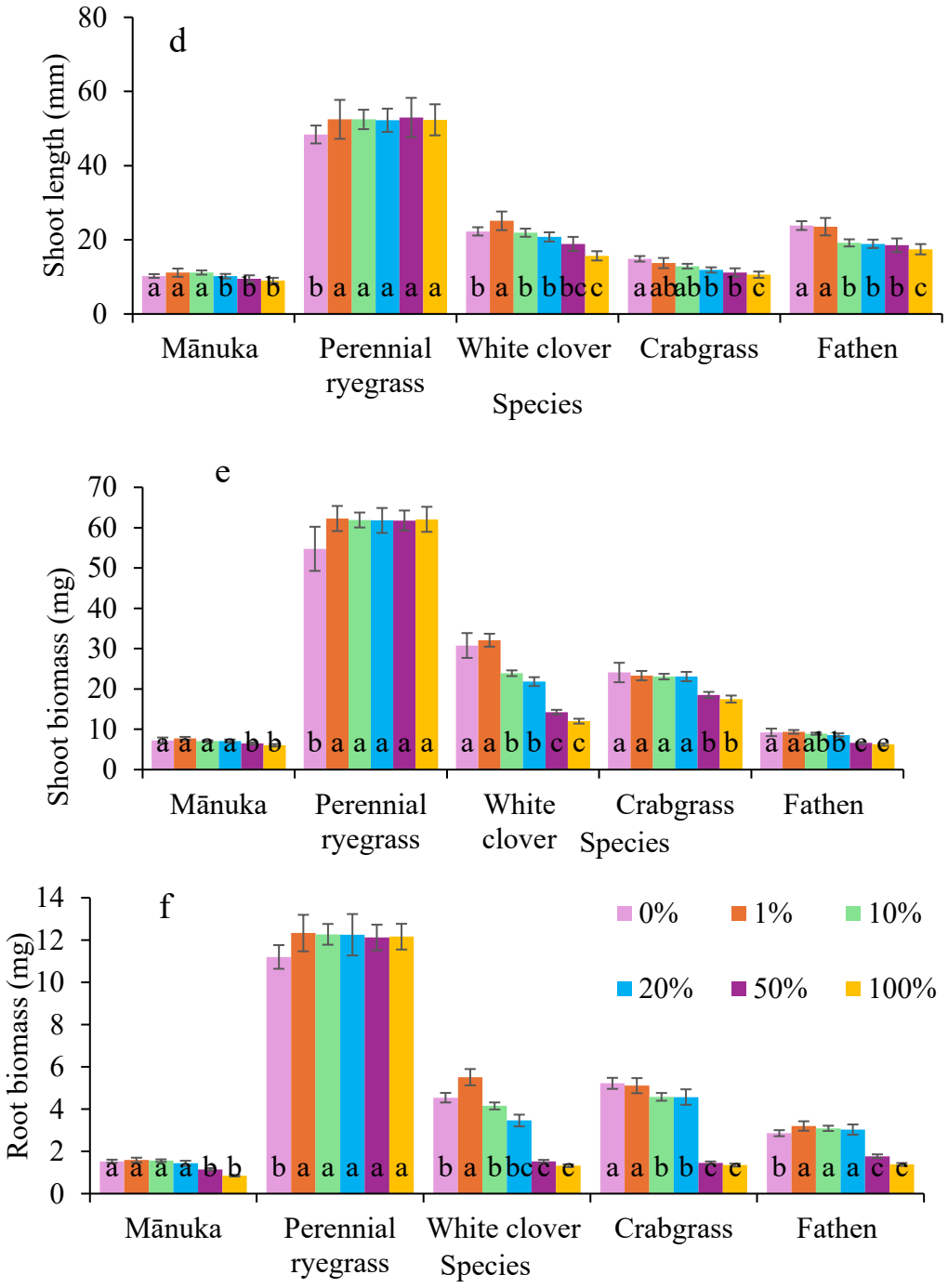


Figure 12. Effects of different concentrations (0, 1, 10, 20, 50, or 100%) of MRE on the germination and seedling growth traits of five target species using GLMs 'within each species' comparisons of a) MGT, b) germination, c) root length, d) shoot length, e) root biomass, and f) shoot biomass. Different letters inside the bars denote significant differences among concentration gradients for each species ($P < 0.05$). The values represent the LS means ± SE, with 5 replicates per treatment.

The germination and seedling growth data were used to assess the allelopathic response index (ARI) to quantify the allelopathic activity of MRE. Crabgrass, fathen, and white clover showed progressive inhibition with increasing extract concentration, whereas perennial ryegrass exhibited a slight stimulatory effect across concentration gradients (Table 9). At low concentrations (1–10%), MRE enhanced germination and reduced MGT in most species. However, concentrations above 20% led to moderate to severe suppression of germination and a corresponding increase in MGT. Perennial ryegrass recorded positive ARI values (0.001−0.100), indicating a mild stimulatory influence on both germination and seedling growth. In contrast, concentrations between 20% and 100% induced significant inhibitory effects on all the traits except perennial ryegrass, whereas mānuka shifted toward inhibition in MGT, root biomass, and shoot length at relatively high concentrations.

Table 9. Allelopathic response indices of various species in response to different MRE concentrations

		Means ± SEs Allelopathic Response index				
Parameter	C (%)	Crabgrass	Fathen	Mānuka	Ryegrass	Clover
MGT	1	0.04±0.05	0.32±0.03	0.14±0.01	0.19±0.01	0.22±0.01
	10	0.00±0.01	0.01±0.02	0.03±0.01	0.04±0.02	0.01±0.03
	20	-0.08±0.04	-0.01±0.02	0.00±0.03	0.04±0.01	-0.02±0.07
	50	-0.61±0.12	-0.42±0.03	-0.10±0.01	-0.05±0.02	-0.33±0.10
	100	-0.64±0.11	-0.44±0.06	-0.16±0.01	-0.07±0.02	-0.46±0.12
Germination	1	0.04±0.04	0.04±0.04	0.03±0.01	0.01±0.02	0.08±0.04
	10	-0.03±0.04	-0.03±0.04	0.01±0.02	0.03±0.01	-0.08±0.08
	20	-0.31±0.02	-0.05±0.02	0.00±0.012	0.03±0.01	-0.41±0.06
	50	-0.43±0.06	-0.28±0.03	-0.02±0.04	0.03±0.01	-0.55±0.10
	100	-0.47±0.10	-0.33±0.04	-0.03±0.01	0.02±0.02	-0.52±0.08
Root biomass	1	0.00±0.02	0.08±0.05	0.04±0.02	0.09±0.03	0.14±0.09
	10	-0.16±0.08	0.03±0.01	0.07±0.03	0.10±0.02	-0.02±0.07
	20	-0.140.06	0.02±0.02	0.01±0.01	0.08±0.03	-0.32±0.10
	50	-0.47±0.12	-0.12±0.09	-0.29±0.02	0.07±0.03	-0.48±0.17
	100	-0.43±0.14	-0.15±0.13	-0.26±0.03	0.08±0.02	-0.56±0.14
Shoot biomass	1	0.03±0.01	0.00±0.03	0.09±0.02	0.12±0.04	0.04±0.07
	10	-0.04±0.02	0.05±0.02	0.08±0.05	0.12±0.07	-0.30±0.10
	20	-0.05±0.02	-0.08±0.02	0.05±0.05	0.11±0.06	-0.46±0.09
	50	-0.12±0.03	-0.42±0.06	0.01±0.03	0.11±0.01	-0.34±0.14
	100	-0.37±0.02	-0.5±0.06	0.02±0.04	0.12±0.02	-0.55±0.24
Root length	1	0.03±0.03	0.01±0.02	0.02±0.04	0.02±0.01	0.05±0.06
	10	-0.03±0.01	0.01±0.02	0.04±0.03	0.02±0.01	-0.02±0.01
	20	-0.31±0.02	-0.03±0.01	0.00±0.03	0.03±0.02	-0.29±0.01
	50	-0.35±0.04	-0.28±0.02	-0.28±0.07	0.02±0.01	-0.59±0.11
	100	-0.41±0.03	-0.29±0.03	-0.34±0.14	0.03±0.02	-0.61±0.13
Shoot length	1	0.09±0.02	0.01±0.02	0.00±0.01	0.08±0.01	0.11±0.05
	10	-0.04±0.01	-0.03±0.02	0.04±0.02	0.07±0.03	-0.02±0.01
	20	-0.25±0.03	-0.27±0.02	0.03±0.02	0.07±0.02	-0.08±0.02
	50	-0.32±0.05	-0.29±0.03	-0.10±0.03	0.08±0.01	-0.58±0.11
	100	-0.39±0.07	-0.36±0.03	-0.07±0.03	0.08±0.01	-0.62±0.09

C = concentration, deep red ≤ -0.50 indicates strong inhibition, whereas deep blue ≥ 0.25 indicates a potential stimulatory effect on target species.

5.3.4 Plant–plant interactions on germination and seedling growth

Assays using target seeds planted in the same soil as potted moringa plants also revealed significant variations in germination and seedling growth among response species (See Appendix 2.2). Perennial ryegrass and mānuka seed germination were not significantly affected. However, their root and shoot lengths and root and shoot biomass were significantly enhanced compared to the control treatment. In contrast, crabgrass, fathen, and white clover showed strong allelopathic inhibitory effects, with significant reductions in germination and suppressed root and shoot development ($p < 0.001$). Both moringa provenances (t and g) followed similar response patterns without significant differences between provenances in terms of their germination and seedling growth traits.

The ARI analysis further revealed species-specific effects on the plant–plant interactions between moringa and the target species. Crabgrass and fathen showed inhibitory responses across all the evaluated growth parameters, with the strongest suppression observed for root biomass, whereas white clover displayed significant reductions in root length and accumulation of biomass. In contrast, mānuka showed neutral to stimulatory response, as revealed by positive ARI values across all growth parameters in shoot length and root biomass, indicating a potential growth stimulation effect. However, tolerance to allelopathic effects was observed in perennial ryegrass, where responses were minimal. These allelopathic patterns indicate that MREs exert differential allelopathic pressure across species, and such species-specific inhibition against key pasture legumes and selected weed species could manifest under natural field conditions.

Table 10 present ARI values derived from the in-pot plant-plant interaction experiment, where moringa plants released allelochemicals directly into the shared substrate that allowed both chemical and rhizosphere-mediated interactions to influence target species

performance. These ARIs are stronger than those observed in the extract-based assays in Table 9 which reflect the combined effects of continuous root exudation and longer exposure duration. However, both experiments showed similar species-specific trends, with strong inhibition in white clover, fathen, and crabgrass, and neutral to mildly stimulatory responses in mānuka and perennial ryegrass. Nevertheless, the magnitude of inhibition was greater in the in-pot system than in extract-based assays. While root extracts capture concentration-dependent allelopathic effects, the in-pot assay more closely reflects the cumulative allelopathic pressure likely to occur under natural filed conditions. Most importantly, despite differences in assay type, neither experiment detected significant provenance-specific differences in allelopathic potency.

Table 10. Allelopathic response indices (RIs) of crabgrass, fathen, mānuka, ryegrass, and white clover to the root exudates of two moringa provenances.

		Means ± SEs Allelopathic Response Index				
Parameter	P	Crabgrass	Fathen	Mānuka	Ryegrass	Clover
Germination	g	-0.52±0.11	-0.38±0.03	0.12±0.02	0.01±0.01	-0.25±0.03
	t	-0.59±0.12	-0.35±0.02	0.13±0.03	0.02±0.02	-0.22±0.03
Root length	g	-0.56±0.05	-0.13±0.10	0.24±0.06	0.00±0.01	-0.08±0.01
	t	-0.55±0.11	-0.14±0.05	0.23±0.04	0.00±0.01	-0.07±0.02
Shoot length	g	-0.67±0.03	-0.18±0.02	0.39±0.02	0.13±0.05	-0.53±0.05
	t	-0.69±0.06	-0.16±0.01	0.41±0.02	0.12±0.03	-0.57±0.03
Root biomass	g	-0.58±0.15	-0.59±0.09	0.36±0.05	0.11±0.06	-0.4±0.19
	t	-0.54±0.12	-0.61±0.14	0.34±0.02	0.10±0.06	-0.37±0.34
Shoot	g	-0.29±0.04	-0.53±0.04	0.31±0.03	0.1±0.04	-0.52±0.05
biomass	t	-0.26±0.12	-0.5±0.03	0.32±0.01	0.13±0.02	-0.51±0.11

P = provenances, deep red means the response index is ≤ -0.5 (signalling strong inhibition), and deep blue means ≥ 0.3 (potential promotion/stimulation effects).

5.4 Discussion

The results of the present study revealed that moringa root allelochemicals have biological effects on other plants in a species-specific and concentration-dependent manner. The differential response to allelochemical concentrations among target species supports biochemical interactions between plants, which are mediated by the release of secondary metabolites in naturally competitive ecosystems (Hierro & Callaway, 2021). Germination and seedling growth suppression at relatively high concentrations (50–100%) concur with the dose-dependent principle in which the phytotoxic effect intensifies with increasing concentrations (El-Rokiek et al., 2022). In the context of New Zealand's pastoral systems where perennial ryegrass and white clover form a cornerstone of productive pastures (Cartmill & Donaghy, 2024; Romera et al., 2017), the introduction of moringa could shift the competitive balance among species, potentially altering plant community composition in this agroecosystem.

The highly significant interaction effect between species and concentration confirmed that species respond differently to allelopathic stress (Hierro & Callaway, 2021). These interactions further indicate varying sensitivities or tolerances that may influence competitive hierarchies and species coexistence (Zhang et al., 2021). Certain species may exhibit detoxification mechanisms or protective root exudates that may buffer the effects of allelochemical inhibition, hence conferring a competitive advantage under allelopathic stress (Latif et al., 2017). Among the treatments, perennial ryegrass presented the highest germination rate and the most vigorous seedling traits. This may indicate an inherent tolerance to allelochemicals through root-mediated detoxifying mechanisms or by microbiome interactions, as proposed by Scavo et al. (2019). In contrast, the growth of white clover, fathen, and mānuka were significantly suppressed at relatively high extract

concentrations, indicating increased sensitivity or reduced tolerance to one or more bioactive compounds found in moringa roots.

The sensitivity of white clover in this study aligns with the vulnerability of legumes to phenolic acids and flavonoids, which disrupt root physiology and nodulation at relatively high concentrations (Đorđević et al., 2022). In contrast, perennial ryegrass exhibited resilience despite exposure, which may reflect inherent structural adaptations traits such as stronger cell wall integrity or root exudation patterns that could hypothetically buffer allelochemical stress (Jones et al., 2025). This selective inhibition could bias species composition towards ryegrass dominance at the expense of clover, with downstream consequences for nitrogen fixation and long-term soil fertility in pastoral systems.

The allelopathic capacity of moringa has traditionally been attributed to flavonoids and phenolic acids from leaf extracts (Zulfiqar et al., 2020). However, metabolomic profiling combined with pathway enrichment analysis has started to unveil how specific chemical classes of root allelochemicals may function in ecological interactions.

The absence of significant separation between provenance_g and provenance_t in the PCA as shown in figure 9 demonstrates the high degree of similarity in their allelochemical profiles. This chemical convergence likely stems from genotypic similarities between the two provenances and emphasises the central role of intraspecific genetic relatedness in shaping the expression of allelochemicals involved in plant–plant chemical interactions (Inderjit & Duke, 2003; Kong et al., 2024). Since plants were grown under the same conditions, it is not surprising that no differences were observed. However, future studies should investigate how moringa root allelochemicals respond to environmental variation.

Several allelochemicals, including phenolics (ferulic, caffeic, and chlorogenic acids), flavonoids (apigenin, naringenin, and isoquercetin), and coumarins (umbelliferone and 4-hydroxycoumarin), were annotated in moringa roots. These compounds are known to impede germination and initial seedling development by altering hormonal signalling pathways and redox homeostasis in susceptible plant species (El-Rokiek et al., 2022; Perveen et al., 2021). In this study, the decline in germination and seedling traits across most target species (except ryegrass) highlights the inhibitory nature of allelochemicals that disrupt hormonal regulation, cell elongation, and impaired nutrient mobilisation (Hussain & Reigosa, 2021; Zulfiqar et al., 2020). The reduction in root and shoot length could be attributed to the presence of phenylpropanoid derivatives, which are known to interfere with essential cellular processes, including reactive oxygen species (ROS), homeostasis, and auxin transport, leading to oxidative stress and reduced cell elongation (Bachheti et al., 2020; Yan et al., 2016).

The contrasting responses observed between the extract bioassays and the pot experiment demonstrate that the allelopathic effects of moringa are influenced by the mode of allelochemical exposure. Germination and seedling growth were strongly inhibited in the extract bioassays, where target species were directly exposed to defined concentrations of water-soluble compounds. In contrast, the pot experiment produced comparatively weaker or species-specific responses despite the presence of well-established moringa plants. These differences suggest that the biological activity of allelochemicals under natural conditions is moderated by soil processes, including adsorption, microbial degradation, dilution, and root-mediated release, which collectively influence their bioavailability and persistence (Inderjit and Duke, 2003; Cheng and Cheng, 2015). Consequently, the extract bioassays represent the maximum allelopathic potential of

moringa, whereas the pot experiment provides a more realistic assessment of plant–plant interactions under soil conditions.

The enrichment of the phenylpropanoid and flavonoid pathways highlights their functional role in mediating the observed interspecific variability in growth inhibition. Hydroxycinnamic acids and flavonols derived from these pathways can disrupt amylase activity during germination, auxin transport, and cell wall expansion in young seedlings (Bu et al., 2018; Hima et al., 2024). These mechanisms constrain radicle emergence and seedling establishment, as observed in white clover in both laboratory and glasshouse experiments. L-Phenylalanine and caffeic acid are the central intermediaries in the phenylpropanoid biosynthesis pathway, which regulates the production of phenolics that serve as allelochemicals (Tak et al., 2023). These pathways may explain the strong suppression and inhibition of germination and seedling growth in white clover, fathen, and crabgrass and the lack of measurable effects on ryegrass and mānuka, further revealing species-specific allelopathic interactions. Compounds enriched in coumarin, benzoic, and indolic metabolism are known to inhibit germination and root elongation in small-seeded annuals (Zhang et al., 2023).

Pathway analysis further revealed that moringa synthesises glucosinolates widely reported in moringa tissues (Lopez-Rodriguez et a., 2020; Borgonovo et al., 2020). The annotation of 1-isothiocyanato-9-(methylsulfinyl)-nonane in root extracts supports the presence of bioactive isothiocyanates generated through myrosinase-mediated hydrolysis, compounds that are known to exert phytotoxic and signalling effects in other related species such as brassicales (Di Gioia et al., 2018). Beyond glucosinolates, significant enrichment of flavonoids, flavanol, isoflavonoid pathways concur with previous studies in which moringa accumulates diverse phenolic acids (e.g., caffeic,

chlorogenic, p-coumaric) and flavonoid glycosides (quercetin, kaempferol), all of which are implicated in allelopathic interference and hormonal modulations (Kamanga et al., 2025; Buthelezi et al., 2013). Additional enrichment of terpenoid, tyrosine, and isoquinoline-alkaloid pathways suggests contributions from broader secondary metabolites classes, which aligns with prior evidence of complex phytochemical defence in moringa (Perveen et al., 2021; El-Rokiek et al., 2022). Henceforth, the glucosinolates, isothiocyanates, phenolic acids, and flavonoids identified in the present study provide a coherent biochemical basis for the inhibitory effects observed in the root-extracts and in-pot assays, which may support a multi-compound allelopathic mechanism.

In addition to direct phytotoxicity, the enrichment of these compounds may contribute to the competitive exclusion of some species through micronutrient sequestration. For example, several phenylpropanoid derivatives exhibit strong chelating affinities for essential metals such as Fe^{3+} and Zn^{2+} (Anjitha et al., 2021; Chen et al., 2022). This may limit nutrient uptake in cooccurring species such as white clover, causing a detrimental effect on seedling growth. Given the high nutrient demands of white clover (for nitrogen fixation), fathen (fast-growing annual species), and crabgrass (competitive C4 grass), (Abbasi & Khan, 2004; Alshallash, 2018; Oreja et al., 2025), micronutrient limitation could be a significant contributor to the observed growth suppression. Moringa, through allelopathic activity, could further disrupt perennial ryegrass–white clover associations that underpin pastoral productivity and alter the balance between native and introduced species (Effah & Clavijo McCormick, 2024). These chemically mediated interactions demonstrate that while moringa has potential as a beneficial crop, it may have negative impacts on native and pastoral species emphasizing the complex ecological effects of introducing alien species on native and agricultural ecosystems (Galappaththi et al., 2023; Weidenhamer and Callaway, 2010; Ewel et al., 1999).

5.5 Conclusion

Moringa root allelochemicals elicit concentration-dependent and species-specific effects on other native and introduced plant species. White clover, a key pasture species in New Zealand, was the most negatively affected among the tested species. Hence, the utilisation of moringa within clover pastures may not be recommended. Perennial ryegrass, on the other hand, showed no significant response to root extracts or exudates, making it more suitable for mixed cropping. Moringa was also observed to have an inhibitory effect on some common weeds, which may add value to its use in some farming systems. The enrichment analysis indicated that moringa allelochemicals are dominated by phenylpropanoids, benzenoids, and related aromatic derivatives that can influence biosynthesis pathways, transport mechanisms, and micronutrient competition. The identification of these allelopathic compounds highlights the importance of evaluating moringa not only as a crop species but also as a potential ecological driver that requires integrated management strategies to balance its agronomic value with potential risks to biodiversity and ecosystem function. Further studies should prioritise the isolation of individual compounds and further testing on pastoral, weed, and native species across different environmental contexts to disentangle their specific roles in plant growth and plant population dynamics and explore their potential uses in agriculture.

5.6 Acknowledgements

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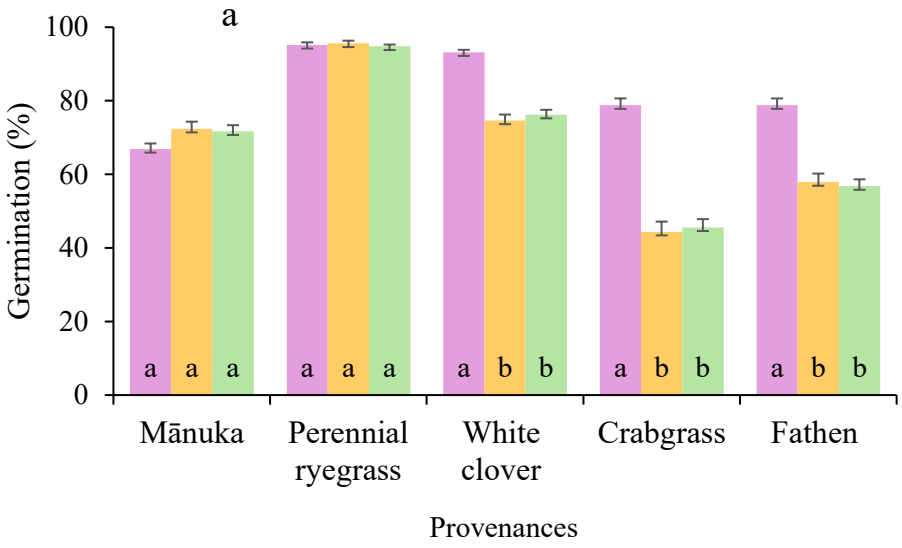
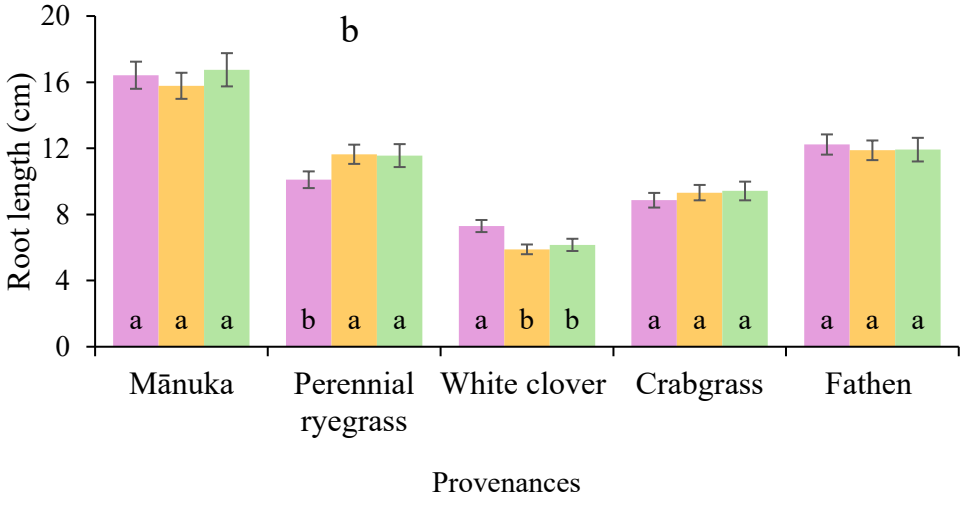
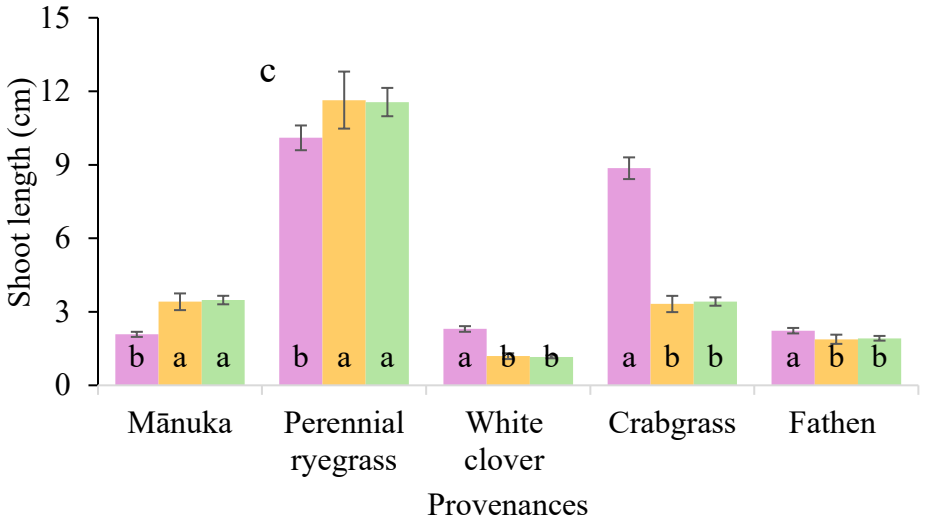
5.8 Appendix 2: Supplementary information to chapter 5

Appendix 2.1 List of 37 annotated potential allelopathic metabolites from moringa root extracts whose level 3 identification confidence was determined via LC–MS/MS analysis.

Class/Sub/super class	Compound Name	KEGG	Stream	m/z	rt.
Cinnamic	Trans-Cinnamic acid	C00423	C18 +	149.0595	2.34
Cinnamic	Trans-Ferulic acid	C01494	C18 +	195.1126	7.95
Cinnamic	4-Methoxycinnamic acid	-	C18 +	179.0701	8.3
Phenols	Salicylic acid	C00805	C18 +	139.0387	8.85
Phenol	Syringic acid	C10833	C18 +	199.0656	8.85
Phenol	4-Hydroxybenzoic acid	C00156	C18 +	139.0387	8.85
7-hydroxycoumarins	6-Methoxy-7-hydroxycoumarin	-	C18 +	193.0492	8.15
Flavanol c.	Quercetin	C00389	C18 +	303.0493	9.17
Flavonoid	Kaempferol-3-glucoside	C12249	C18 +	449.1073	7.36
Flavonoid	Kaempferol	C05903	C18 +	287.0544	9.49
Flavonoid	Luteolin	C01514	C18 +	287.0544	9.49
Isoflavonoids	Genistein	C06563	C18 +	271.0596	8.44
Isoflavonoids	Daidzein	C10208	C18 +	255.0647	9.2
Quinolines	4-Hydroxyquinoline	C19434	C18 +	146.0598	9.66
Hydroxy fatty acids	3-Hydroxy-3-methylglutaric acid	C03761	C18 +	163.0598	8.16
Flavones	Apigenin	C01477	C18 +	271.0596	8.44
Phenol lipids	(+)-Costunolide	C09382	C18 +	233.1532	8.93
Indole carboxylic acids	Indole-3-carboxylic acid	C19837	C18 +	166.0347	8.05
Benzenoids	Emodin	C10343	C18 +	271.0596	8.43
Benzenoids	Benzoic acid	C00180	C18 +	123.044	8.30
Benzoyl derivative	2,4,5-Trimethoxybenzaldehyde	-	C18 +	197.0806	7.55
Glucosinolates	1-Isothiocyanato-9-(methylsulfinyl)-nonane	-	C18 +	248.1124	7.32
Flavonoid	Quercetin-3-Rhamnoside	C01750	C18-	447.0923	8.77
Flavonoid	Naringenin-7-O-glucoside	-	C18-	433.1129	8.46
Flavonoid	Quercitrin	C01750	C18-	447.0923	8.77
Flavonoid	Tricin	C10193	C18-	329.0613	8.85
Flavonoid	Myricitrin	C10108	C18-	463.0873	8.46
Alcohols and polyols	Pantothenic acid	C00864	C18-	218.1024	2.12
Benzoic acids	4-Aminobenzoic acid	C00568	C18-	136.0391	5.59
Phenols	2-Acetamidophenol	-	C18-	150.0548	6.99
Alpha-Amino acid	DOPA (3,4-Dihydroxy-L-phenylalanine	C00355	Hilic POS	198.076	2.47
Phenylpropanoids	Chlorogenic acid	C00852	C18-	353.0869	6.98
Alpha-Amino acid	L-Tyrosine	C00082	Hilic POS	182.081	15.05
Cinnamic acids	4-Coumaric acid	C00811	Hilic NEG	163.0391	1.96
Benzenediol	Pyrocatechol	C15571	Hilic NEG	109.0284	2.17
Alpha-Amino acid	L-Valine	C00183	Hilic NEG	116.0706	14.73


a
Germination (%)
100
80
60
40
20
0
a a a
a a a
a b b
a b b
a b b
Mānuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Provenances


b
Root length (cm)
20
16
12
8
4
0
a a a
b a a
a b b
a a a
a a a
Mānuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Provenances


c
Shoot length (cm)
15
12
9
6
3
0
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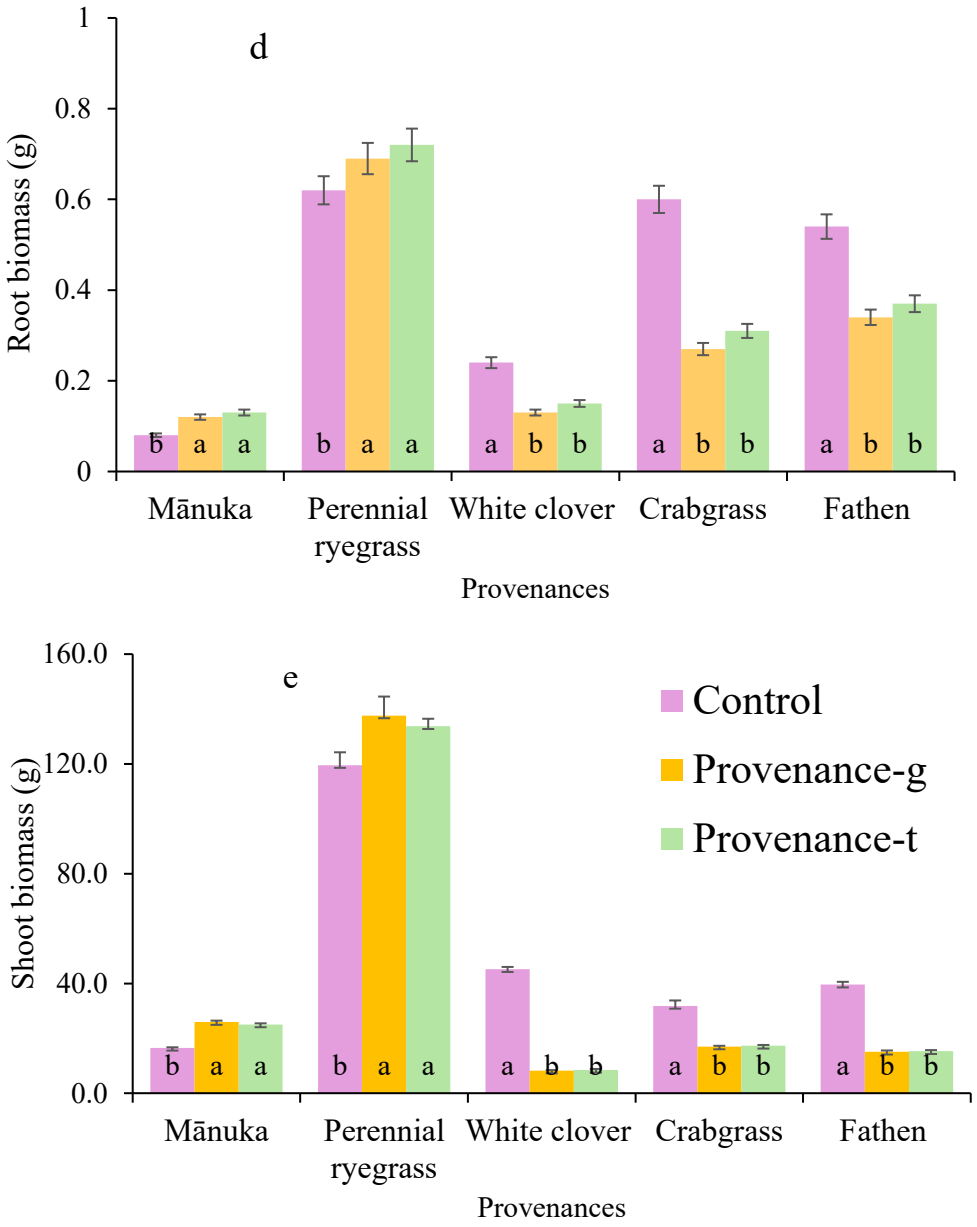
a b b
a b b
a b b
Mānuka
Perennial ryegrass
White clover
Crabgrass
Fathen
Provenances



Appendix 2.2 Effects of moringa root exudates on (a) germination, (b) root length, (c) shoot length, (d) root biomass, and (e) shoot biomass of response species in a plant–plan

Statement of Contribution to Chapter 6

MASSEY UNIVERSITY
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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Blair Moses Kamanga
Name and title of main supervisor:	Dr Donita L. Cartmill
In which chapter is the manuscript/published work?	6

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

BMK and ACM conceived the idea, and BMK designed the research. BMK and PB conducted the laboratory and glasshouse experiments, methodology, investigation, formal analysis, and data curation. BMK conducted final data analysis and wrote the first draft of the manuscript with exclusive inputs including writing, review and editing, supervision, resources and funding acquisition from ACM, CM, and DLC.

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Chapter 6

Cold stress–induced physiological and metabolic reprogramming in *Moringa oleifera* L.: Implications for adaptation and cultivation in temperate regions

Moringa plants in plant growth chamber. Photo credits: ***Blair Moses Kamanga***

This chapter investigates the adaptation mechanisms in moringa by evaluating the physiological and metabolic changes in moringa in response to cold stress and its implications for cultivation in New Zealand are assessed. Untargeted metabolomics profiling under four temperature regimes of 25, 15, 10, and 4°C is conducted. The chapter is presented in the style of the journal *Physiology and Molecular Biology of Plants*. This chapter was submitted for publication to journal *Physiology and Molecular Biology of Plants* as:

Kamanga, B.M., Barrett, P., Cartmill, D.L., McGill, C., and Clavijo McCormick A. Cold stress triggers physiological and metabolic changes in *Moringa oleifera* Lam.: Implications for adaptation and cultivation in temperate regions. *Physiology and Molecular Biology of Plants, 2026*.

Abstract

Cold stress is a major abiotic constraint limiting the distribution and productivity of tropical and subtropical crops such as *Moringa oleifera* L. (moringa) in temperate regions. To elucidate moringa's adaptation mechanisms to cold stress, photosynthetic performance, membrane integrity, and metabolite profiles were assessed under four temperature regimes (25, 15, 10, and 4°C) using physiological, biochemical, and metabolomics approach. Net photosynthesis, stomatal conductance, and transpiration rates decreased progressively with decreasing temperature and were almost completely inhibited at 4°C. The membrane stability index decreased at 4°C, accompanied by a sharp increase in membrane injury. Untargeted LC–MS metabolomic profiling detected 5,322 metabolite features, of which 47 metabolites were putatively annotated based on accurate mass, retention time, and MS/MS spectral matching against the public MassBank database. Temperature-dependent metabolic reprogramming involved coordinated changes in metabolites associated with amino acid metabolism, carbohydrate metabolism, lipid remodelling, phytohormone signalling, and glucosinolate metabolism, demonstrating that cold acclimation is regulated through the coordinated interaction of multiple metabolic pathways rather than isolated biochemical responses. However, the decrease in photosynthetic capacity and severe membrane injury at 4°C revealed moringa's inherent chilling sensitivity. The identification of protective metabolites such as sucrose, proline precursors, and flavonoids highlights targets for breeding and metabolic engineering to improve cold stress tolerance to support moringa's expansion and cultivation in temperate environments.

Keywords: Adaptations; cold stress; osmolytes; membrane stability; photosynthetic activity; water use efficiency

6.1 Introduction

Moringa oleifera L. (moringa) is a fast-growing perennial tree widely cultivated across tropical and subtropical regions due to its strong adaptation to drought, broad soil tolerance, and low-input production requirements suitable for sustainable small scale farming systems (Anwar et al., 2007; Palada et al., 2019). The species is valued for its nutrient rich foliage that contain high protein, calcium, potassium, and vitamins, which contributes to its widespread application in mitigating micronutrient deficiencies (Bancessi et al., 2020). In addition to edible leaves, flowers, and pods, moringa provides multifunctional benefits through seed derived water purification, medicinal uses of the bark and roots, and its role as a green manure in regenerative agriculture (Palada et al., 2019).

Cold stress is a major abiotic factor limiting the distribution and productivity of tropical and subtropical crops in temperate regions. During vegetative growth, it causes physical damage to plant tissues and disrupt metabolic activities, leading to stunted growth and extensive crop losses (Khan et al., 2022). However, plants have evolved distinctive adaptation mechanisms by shifting their morphological, physiological and metabolic pathways to achieve new homeostatic states to survive and thrive under adverse weather conditions (Salam et al., 2023; Zhu, 2016). Temperature has been identified as a major determinant for the successful production of moringa, with extreme or severe frosts limiting its growth and geographical distribution (El Bilali et al., 2024).

While moringa is an active producer of numerous bioactive compounds that contributes to its ecological success (Kamanga et al., 2025), it is not known how its metabolism responds to cold stress. However, primary metabolites (PMs) play essential roles in plant growth and adaptation to abiotic stresses (Biswas et al., 2021). Under cold stress, these

metabolites contribute to osmotic adjustment, membrane stabilization, maintenance of cellular energy homeostasis and reactive oxygen species scavenging, thereby enhancing plant tolerance to low temperatures (Biswas et al., 2021). Cold acclimation in plants is associated with the accumulation of soluble sugars and proteins that function as compatible solutes to enhance cold tolerance (He et al., 2023; Hincha et al., 2012).

Soluble sugars, including glucose, sucrose, fructose, and galactose, maintain osmotic potential, promote water retention capacity, eliminate damage from membrane structures, and prevent the death of plants (Ding et al., 2019; Sami et al., 2016). Additionally, proline has been reported to play significant roles in the response to environmental stressors by maintaining osmotic balance and regulating osmotic pressure, protein stabilisation, and hydration to improve cold tolerance (Ghosh et al., 2022; Guo et al., 2022). Hence, characterising changes in these metabolites provides valuable insights into the metabolic mechanisms underlying cold stress adaptation in moringa.

Plants not only adjust their primary metabolism in response to abiotic and biotic stresses but also induce the biosynthesis of diverse secondary metabolites (SMs) (Biswas et al., 2021). Together with metabolic reconfiguration, these compounds contribute to physiological modifications such as ion and water homeostasis, the regulation of stomatal conductance, and the activation of antioxidant and detoxification enzymes to increase stress tolerance (He et al., 2023; Hincha et al., 2012). Harsh environmental stress triggers the formation and synthesis of different SMs, including flavonoids, isoflavonoids, phenolics, anthocyanins, terpenoids, fatty acyls, and alkaloids (Dawood et al., 2022). These factors are associated with cold stress tolerance in various plant species and function as antioxidants, osmoprotectants, and signalling molecules to protect against cold stress in plants (Jahed et al., 2023; Satyakam et al., 2022).

Under cold stress, plants undergo extensive metabolic reprogramming to counteract impaired photosynthesis, membrane destabilisation, oxidative damage, and reduced growth. Although these adaptive mechanisms have been widely documented in plants, the physiological and metabolomic basis of cold stress tolerance in moringa remains poorly understood (Kamanga et al., 2026). This limited understanding of the metabolic mechanisms governing cold acclimation in moringa remains a major constraint to its successful cultivation beyond tropical and subtropical regions. We hypothesised that progressive cold stress induces coordinated physiological and metabolic adjustments that contribute to membrane protection and enhanced cold tolerance in moringa. To test this hypothesis, physiological, biochemical, and untargeted metabolomic analyses were conducted to characterise the adaptive responses of moringa during prolonged cold stress and identify the metabolic pathways associated with cold acclimation.

6.2 Materials and methods

6.2.1 Plant material and growth conditions

This experiment was conducted between July and November 2024 in a glasshouse at the Plant Growth Units (PGU) and the Plant Science Laboratory, Massey University, Palmerston North, New Zealand (-40.37807°S, 175.61362°E). Moringa seeds (PKM-2) obtained from Tamil Nadu, India, were germinated and grown under glasshouse conditions (25 ± 2°C, 50–65% RH) for 60 days prior to the initiation of cold stress treatments. Cold stress treatments were conducted in reprogrammable plant growth chambers (Contherm Biosyn Series 6050, Canada) under a 16 h light/8 h dark photoperiod with a photon flux density of 511 μmol m-2 s-1 and 50% RH. The temperature regime treatments were applied following the procedures of Ljubej et al. (2021), with minor modifications to the number of plants and the specific temperature conditions used.

The temperature treatments were selected to represent progressively increasing levels of chilling stress relative to the optimum growth temperature of moringa (approximately 25°C). Preliminary observations indicated that plants maintained at 20°C exhibited no visible symptoms of chilling injury and showed physiological responses comparable to those grown under the control temperature. Consequently, 20°C was not included in the final experiment because it did not impose sufficient stress to differentiate cold-responsive physiological or metabolic changes. Instead, constant temperatures of 15°C, 10°C, and 4°C were selected to represent mild, moderate, and severe chilling conditions, respectively, thereby enabling the progressive evaluation of cold stress responses.

Briefly, three chilling treatments were applied: (i) 15±1°C for 7 days, (ii) 10°C±1 for 7 days, and (iii) 4±°C for 7 days. Plants assigned to the low-temperature treatment were transferred from control growth conditions to a controlled-environment chamber maintained at 15, 10, and 4°C. To reduce transient temperature shock and better reflect progressive cold exposure, plants were acclimated by reducing the temperature from 25°C to the target temperature at a rate of 2°C h^{-1} before the experimental treatments. Plants were then maintained under cold conditions for the designated exposure duration before physiological and metabolomic analyses were conducted. The control plants were maintained under glasshouse conditions at 25±2°C. Each treatment consisted of five biological replicates, with one plant per replicate used for downstream analyses.

6.2.2 Determination of photosynthetic activities

Photosynthetic activities were measured to evaluate the response of moringa plants to cold stress using Li-COR 6800 portable photosynthesis system (Li-COR Biosciences, Lincoln, USA). Measurements were taken from five fully opened and expanded leaves from the top of the plant after cold treatment. The net photosynthetic rate (Pn), stomatal

conductance (Gs), intercellular CO2 concentration (Ci), and transpiration rate (Tr) were measured at a photosynthetic photon flux density (PPFD) of 1000 µmol-m-2 -S-1 provided by LEDs emitting blue and red-light sources. The leaf area was calculated and input into the Li-COR 6800. Owing to the morphology of the moringa leaves, which are tripinnate and each leaflet has the shape of an ellipse, the area was determined via the formula Area = π ½ab, where a and b are the width and length of the leaflet, respectively. The leaf area was individually adjusted to produce accurate measurements of photosynthesis and stomatal conductance. Photosynthetic measurements were taken between 10:00 am and 1:00 pm.

6.2.3 Determination of leaf chlorophyll content

Photosynthetic activities were measured to evaluate the response of moringa plants to cold stress using Li-COR 6800 portable photosynthesis system (Li-COR Biosciences, Lincoln, USA). Measurements were taken from five fully opened and expanded leaves from the top of the plant after cold treatment. The net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO2 concentration (Ci), and transpiration rate (Tr) were measured at a photosynthetic photon flux density (PPFD) of 1000 µmol-m-2 -S-1 provided by LEDs emitting blue and red-light sources. The leaf area was calculated and input into the Li-COR 6800. Owing to the morphology of the moringa leaves, which are tripinnate and each leaflet has the shape of an ellipse, the area was determined via the formula Area = π ½ab, where a and b are the width and length of the leaflet, respectively. The leaf area was individually adjusted to produce accurate measurements of photosynthesis and stomatal conductance. Photosynthetic measurements were taken between 10:00 am and 1:00 pm.

6.2.4 Determination of leaf chlorophyll content

Relative chlorophyll content of leaves was determined by a non-destructive method according to Zhang et al. (2022), with minor modifications on the number of leaves using a portable chlorophyll meter (SPAD-502, Minolta, Japan). From each treatment, three (3) leaves were randomly selected from different canopy orientations to minimise positional leaf selection bias. Three SPAD readings were taken per leaf, and the average of these three measurements was recorded as the SPAD value for that leaf.

6.2.5 Determination of the membrane integrity of moringa leaves

The membrane stability index (MSI) was determined through electrolyte leakage to assess the level of cell membrane stability and damage to leaves due to cold stress. To measure electrolyte leakage, protocols outlined by Yang et al. (2023) were used with minor modifications to the number of leaves. Briefly, five grams (5 g) of leaves were pooled randomly from each plant for each treatment. The leaves were rinsed with demineralised water and subsequently floated in 25 ml of demineralised water for 24 hours at room temperature before initial measurement of electrolyte leakage (Lt) of the bathing solution was performed using an electrical conductivity meter (HQ Series portable meter, HACH). The samples were subsequently autoclaved at 121°C for 20 minutes to ensure complete membrane disruption. After cooling to room temperature, the final electrolyte leakage (Ltm) of the same solution was recorded. The MSI was estimated following the equation of Khanna-Chopra and Selote (2007).

$$\mathrm{MSI} = 1 - \left(\frac{\mathrm{L}t}{\mathrm{L}tm}\right)$$

where L_t is the initial electrolyte before autoclaving, and L_{tm} is the final electrolyte leakage.

The degree of membrane injury (MI) was estimated as the ratio of the MSI of cold-stressed plants (MSId) to the MSI of control treatment plants (MSIc) (Dhanda et al., 2004).

$$\text{MI}\ (\%) = 1 - \left(\frac{MSId}{MSIc}\right) x\ 100$$

6.2.6 Biological sample preparation and LC–MS analysis

Metabolite extraction and LC–MS analysis were performed following the procedures of Kamanga et al. (2026), with minor modifications. Briefly, 200 mg of fully expanded leaves were harvested, wrapped in aluminium foil, snap-frozen in liquid nitrogen, stored at -80°C, freeze-dried, held at -20°C, and ground to 50–150 µm. For extraction, 50±3 mg of powdered tissue was combined with a 2.5 mm glass bead and 800 µL of prechilled chloroform: methanol (1:1, v/v), homogenised for 5 minutes at 25 Hz (Retsch MM400), and incubated at -20°C for 1 hour. After phase separation with 400 µL of HPLC-grade water and centrifugation (11,000 rpm, 15 min, 4°C), a 250 µL aliquot was collected for both reverse-phase C18 and HILIC analyses. Aliquots from each treatment were pooled to generate quality-control (QC) samples, which were subsequently dried under nitrogen (3.5 L min-1) and stored at -80°C. Prior to LC–MS analysis, the samples and QCs were reconstituted in CAN:H2O (1:9, v/v) with an internal standard (MSK-QC-KIT; 10 µL mL-1), with 1:9 (v/v) for C18 and 1:1 (v/v) for HILIC, vortexed, and transferred to glass inserts.

Chromatographic separation and mass spectrometric detection of polar and semipolar metabolites were conducted on a Thermo UltiMate 3000 UHPLC system coupled to a Q Exactive Focus Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, USA) equipped with a heated electrospray ionisation (HESI) source operating in positive and

negative modes. Semipolar compounds (5 µm injection) were separated on a Hypersil Gold C18 column (100 × 2.1 mm, 1.9 µm) at 25°C via a 350 µm min-1 gradient of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), which was eluted from 5% to 100% B over 18 minutes. Polar metabolites were analysed on a ZIC-pHILIC column (100 × 2.1 mm, 5 µL; Merck, Germany) at 150 µL min-1 with 10 mM ammonium formate (pH ~4) in water (A) and 97% acetonitrile (B). Full-scan spectra (m/z 60–900) were acquired at 70,000 resolutions with data-dependent MS2 at 17,000 resolutions (isolation window 1.5 m/z, collision energy 25 eV). The source voltages were 3.5 kV and -3.5 kV for positive and negative modes, respectively, with capillary and auxiliary temperatures of 320°C and 350°C and a sheath/auxiliary/sweep gas setting of 35/8/1 arbitrary units. All injections were performed at 4°C, with instrument stability monitored via solvent blanks, amino acid standards (A9906; Sigma–Aldrich), and QC samples at regular intervals, and the data were processed via Xcalibur.

6.2.5 Data processing and statistical analyses

The Thermo-raw files from C18 and HILIC (positive/negative) modes were converted to mzXLM format using MSConvert GUI (Adusumilli & Mallick, 2017), baseline-filtered in MZmine, and processed in XCMS online (xcmsonline.scripps.edu) for peak detection, alignment, and feature extraction. The C18 peak-peaking parameters were set as follows: m/z tolerance, 10 ppm; peak width, 5–20 s; mzdiff, 0.001; S/N threshold, 20; prefilter intensity, 1 × 104; and noise filter, 8 × 103. The resulting feature tables (m/z, retention time, and intensity) were filtered against blanks using QC vs blank t tests ($p > 0.05$ were removed) and then imported into MetaboAnalyst 6.0 for integrity checks, RSD-based filtering (RSD < 30% in the QC samples), and autoscaling (mean-centered and standard deviation (SD)-normalised) following the validation of normality. Statistical analysis was

conducted in R v4.4.2 (R-CoreTeam, 2024) using one-way ANOVA with Tukey's post hoc test ($p < 0.05$) to assess differentially abundant metabolite accumulation across treatments.

Untargeted metabolomic profiling was performed using complementary HILIC and reversed-phase C18 chromatographic separations operated in both positive and negative electrospray ionisation modes. Raw LC–MS data were processed through peak detection, alignment, deconvolution, and feature extraction prior to statistical analysis. A total of 5,322 metabolite features were detected across all samples. Metabolite annotation was performed by comparing accurate mass-to-charge ratio (m/z), retention time, and MS/MS fragmentation spectra against the public MassBank spectral database. Based on spectral similarity, 47 metabolites were putatively annotated according to the Metabolomics Standards Initiative (MSI) Level 2 and 3 criteria and subsequently classified into their respective biochemical classes for biological interpretation. The remaining detected features were not assigned because of the limited availability of reference spectra for many plant metabolites in public databases, a common limitation of untargeted LC–MS metabolomics (Li et al., 2023).

Prior to multivariate analysis, metabolite peak intensities were normalized to reduce systematic analytical variation, log-transformed to improve data normality and stabilize variance, and Pareto scaled to increase the contribution of metabolites with intermediate variance while reducing the influence of highly abundant compounds. Principal component analysis (PCA) was subsequently performed using all detected metabolite features to evaluate treatment-dependent metabolic variation. Differential metabolite accumulation among temperature treatments was assessed using one-way analysis of variance (ANOVA), and metabolites with $P < 0.05$ were considered significantly affected by temperature. Where appropriate, false discovery rate (FDR) correction was applied to

account for multiple comparisons. The percentage of variance explained by each principal component was evaluated using scree plots to assess the contribution of individual principal components to the total variance and determine whether the first two principal components adequately represented the dominant patterns of the metabolic variations.

6.3 Results

6.3.1 Photosynthetic activity and membrane integrity of moringa plants in response to cold stress

Cold stress influenced the photosynthetic performance and gas exchange of moringa plants, with cold-stressed plants showing a steep decline in most of the photosynthetic activities measured, i.e., net photosynthesis, stomatal conductance, and transpiration rate. This further impaired carbon dioxide diffusion and assimilation, as revealed by increased accumulation of intercellular CO_2 at 4°C and 10°C and compromised water use efficiency at 4°C (Figure 13).

6.3.2 Effect of temperature on relative chlorophyll content

The effects of cold stress on leaf relative chlorophyll content (SPAD) are shown in figure 14. Temperature significantly affected relative chlorophyll content ($p < 0.05$), with SPAD increasing progressively as temperature increased. Leaves maintained at 4°C for seven days showed the lowest chlorophyll content which indicated a significant reduction chlorophyll reduction under chilling stress. The highest chlorophyll contents were observed at 15°C and 25°C, with no significant differences between these treatments. Chilling stress at 4°C suppressed chlorophyl accumulation, whereas moderate to optimal temperatures (15-25°C) maintained significantly higher SPAD values.

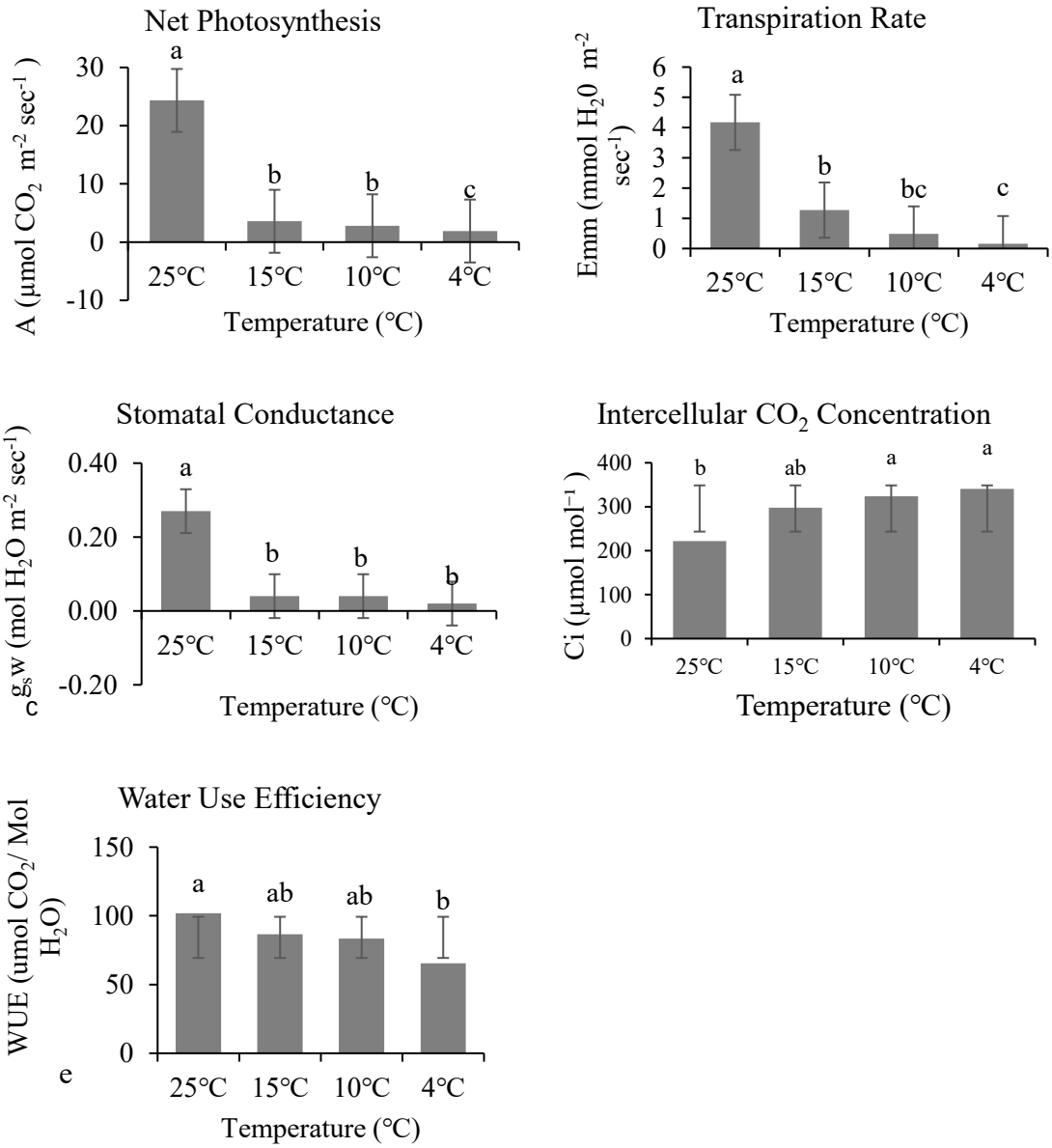


Figure 13. Effects of temperature on the photosynthetic activities of moringa plants. a) net photosynthesis, b) transpiration rate, and c) stomatal conductance were highest at 25°C but declined sharply under cold stress, d) WUE decreased significantly only at 4°C, whereas the e) intercellular CO_2 concentration increased at 4°C, and f) membrane stability index (MSI) and degree of membrane injury (MI) in moringa leaves exposed to different temperature regimes. Different letters at the top of each bar indicate significant differences among treatments ($p < 0.05$) and the values represent the means ± SEs.

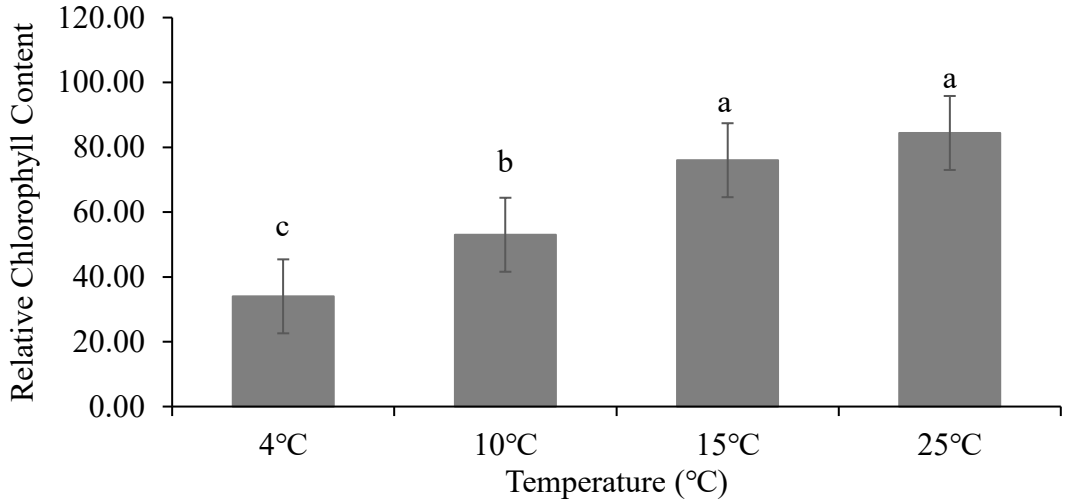


Figure 14. Effect of temperature on relative chlorophyll content (SPAD units) in moringa leaves exposed to four temperature regimes(4, 10, 15, and 25°C), and chlorophyll content was determined non-destructively using a SPAD-505 chlorophyll meter. Bars represent mean ± standard error (SE). Different letters above the bars indicate significant differences among temperature treatments according to Tukey's honestly significant difference (HSD) test at $p < 0.05$.

6.3.3 Effect of temperature on membrane integrity of moringa leaves

Temperature significantly affected membrane integrity, as reflected by reciprocal changes in membrane stability index (MSI) and membrane injury (MI) (Figure 16). MSI decreased progressively with decreasing temperature, from 72.8% at 25°C to 23.2% at 4°C, whereas MI increased from 2.2% to 67.3% over the same temperature range. An intermediate response was observed at 10°C, where MSI (41.3%) and MI (42.1%) were comparable, while exposure to 15°C resulted in higher MSI (57.1%) and lower MI (20.9%) than at 10°C. The highest membrane stability and lowest membrane injury occurred under the control temperature (25°C), whereas the opposite pattern was observed at 4°C. All temperature treatments differed significantly for both MSI and MI ($P < 0.05$).

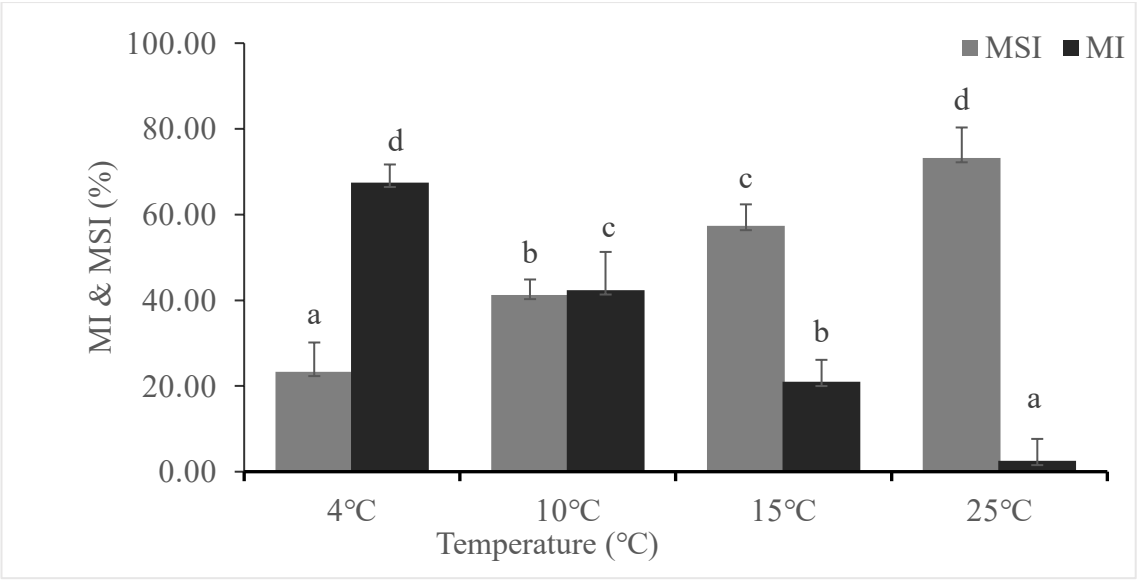


Figure 15. Temperature-dependent changes in membrane stability index (MSI) and membrane injury (MI) in moringa leaves. MI increased progressively with decreasing temperature, rising from approximately 2% at 25°C to 67% at 4°C, indicating greater membrane damage under cold stress. Conversely, MSI decreased significantly with decreasing temperature, declining from approximately 73% at 25°C to 23% at 4°C, reflecting reduced membrane integrity at lower temperatures. Bars represent the mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters above the bars indicate significant differences among temperature treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

6.3.4 Metabolite identification and profiling in moringa plants

Principal component analysis (PCA) based on all detected metabolites revealed distinct clustering of samples according to temperature treatment across the HILIC positive, HILIC negative, C18 positive, and C18 negative datasets (Figure 16). The stronger discrimination observed in HILIC ionisation mode indicates the presence of polar metabolites such as amino acids, organic acids, and soluble sugars. The first two principal components explained 31.7–48.2% of the total variance across the four datasets and consistently discriminated the temperature treatments.

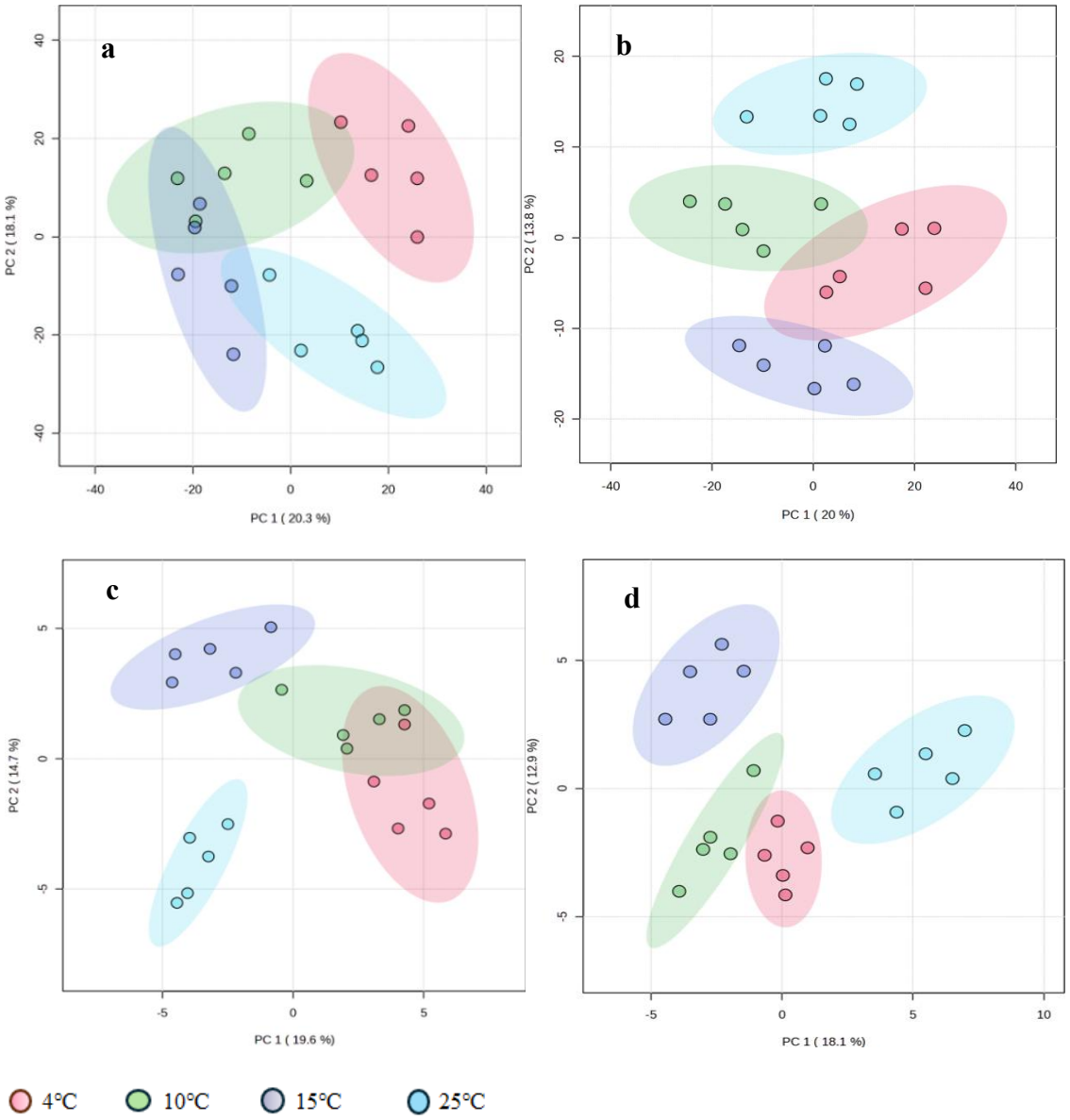


Figure 16. Principal component analysis (PCA) score plots of metabolites detected in the (a) HILIC positive; F = 39.889, R2 = 0.7938, p < 0.001; (b) HILIC negative; F = 40.993, R2 = 0.8841, p < 0.001; (c) C18 positive; F = 19.276, R2 = 0.7831, p < 0.001; and (d) C18 negative; F = 14.287, R2 = 0.7280, metabolomic datasets under different temperature treatments (25, 15, 10, and 4°C). PCA was performed using all detected metabolites following normalization, log transformation, and Pareto scaling. The percentage of variance explained by PC1 and PC2 is indicated on the corresponding axes. The distribution of variance explained by subsequent principal components is presented by scree plots in Appendix 3.1.

Scree plot analysis showed that the remaining variance was progressively distributed among subsequent principal components, with cumulative variance reaching 71.1–81.5% by PC8 (Appendix 3.1). The present findings demonstrate that the first two principal components effectively captured the major treatment-associated metabolic variation while additional principal components accounted for the remaining variability.

Untargeted LC–MS metabolomic profiling detected a total of 5,322 metabolite features across the HILIC and C18 chromatographic platforms operated in both positive and negative ionisation modes. Following metabolite annotation, 47 metabolites were putatively annotated based on accurate mass (m/z), retention time, and MS/MS spectral matching against the MassBank database. These annotated metabolites exhibited distinct temperature-dependent accumulation patterns and were associated with diverse biochemical pathways involved in the cold stress response.

In addition to the clear separation of metabolite profiles among temperature treatments revealed by PCA, the annotated metabolites provided biological insight into the metabolic reprogramming underlying cold acclimation in moringa. Among the 47 metabolites, 32 were annotated with Metabolomics Standards Initiative (MSI) level 3 identification confidence (Appendix 3.2), whereas 15 achieved MSI level 2 confidence, as confirmed by spectral library matching (Table 11). Most of these compounds are dominated by broad reconfiguration of secondary metabolism, amino acid pathways, polyamine biosynthesis, energy metabolism, and osmolyte accumulation in response to cold stress in moringa plants.

Table 11. Metabolites associated with cold tolerance in moringa leaves with level 2 confidence were identified via LC–MS/MS spectral library matching.

Class/subclass	Compound Name	KEGG	Stream	m/z	rt.	Fragments
Cinnamic acids	caffeic acid	C01481	C18 POS	181.0491	7.70	163.0381, 135.0441
Cinnamic acids	p-Coumaric acid	C00811	C18 POS	165.0543	12.70	123.0443
Flavonoids	Kaempferol	C05903	C18 POS	287.0543	11.37	91.0421
Benzoic acid	Salicylic acid	C000805	C18 POS	139.0387	12.70	111.0442, 83.0492, 69.0335
Fatty acids	γ-Linolenic acid	C06426	C18 POS	279.2312	13.64	262.2261, 261.2214, 243,2119
Phenylpropanoids	Luteolin	C01514	C18 NEG	285.0405	11.37	285.0399
Flavonoids	Cyanidin-3-glucoside	C08604	C18 NEG	448.0968	8.63	447.0838
Flavonoids	Catechin(+)	C06562	C18 NEG	289.0717	8.30	245.0845
Hydroxy acids	D-(+)-Malic acid	C00497	C18 NEG	133.0133	0.89	115.0028, 72.9935
Amino acids	Phenylalanine	C00079	H- POS	166.0858	14.15	121.085
Indolyl carboxylic acid	L-Tryptophane	C00078	H-POS	205.0967	14.66	188.0725
Amino acids	L-Aspartic acid	C00049	H-POS	134.0445	16.43	116.0336, 88.0387
Amino acids	γ-Aminobutyric acid	C00334	H-POS	104.0707	17.58	87.0450, 86.0609, 68.0348
Amino acids	L-Citrulline	C00327	H-POS	176.1026	16.75	159.0751, 133.0705, 115.0866
Carboxylic acid	Succinic acid	C00042	H-NEG	117.0182	2.55	99.0088, 73.0295

Note: Compound identification followed the level 2 criteria of the metabolomics standards initiative (MSI), rt. = retention time, m/z = mass-to-charge ratio.

6.3.3 Changes in primary and secondary metabolites in response to cold stress

Cold stress induced significant shifts in both the primary and secondary metabolism of moringa. Among the primary metabolites, several amino acids, including tryptophan, arginine, tyrosine, glutamate, citrulline, and proline-related compounds, accumulated significantly at 4°C (Figure 18), while their levels decreased progressively with increasing temperature. Carbohydrates and osmolytes such as glucose, sucrose/galactose, sorbitol, and glucose-6-phosphate were elevated at 4°C (Figure 19). Furthermore, organic acids associated with the TCA cycle, i.e., 2-ketoglutarate, malate, and succinate, are also

modulated by temperature. Additionally, the levels of polyamines and their derivatives, i.e., putrescine, agmatine, and gamma-aminobutyric acid (GAMA), were also increased under cold stress.

Similarly, secondary metabolites presented significant cold-induced alterations under cold stress. Flavonoids, including quercetin, kaempferol, luteolin, vitexin, myricetin-3-O-rhamnoside, catechin, epicatechin, and tricin (Appendix 3.3), together with phenolic acids, including caffeic, ferulic, p-coumaric, sinapic, and salicylic acids (Appendix 3.4), were also enriched at lower concentrations. The levels of stress-related hormones significantly changed, i.e., abscisic acid increased at 4°C, whereas indole-3-acetic acid and tyramine presented variable responses, which aligns with their regulatory roles in cold signalling and growth adjustment.

6.3.4 Metabolite pathway enrichment analysis

Pathway enrichment analysis revealed significant temperature-dependent metabolic reconfiguration in moringa plants (Figure 17). There were major rearrangements in the central amino acid pools across treatments, with alanine, aspartate and glutamate metabolism having the most strongly impacted pathway (0.66). The results further revealed broad shifts in soluble carbohydrate turnover, with starch and sucrose metabolism being the second most impacted pathway (0.47). Phenylpropanoid biosynthesis was also strongly enriched (0.41). Other significantly enriched pathways included arginine/proline metabolism, arginine biosynthesis, flavone/flavanol biosynthesis, and central carbon metabolism pathways, such as the TCA cycle and glyoxylate/dicarboxylate metabolism.

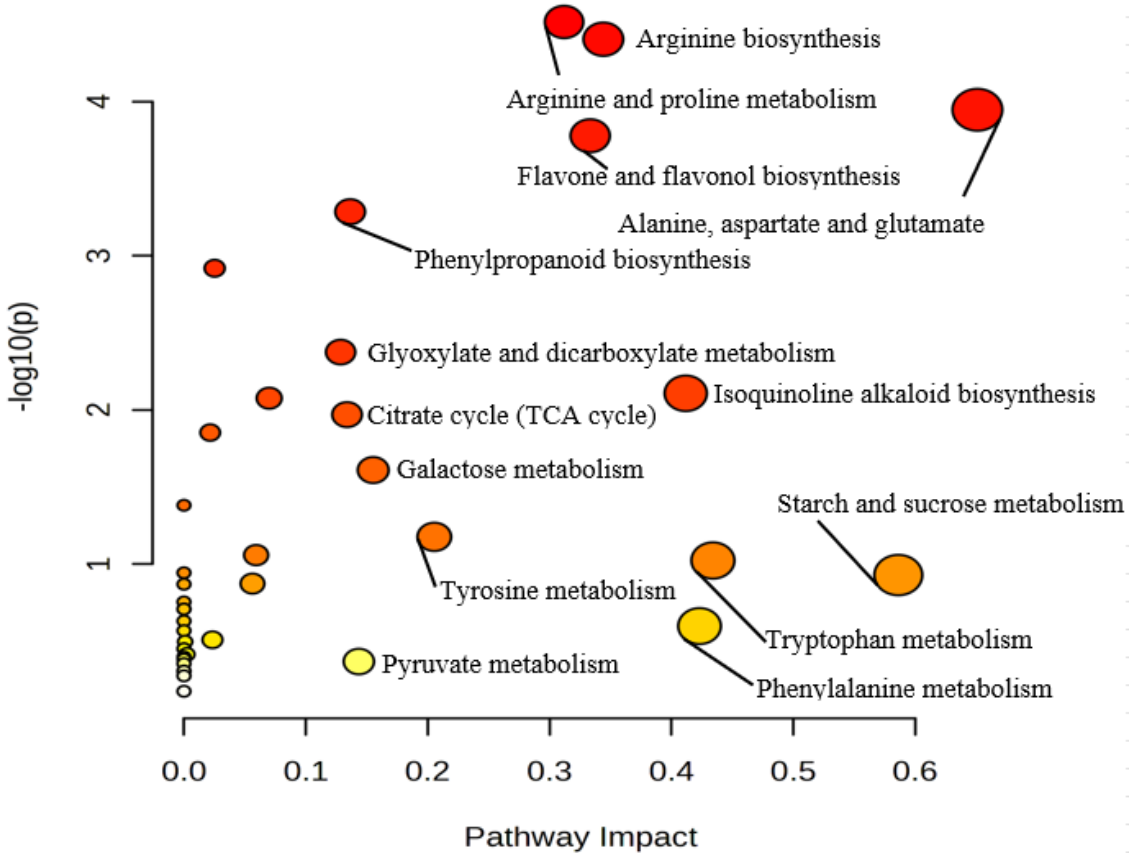
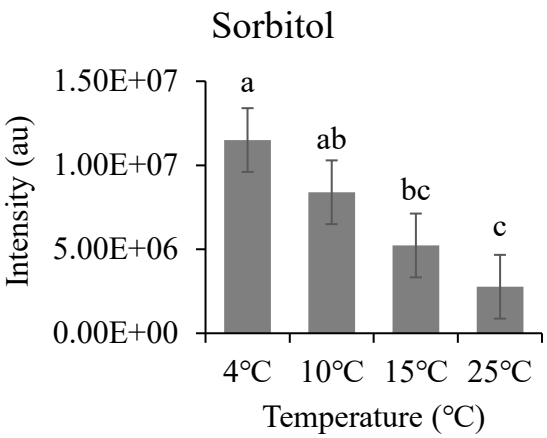


Figure 17. Pathway enrichment analysis of metabolites in moringa leaves exposed to four temperature regimens (25, 15, 10, and 4°C). The circle size indicates pathway impact, and the colour scale represents significance, i.e., deep red represents high significance ($-\log_{10}p$). The most impacted pathways included alanine, aspartate, and glutamate metabolism (0.60); starch and sucrose metabolism (0.47), and isoquinoline alkaloid metabolism (0.41). Enrichments with pathway impacts greater than 0.1 were identified.


Sorbitol
1.50E+07
1.00E+07
5.00E+06
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Intensity (au)
a
ab
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c
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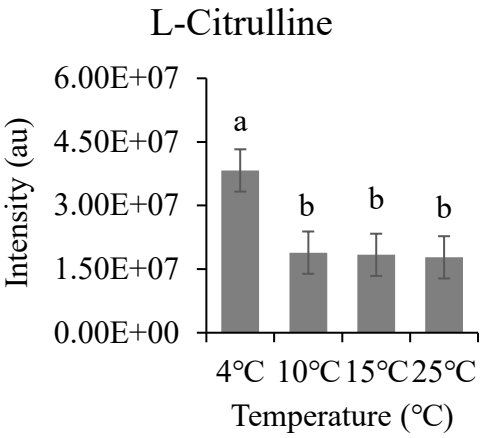
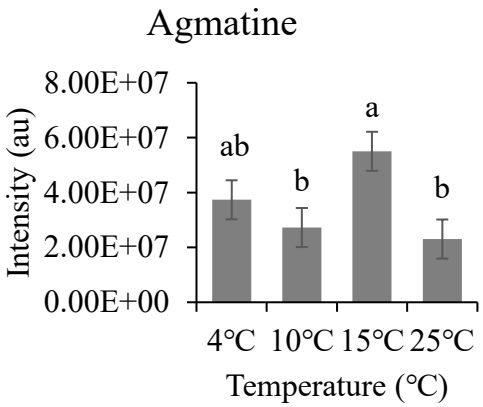
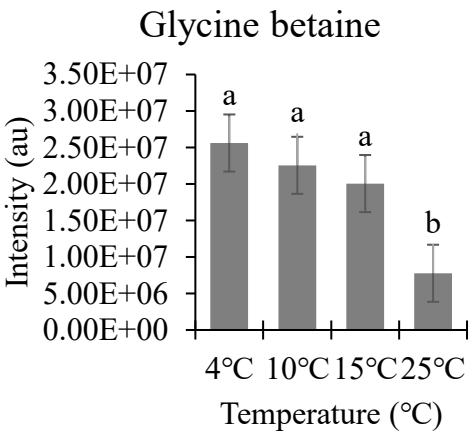
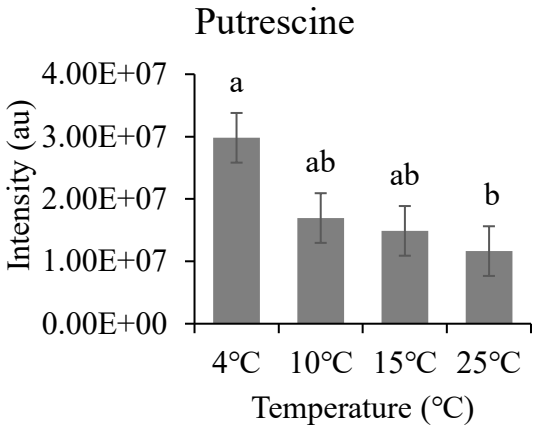
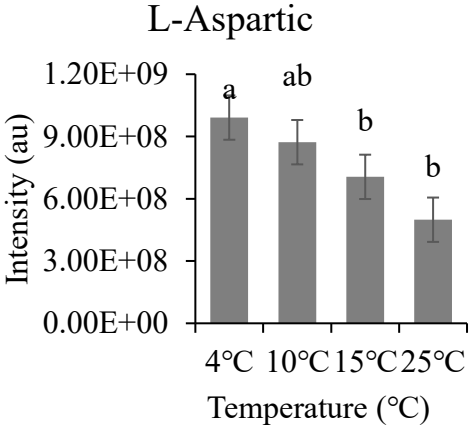
Temperature (°C)


L-Citrulline
6.00E+07
4.50E+07
3.00E+07
1.50E+07
0.00E+00
Intensity (au)
a
b
b
b
4°C 10°C 15°C 25°C
Temperature (°C)


Agmatine
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Intensity (au)
ab
b
a
b
4°C 10°C 15°C 25°C
Temperature (°C)


Glycine betaine
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
Intensity (au)
a
a
a
b
4°C 10°C 15°C 25°C
Temperature (°C)


Putrescine
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Intensity (au)
a
ab
ab
b
4°C 10°C 15°C 25°C
Temperature (°C)


L-Aspartic
1.20E+09
9.00E+08
6.00E+08
3.00E+08
0.00E+00
Intensity (au)
a
ab
b
b
4°C 10°C 15°C 25°C
Temperature (°C)

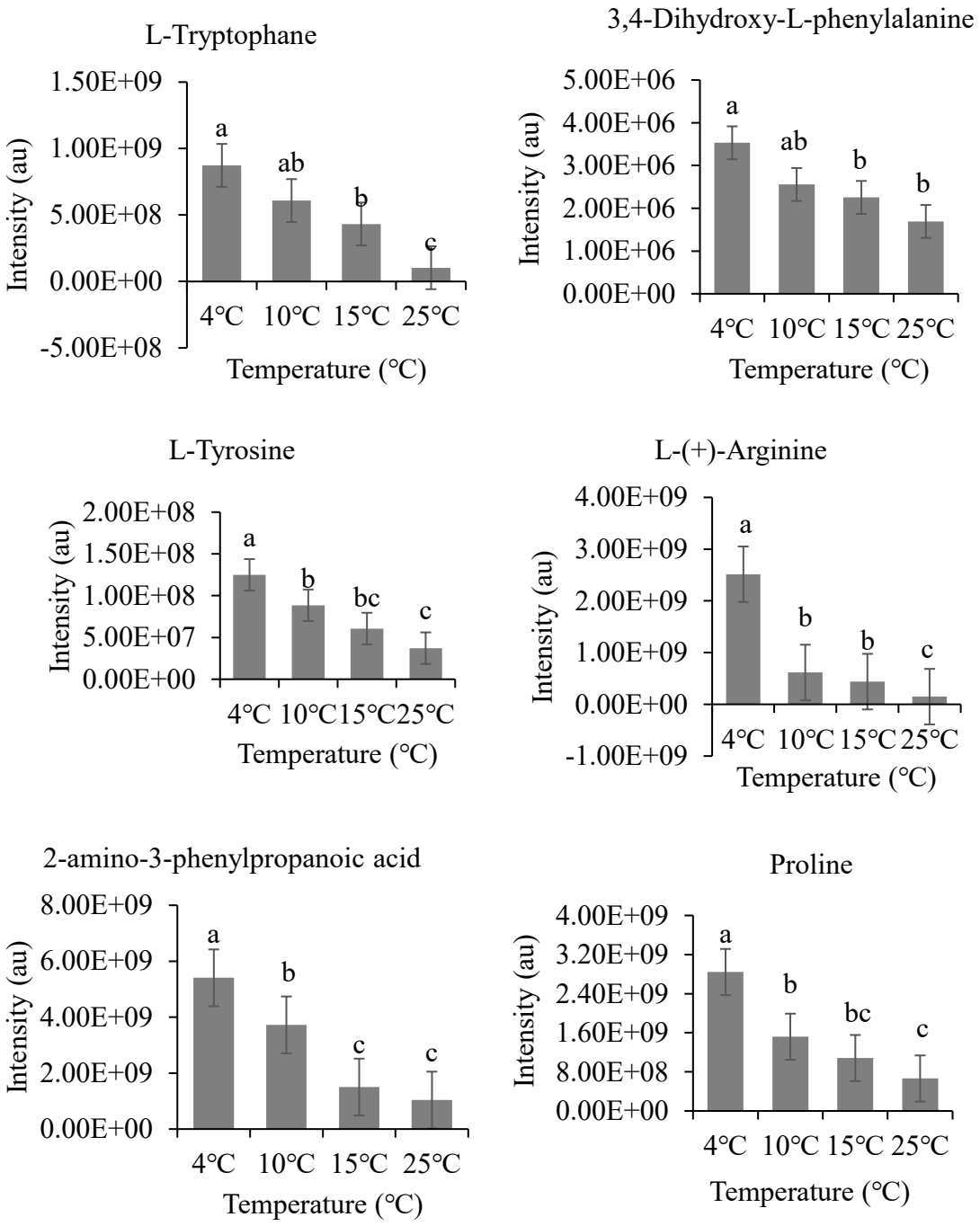
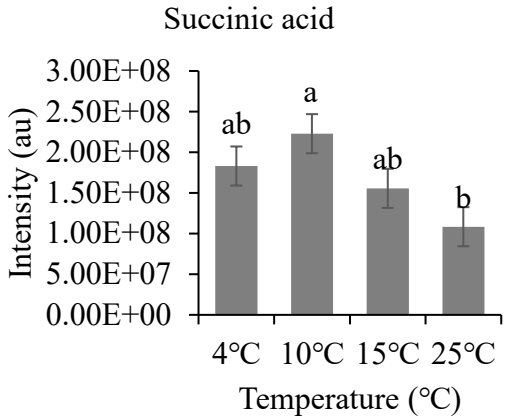
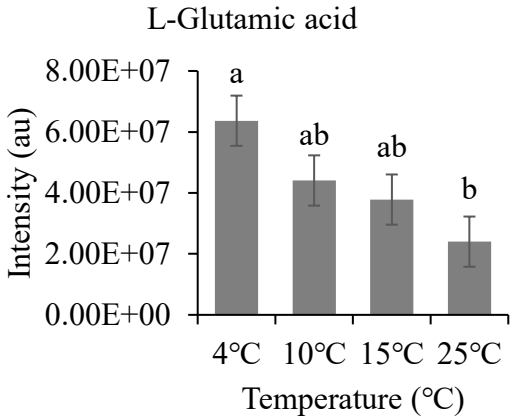
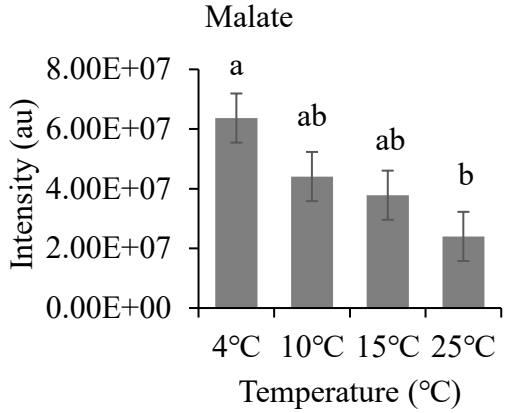
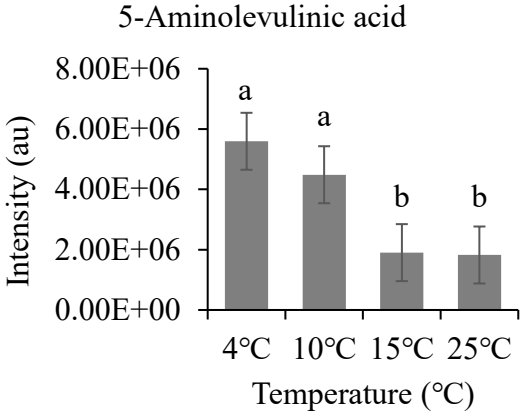


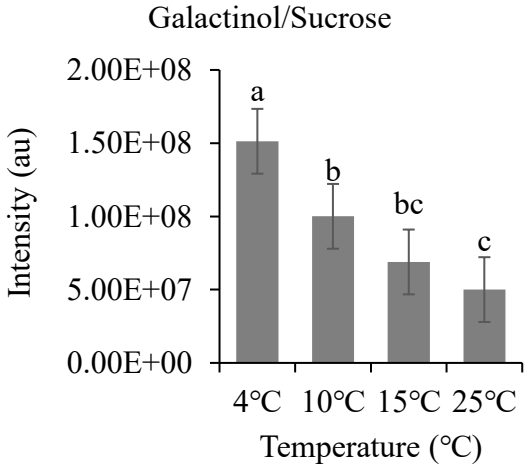
Figure 18. Relative abundance of metabolites identified in HILIC positive ionisation mode. Sugars and osmolytes (sorbitol, galactinol/sucrose, glucose, and glucose-6-phosphate), amino acids (aspartate, tyrosine, tryptophan, arginine, and citrulline), and polyamines (putrescine, agmatine, and GABA) highly accumulated at 4°C of osmotic adjustment and nitrogen metabolism under cold stress. Different lowercase letters above the bars indicate significant differences among temperature treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

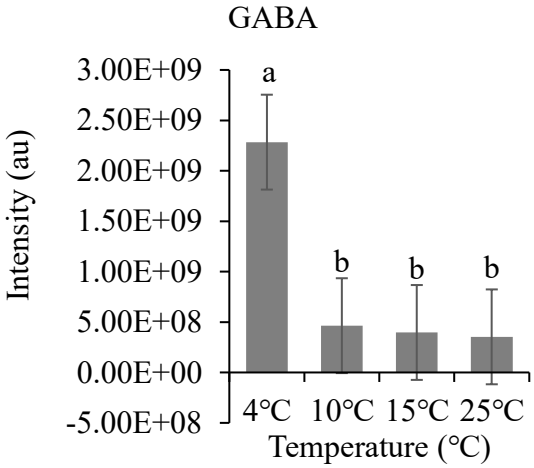

Succinic acid
3.00E+08
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
Intensity (au)
ab
a
ab
b
4℃ 10℃ 15℃ 25℃
Temperature (℃)


L-Glutamic acid
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Intensity (au)
a
ab
ab
b
4℃ 10℃ 15℃ 25℃
Temperature (℃)


Malate
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Intensity (au)
a
ab
ab
b
4℃ 10℃ 15℃ 25℃
Temperature (℃)


5-Aminolevulinic acid
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Intensity (au)
a
a
b
b
4℃ 10℃ 15℃ 25℃
Temperature (℃)


Galactinol/Sucrose
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
Intensity (au)
a
b
bc
c
4℃ 10℃ 15℃ 25℃
Temperature (℃)


GABA
3.00E+09
2.50E+09
2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
-5.00E+08
Intensity (au)
a
b
b
b
4℃ 10℃ 15℃ 25℃
Temperature (℃)

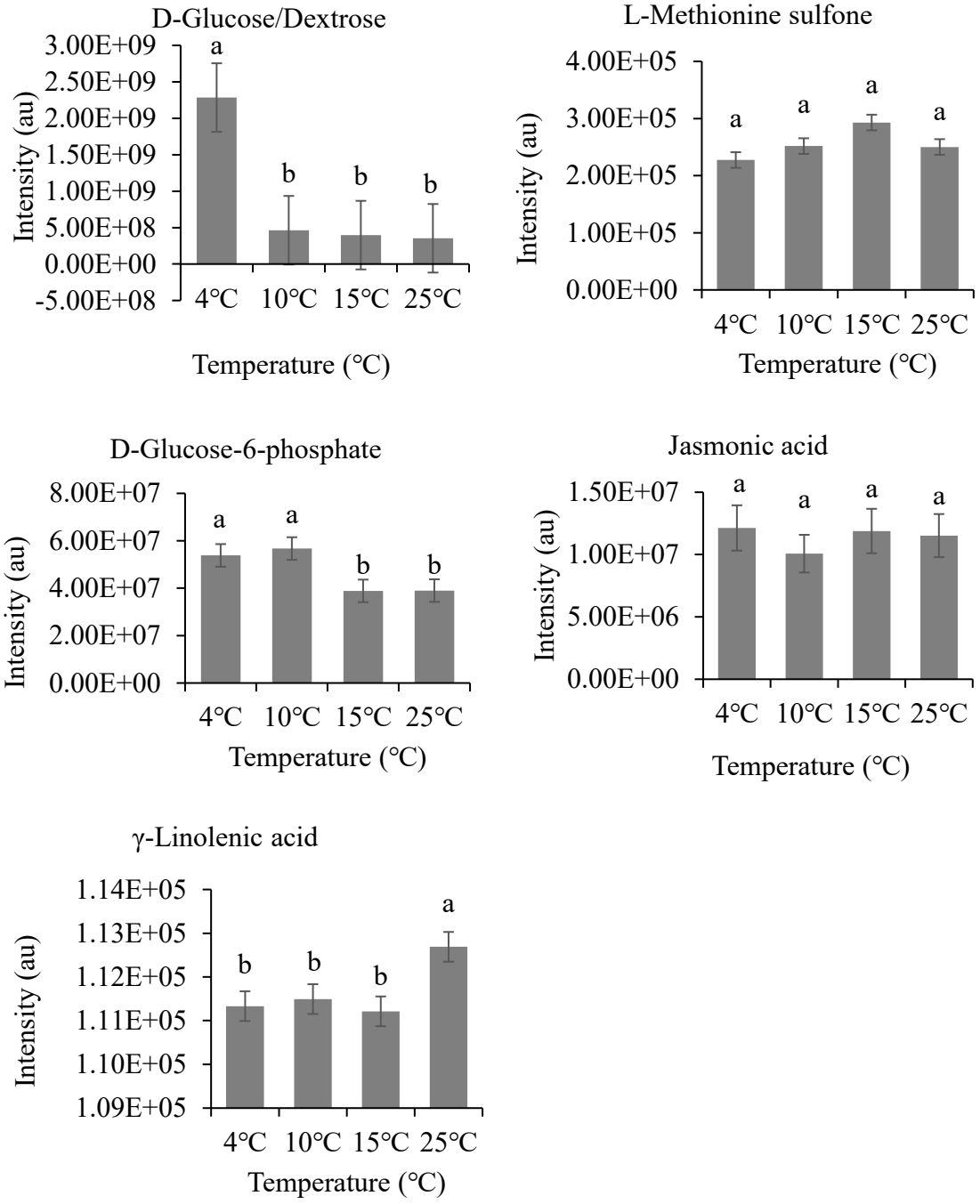


Figure 19. Relative abundance of metabolites detected in HILIC negative ionisation mode. The compounds included organic acids (succinate, glutamate, methionine sulfone), sugars (glucose, sucrose/galactinol), amino acids (5-aminolevulinic acid, L-DOPA), and secondary metabolites (luteolin), indicating metabolic reprogramming for osmoprotection and redox balance. Different lowercase letters above the bars indicate significant differences among temperature treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

6.4 Discussion

Cold stress is a major abiotic factor limiting the distribution and cultivation of tropical and subtropical crops in temperate regions (Eremina et al., 2016; Soualiou et al., 2022). The present study demonstrated that moringa is sensitive to chilling stress, as reflected by coordinated changes in physiology, membrane integrity, and metabolic reconstitution.

6.4.1 Physiological changes in moringa plants in response to cold stress

The photosynthetic activity decreased sharply under chilling stress, with the net photosynthesis, transpiration rate, and stomatal conductance decreasing at 10–15°C and nearly disappearing at 4°C. While stomatal limitation contributes to reduced CO2 uptake, the near collapse of assimilation at 4°C indicates that nonstomatal factors such as impaired enzyme activity, photoinhibition, and disruption of electron transport are major constraints under severe cold stress (Ali et al., 2022). The results of the present study indicate that low temperature (4°C) induces significant cold stress, whereas temperatures of 10°C and 15°C trigger intermediate responses, with 25°C reflecting normal physiological functioning. These multivariate patterns further emphasise the sensitivity of moringa cultivars to low temperature and provide evidence for a potential threshold of approximately 10–15°C, below which the cold stress response is activated.

The water use efficiency remained relatively stable at 10–15°C, which may suggest partial metabolic adjustment. However, the decline at 4°C indicates a breakdown in the coordination of carbon fixation and water regulation, which concurs with signs of chilling sensitivity in other tropical and temperate crops (Lukatkin et al., 2012; Qian et al., 2024). The progressive increase in Ci with decreasing temperature indicates that cold stress limits biochemical CO2 assimilation rather than stomatal conductance. At 25°C, the relatively low Ci revealed an efficient carboxylation capacity, whereas at 15–4°C,

relatively high Ci values suggest that a decrease in Rubisco activity impaired the regeneration of ribulose-1,5-bisphosphate and restricted electron transport under chilling stress (Ali et al., 2022).

Such biochemical constraints reduce the capacity for CO_2 fixation, which leads to its accumulation in the intercellular spaces despite sufficient stomatal openness. This response is critical for tropical and subtropical species, which lack strong cold acclimation mechanisms compared with temperate taxa, which can adjust enzymatic kinetics and membrane fluidity under cold stress (Zhou et al., 2025). This condition may pose a significant challenge in the establishment of moringa in temperate regions, especially in places such as South Island of New Zealand, whose temperature falls below 0°C during winter periods (NIWA, 2023).

Although 4°C is commonly used to impose chilling stress in experimental studies, it also represents a physiological stressful temperature for many temperate plant species, which respond by activating cold acclimation mechanisms such as osmotic adjustment, antioxidant defence, and membrane destabilisation (Preston & Sandve, 2013; Sandve et al., 2011). However, temperate species generally exhibit greater membrane stability and lower electrolyte leakage than tropical species under comparable chilling conditions because of their superior capacity for cold acclimation (Singh et al., 2026). In the present study, the decline in MSI and corresponding increase in MI at 4°C indicate that moringa, a species that grows best in tropical and subtropical areas, has limited tolerance to severe chilling despite evidence of physiological acclimation at 10-15°C. While these findings suggest that moringa may withstand moderate chilling, prolonged exposure to temperatures near 4°C would likely compromise growth and productivity, highlighting the need for cold-tolerant germplasm and appropriate agronomic management for cultivation in temperate regions.

6.4.2 Effect of cold stress on relative chlorophyll content

Temperature influenced relative chlorophyll content accumulation, with SPAD values increasing progressively from 4°C to 25°C, indicating that chilling conditions substantially impair chlorophyll accumulation (Trzcinska-Danielewicz et al., 2009). Low-temperature exposure is known to disrupt chloroplast ultrastructure, inhibit chlorophyll biosynthesis through downregulation of key enzymes such as protochlorophyllide oxidoreductase, and enhance chlorophyll degradation through oxidative stress (Adhikari et al., 2022; Ding et al., 2019; Hajihashemi et al., 2018). Chilling stress also impairs photosystem II (PSII) efficiency which leads to reduced carbon assimilation and photoinhibition (Wei et al., 2022). The significantly lower SPAD values at 4°C therefore reflect compromised photosynthetic capacity and metabolic activity, whereas the comparable values at 15°C and 25°C suggests that moderate to optimal temperatures sustain chloroplast integrity and pigment stability in plants (Chauhan et al., 2023; Hu et al., 2020).

From an agronomic perspective, these findings have a direct implication for the cultivation of moringa, a thermophilic species adapted to tropical and subtropical climates (Anwar et al., 2007; Palada et al., 2019). The significant decline in chlorophyll content under chilling conditions indicates that moringa is highly susceptible to low temperature stress, which may limit biomass accumulations and leaf quality in temperate regions. Consequently, successful cultivation in such environments would require strategies to mitigate chilling exposure, including greenhouse production, seasonal scheduling to avoid cold periods, mulching to moderate soil temperatures , or the selection of relatively cold-tolerant genotypes (Adhikari et al., 2022). Maintaining temperatures above 15°C

appears critical to preserve chlorophyll content and prolong photosynthetic efficiency and yield potential of moringa in temperate climates.

6.4.3. Membrane stability index and injury of moringa plants

At the structural level, membrane integrity was strongly compromised under cold conditions (Figure 15). The progressive decline in MSI and corresponding increase in MI with decreasing temperature indicate the vulnerability of membranes to chilling-induced destabilisation at 4°C in moringa leaves. These changes highlight the central role of membrane destabilisation in cold-sensitive plants, which is linked to lipid phase transitions, electrolyte leakage, and oxidative damage (Yoshida & Uemura, 1990). This destabilisation not only compromises cell integrity but also amplifies stress through the accumulation of reactive oxygen species (ROS), which further damages lipid bilayers and proteins in plants (Zhou et al., 2025).

The sharp increase in MI at 4°C further indicates that moringa lacks sufficient cold-induced lipid remodelling or protective mechanisms, features which are commonly observed in cold-sensitive species (Jahed et al., 2023; Qian et al., 2024). The present findings confirmed that membrane stability is a key determinant of cold tolerance in moringa. MSI increased progressively with increasing temperature, whereas MI exhibited the opposite trend, reflecting enhanced membrane integrity under less severe cold stress. Furthermore, the gradual increase in MSI between 10 and 15°C may indicate the onset of physiological acclimation, likely mediated by the accumulation of osmolytes and antioxidant metabolites that facilitate osmotic adjustment, alleviate oxidative damage, and preserve membrane integrity (Yoshida & Uemura, 1990). These observations concur with previous studies which demonstrate that MSI and MI are reliable physiological indicators of chilling tolerance and membrane integrity in plants exposed to low-

temperature stress such as basil (*Ocimum bassilicum*) and rice cultivars (Alamgir, 2023; Gupta et al., 2024).

6.4.3 Changes in primary metabolism in response to cold stress

Principal component analysis confirmed that prolonged cold exposure induced distinct metabolic reprogramming in moringa leaves, as evidenced by the clear separation of temperature treatments across all metabolomic platforms. Although the first two principal components explained a moderate proportion of the total variance (31.7–48.2%), this a characteristic trait commonly observed in untargeted metabolomic datasets, where metabolic variation is distributed across numerous correlated metabolites rather than being dominated by a few variables (Miao et al., 2025; Swain-Lenz et al., 2017). Consequently, biologically meaningful sample discrimination can occur even when the cumulative variance explained by PC1 and PC2 is relatively low. The scree plots further demonstrated that additional principal components contributed progressively smaller proportions of the total variance, supporting the robustness of the PCA model and indicating that the observed clustering reflected coordinated metabolic responses to temperature rather than random variation. Similar variance distributions have been widely reported for complex metabolomic datasets and reflect the high dimensionality and interconnected nature of plant metabolism (Miao et al., 2025; Shuangqian et al., 2023).

Given that HILIC ionisation results in stronger group separation than C18 does, cold-induced changes in moringa are likely driven by shifts in polar metabolites such as amino acids, organic acids, and soluble sugars, which are well-known markers of osmotic and redox adjustments under environmental stress conditions (Biswas et al., 2021; Wink, 2010). These compounds contribute to osmoprotection, redox balance, and cryoprotection to support cellular stability during cold stress (Ali et al., 2022; Wang et al., 2018). In

contrast, the subtle yet significant shifts detected in the C18 mode provide evidence for the remodelling of lipophilic metabolites such as fatty acids and phenolics, which play significant roles in membrane stabilisation and SM biosynthesis (Biswas et al., 2021).

Metabolomic profiling provided mechanistic insights into these physiological and structural impairments. Cold stress induced significant accumulation of PMs (Figure 18).

Amino acids such as tryptophan, tyrosine, arginine, aspartate, glutamate, citrulline, and pyroglutamic acid were enriched at 4°C. Many of these act as osmoprotectants and stabilisers of proteins while also serving as precursors for SMs that scavenge ROS (Guo et al., 2022). For example, glutamate and aspartate were also annotated, these are key nodes in nitrogen metabolism that support proline and GABA biosynthesis, both of which increase osmotic adjustment and redox homeostasis (Guo et al., 2022). Tryptophan, a precursor for auxin and indole indolic compounds, is likely to contribute to growth regulation and stress signalling (Eremina et al., 2016).

Sugars and osmolytes play prominent roles, with sucrose/galactinol, sorbitol, glucose, and glucose-6-phosphate showing strong accumulation under cold conditions. These carbohydrates contribute to osmotic balance, membrane protection, and cryoprotection, whereas sucrose and galactinol serve as precursors of raffinose family oligosaccharides that stabilise the membrane during freeze–thaw stress (Knaupp et al., 2011; Zuther et al., 2004). The elevation of glucose-6-phosphate could be connected with adjustments in glycolysis and the oxidative pentose phosphate pathway, which ensures nicotinamide adenine dinucleotide phosphate (NADPH) production for antioxidant defense (Jiang et al., 2022). Furthermore, the accumulation of organic acids of the TCA cycle, including 2-ketoglutamate, malate, and succinate (Appendix 3.3), was observed. These compounds play significant roles in remodelling central carbon metabolism to sustain energy

production under reduced photosynthetic activity to sustain plant growth and productivity (Xu & Fu, 2022).

Cold stress further induced the accumulation of polyamines, including putrescine, agmatine, and GABA (Figure 19). These compounds are known to stabilise membranes, regulate ion homeostasis, and scavenge ROS (Muthusamy & Lee, 2023). Research has demonstrated that GABA integrates into the "GABA shunt", which provides metabolic flexibility and the addition of energy during stress (Mazzucotelli et al., 2006). This might explain the accumulation of GABA at 4°C. These primary metabolic adjustments emphasise a coordinated attempt by moringa to mitigate osmotic imbalance, sustain energy metabolism, and buffer redox perturbations in a chilling environment.

6.4.4 Changes in secondary metabolism in response to cold stress

Secondary metabolism was significantly influenced in moringa under cold stress conditions, with strong enrichment of flavonoids (quercetin, kaempferol, luteolin, catechin, epicatechin, myricetin-3-O-rhamnoside, tricin, and vitexin) and phenolic acids (caffeic, ferulic, *p*-coumaric, sinapic, and salicylic acids) at relatively low temperatures (**Appendix 3.4**). These compounds are central to plant antioxidant defense mechanisms and function as membrane stabilisers, ROS scavengers, and modulators of redox signalling (Muthusamy & Lee, 2023; Samanta et al., 2011). The increase in these genes under cold stress suggests the activation of the phenylpropanoid pathway, a hallmark of cold stress responses that enhances the protection of membranes and photosystems against oxidative damage (Lukatkin et al., 2012; Zhou et al., 2025). Importantly, the accumulation of quercetin and luteolin in leaves may help mitigate photoinhibition by quenching excess excitation energy, thereby preserving photosystem II efficiency under chilling conditions (Salam et al., 2023; Zhou et al., 2025).

The accumulation of abscisic acid (ABA) under low-temperature exposure indicates its involvement in the cold-stress response of moringa in coordinating stomatal regulation and stress signalling. While the strongest ABA accumulation at 4°C reflects a severe chilling response, changes observed at 10 and 15°C are also physiologically relevant because these temperatures more closely represent moderate chilling conditions that moringa may encounter in temperate regions such as northern New Zealand. ABA accumulation may have contributed to the reduction in stomatal conductance by limiting transpirational water loss; however, this response may also restrict CO_2 diffusion into the leaf, thereby contributing to reduced photosynthetic performance under cold stress (He et al., 2023; Li et al., 2025).

While this ABA-mediated adjustment aligns with its established roles in drought and cold signalling (Raza et al., 2023), in moringa, it appears insufficient to prevent the severe decline in photosynthetic activity that reflects its intrinsic chilling sensitivity. Therefore, the ABA response should be interpreted not only as a marker of severe stress at 4°C, but also as part of a broader temperature-dependent acclimation process that may influence moringa establishment and productivity under temperate growing conditions.

In contrast, indole-3-acetic acid (IAA) and tyramine presented variable responses across the temperature range. This may suggest that the modulation of growth-related pathways is linked to secondary metabolism under stress (He et al., 2023). This hormonal interplay suggests that moringa may deploys ABA-driven protective signalling in parallel with auxin- and amine-associated adjustments (Kane & McAdam, 2023). However, the balance between stress protection and growth regulation remains skewed under severely cold environments, which limits its ability to acclimatise effectively.

6.4.5 Integrating multiple perspectives and metabolite pathway enrichment in moringa

Integrating physiological measurements with untargeted metabolomics and pathway enrichment analysis provides a holistic view of how moringa attempts to cope with declining temperatures (4°C). Cold stress triggers the reconstitution of central metabolic pathways, which aligns with well-established biochemical adjustments underlying cold stress acclimation in plants (Fürtauer et al., 2019; Satyakam et al., 2022). Pathway enrichment of amino acids, i.e., alanine, aspartate, and glutamate metabolism, indicates the redirection of nitrogen flux toward stress adaptive compounds, mainly proline, which functions as both an osmoprotectant and an antioxidant during chilling and freezing stress (Ghosh et al., 2022; Kita et al., 2024; Zhou et al., 2025).

The highly significant impact of alanine could be associated with the suppressive characteristic of mitochondrial respiration under cold stress, where pyruvate is diverted to alanine to maintain metabolic equilibrium (Ding et al., 2019). Concurrent enrichment of phenylpropanoid biosynthesis provides evidence for the activation of antioxidant and structural defence pathways. Derivatives such as flavonoids and lignin precursors contribute to ROS scavenging and reinforce cell walls against freeze-induced mechanical injury (Liu et al., 2018; Patil et al., 2024).

The highly significant pathway of carbohydrate (starch and sucrose) metabolism involves the mobilisation of solute sugars, including sucrose, galactinol, and sorbitol, which function as osmolytes and cryoprotectants to stabilise membranes and proteins under chilling stress (Sami et al., 2016; Wang et al., 2018). The activation of phenylpropanoid and flavonoid biosynthesis supports the accumulation of phenolic compounds whose antioxidant capacity is essential for mitigating ROS and protecting photosystems under

cold stress (Guo et al., 2022; Kebert et al., 2022). These changes illustrate the coordinated response mechanisms in which moringa invests metabolic resources in osmotic adjustment, ROS scavenging, and stabilisation of the cellular structure.

The involvement of isoquinoline alkaloid biosynthesis, tryptophan metabolism, and glyoxylate and dicarboxylate metabolism demonstrates the complex and metabolic breadth of plant responses to cold stress. These pathways extend beyond primary osmotic adjustment and antioxidant defence systems to encompass SM-mediated protection, the modulation of hormone-related signalling networks, and the increased metabolic flexibility required under low-temperature constraints (Ding et al., 2019; Eremina et al., 2016; He et al., 2023). Additionally, isoquinoline- and tryptophan-derived metabolites have been implicated in stress-induced redox regulation and defence signalling, whereas glyoxylate metabolism contributes to the maintenance of carbon flux, respiratory balance, and redox homeostasis when photosynthetic efficiency is attenuated by cold (Biswas et al., 2021; Sana et al., 2025; Tak et al., 2023; Wang et al., 2025). However, these metabolic pathway alterations and biosynthetic adjustments were insufficient to prevent cell membrane injury and increase the photosynthetic efficiency at 4°C. Hence, the limited tolerance of moringa at near-freezing temperatures has agronomic implications; i.e., without genetic improvements or targeted agronomic interventions, the productivity of moringa in temperate environments will remain constrained.

6.5 Conclusion

The results of the present study revealed that moringa plants have a multilayered defence strategy against cold stress. This strategy integrates the physiological downregulation of photosynthesis and stomatal conductance to reduce water loss and maintain WUE under moderate stress; structural destabilisation of membranes under chilling, as evidenced by

decreased MSI and increased MI; and metabolic mechanisms, i.e., the accumulation of osmolytes, amino acids, and antioxidant secondary metabolites. Despite these adjustments, moringa exhibited limited cold tolerance. The decrease in photosynthetic activity and severe membrane destabilisation at 4°C emphasise its inherent chilling sensitivity. Nevertheless, the identification of key protective metabolites, such as proline precursors, sucrose, putrescine, and flavonoids, offers promising targets for breeding and metabolic engineering to improve cold tolerance. This work highlights the potential of integrating metabolomics with other data sources to advance our knowledge of plant responses to stress. Future studies should focus on enhancing the synthesis or regulation of these compounds to increase osmotic adjustment, antioxidant capacity, and membrane stability and/or the selection of provenances with cold tolerance traits to expand the cultivation potential of moringa in temperate environments.

6.6 Acknowledgements

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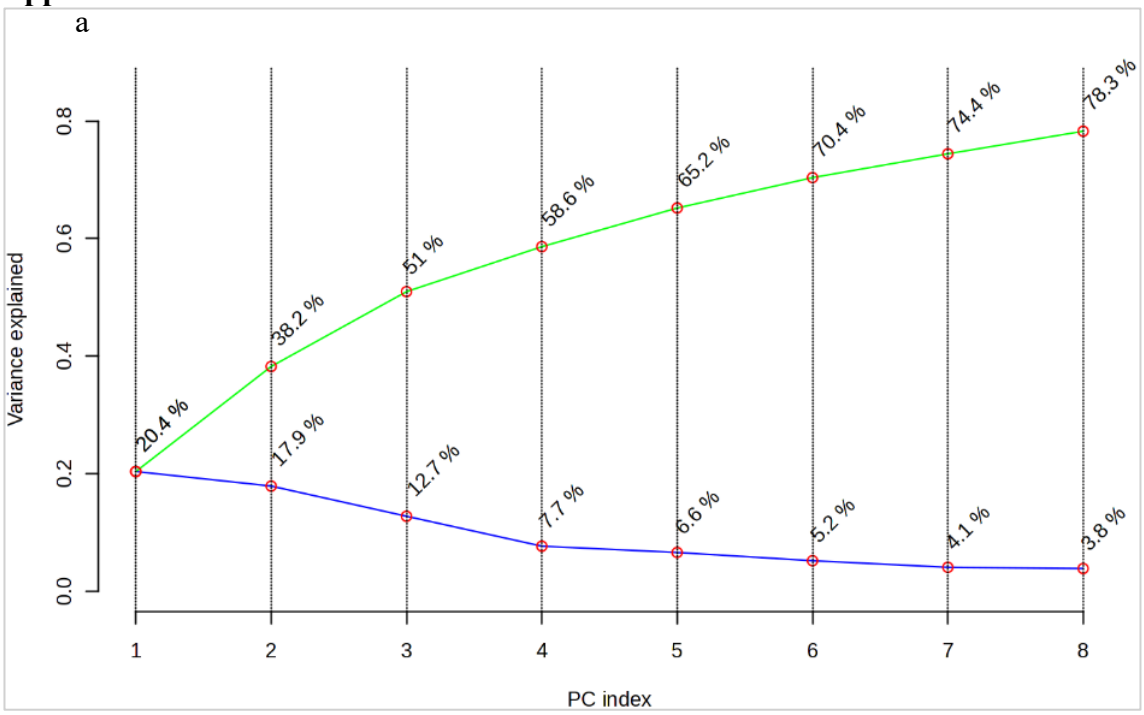
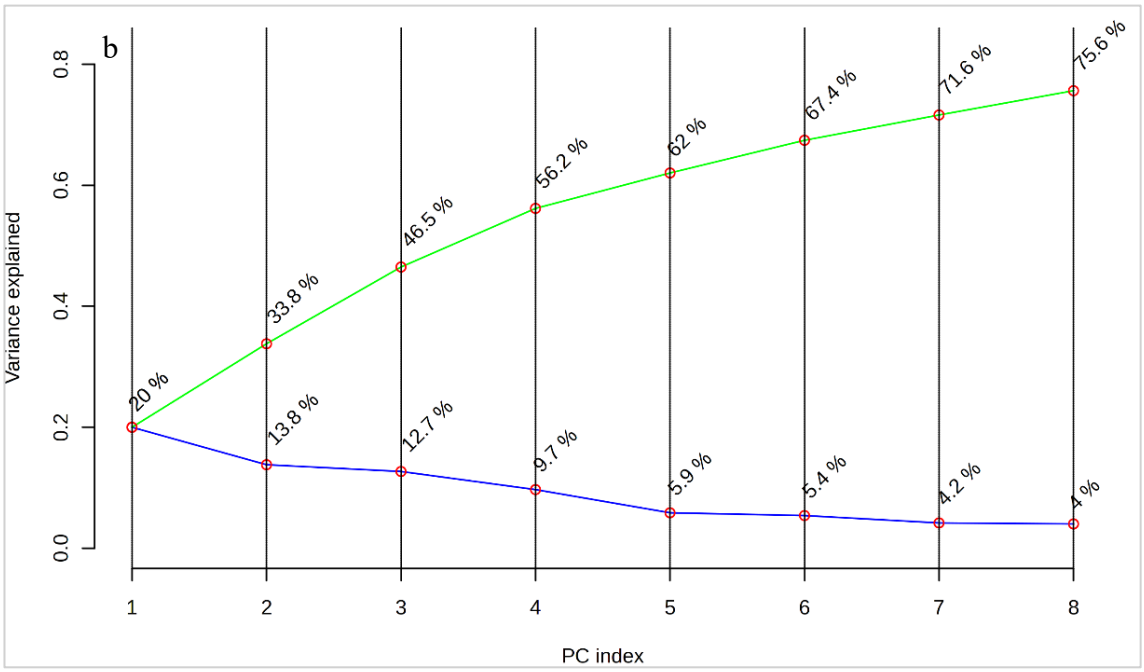
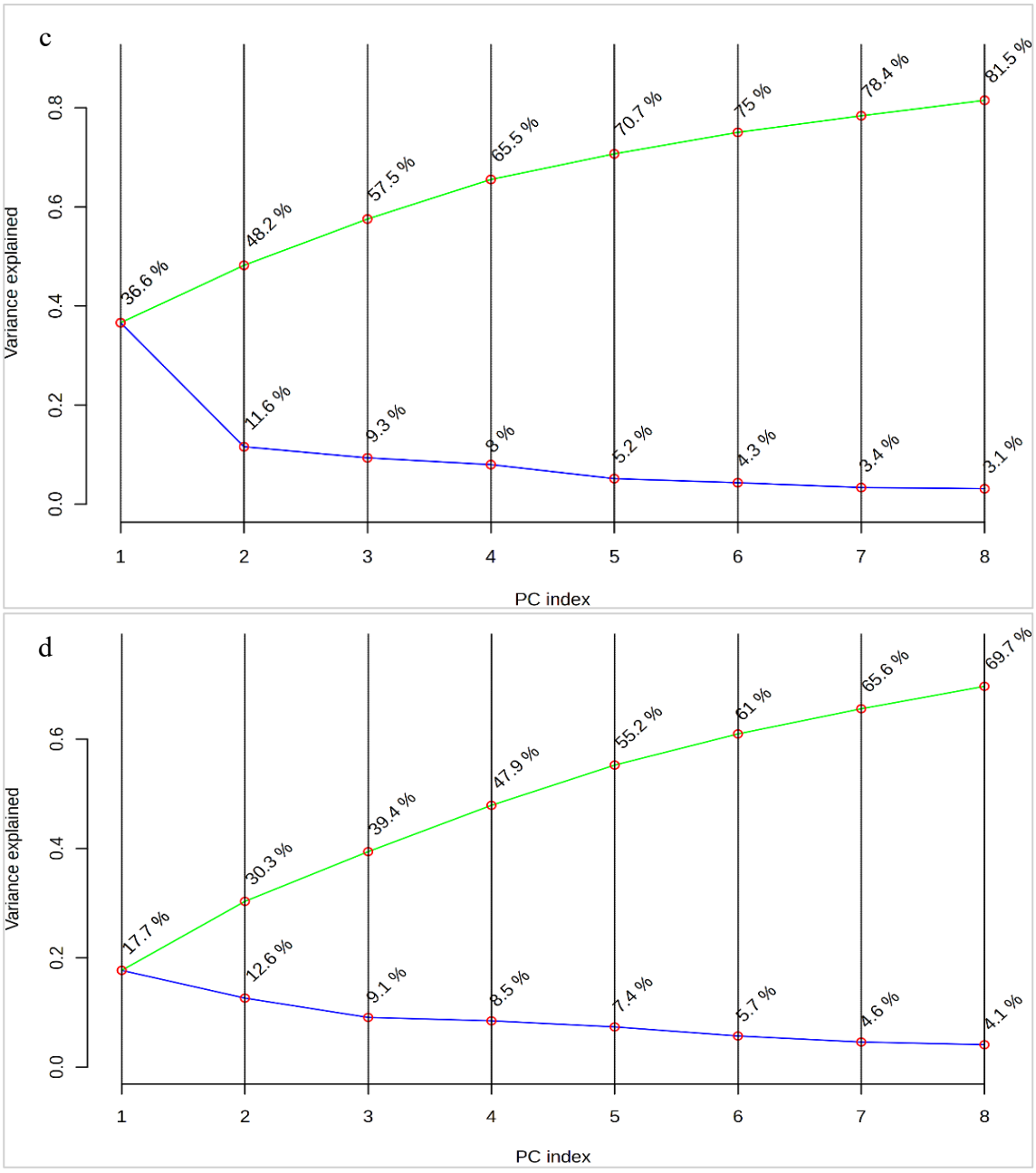
6.8 Appendix 3: Supplementary information

Appendix 3.1







Appendix 3.1 Scree plots showing the percentage of variance explained by individual principal components (blue line) and the cumulative variance explained (green line) for the (a), C18 positive (b), C18 negative (c) HILIC negative (Cd), HILIC positive metabolomic datasets. PCA was performed using all detected metabolites from moringa leaves following normalization, log transformation, and Pareto scaling in MetaboAnalyst 6.0. The distribution of variance across successive principal components indicates that the metabolomic variation was captured by multiple principal components, which reflected the multidimensional nature of the dataset.

Appendix 3.2 Metabolites associated with cold tolerance in moringa leaves with level 3 confidence were identified via LC–MS/MS spectral library matching.

Superclass	Class/subclass	Compound Name	KEGG	Stream	m/z	rt.
Phenylpropanoids	Flavonoid glycosides	Luteolin-7-glucoside	C03951	C18 POS	449.1085	8.75
Phenylpropanoids	Flavonoids	Myricetin-3-O-rhamnoside	C10108	C18 POS	465.1017	7.82
Phenylpropanoids	Flavonoids/Flavans	(+)-Epicatechin	C09727	C18 POS	291.0857	8.29
Phenylpropanoids	Flavonoids/Flavones	Quercetin	C01750	C18 POS	303.0493	10.7
Phenylpropanoids	Cinnamic acids a	Ferulic acid	C01494	C18 POS	195.0648	8.37
Phenylpropanoids	Flavonoid glycosides	Vitexin	C01460	C18 POS	433.1119	8.46
Organoheterocyclic compounds	Indolyl carboxylic acids	Indole-3-acetic acid	C00954	C18 POS	176.0783	9.68
Benzenoids	Phenethylamines	Tyramine	C00483	C18 POS	138.0991	9.58
Phenylpropanoids	Flavonoid glycosides	Tricin	C10193	C18 POS	331.0727	8.02
Organic acids	Gamma-keto acids	2-Ketoglutaric acid	C00026	C18 NEG	145.0133	5.21
Phenylpropanoids	Flavonoids	Chrysoeriol	C04293	C18 NEG	299.0561	10.1
Organoheterocyclic	Indolyl carboxylic acids	Tryptophan	C00078	C18 NEG	203.0821	4.37
Phenylpropanoids	Flavonoid glycosides	Kaempferol-3-O-rutinoside	C21833	C18 NEG	593.1513	8.29
Phenylpropanoids	Flavonoids/Flavans	Fustin	C01378	C18 NEG	287.0561	9.94
Lipids/lipid-like molecules	Prenol lipid	Abscisic acid	C06082	C18 NEG	263.1288	10.5
Phenylpropanoids	Hydroxycinnamic acids	Sinapic acid	C00482	C18 NEG	225.0754	10.0
Lipids/lipid-like molecules	Fatty Acyls/Lineolic	Jasmonic acid	C08491	H-POS	211.1324	2.52
Organic acids	Alpha amino acids	L-(+)-Arginine	C00062	H-POS	175.1186	16.7
Organic acids & derivatives	Alpha amino acids	L-Tyrosine	D00022	H-POS	182.0808	15.1
Organic acids & derivatives	Alpha amino acids	L-DOPA	C00355	H-POS	198.0757	2.54
Organic acids & derivatives	Alpha amino acids	L-Proline	C00148	H-POS	116.0705	14.6
Organic nitrogen	Organonitrogen	Putrescine	C02896	H-POS	89.1075	15.6
Carboxylic acids/derivative	Alpha amino acids	Glycine betaine	C00719	H-POS	118.0862	14.8
Organic oxygen compounds	Carbohydrates	Sorbitol	C00794	H-POS	183.0842	15.0
Organic nitrogen	Organonitrogen	Agmatine	C00179	H-POS	131.1289	15.6
Organic acids & derivatives	Alpha amino acids	L-Methionine sulfone	C00855	H-NEG	180.0328	2.51
Organic acids & derivatives	Alpha amino acids	5-Aminolevulinic acid	C00430	H-NEG	130.0499	2.45
Organic oxygen compounds	Carbohydrates	Galactinol/Sucrose	C01235	H-NEG	341.1085	17.2
Organic oxygen compounds	Carbohydrates	D-Glucose-6-phosphate	C00092	H-NEG	259.0221	17.2
Organic oxygen compounds	Carbohydrates	D-Glucose/Glucose/Dextrose	C00031	H-NEG	179.0552	15.2
Organic acids & derivatives	Alpha amino acids	L-Glutamic acid/L-Glutamate	C00025	H-NEG	146.0448	16.1
Organic acids & derivatives	Hydroxy acids	Malate	C00149	H-NEG	133.0132	7.92

Note: Compound identification followed the level 3 criteria of the metabolomics standards initiative (MSI), rt. = retention time, m/z = mass-to-charge ratio.

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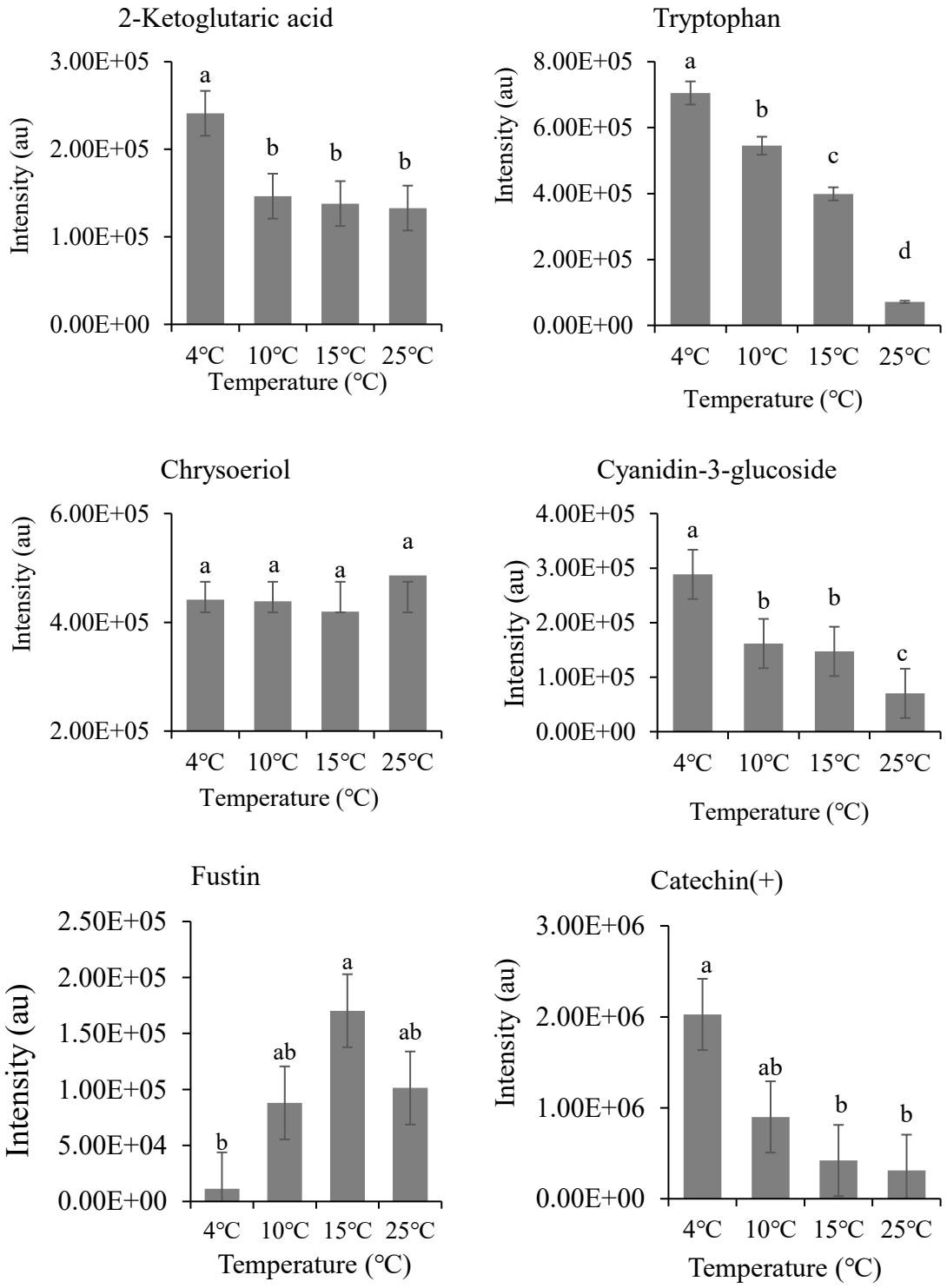
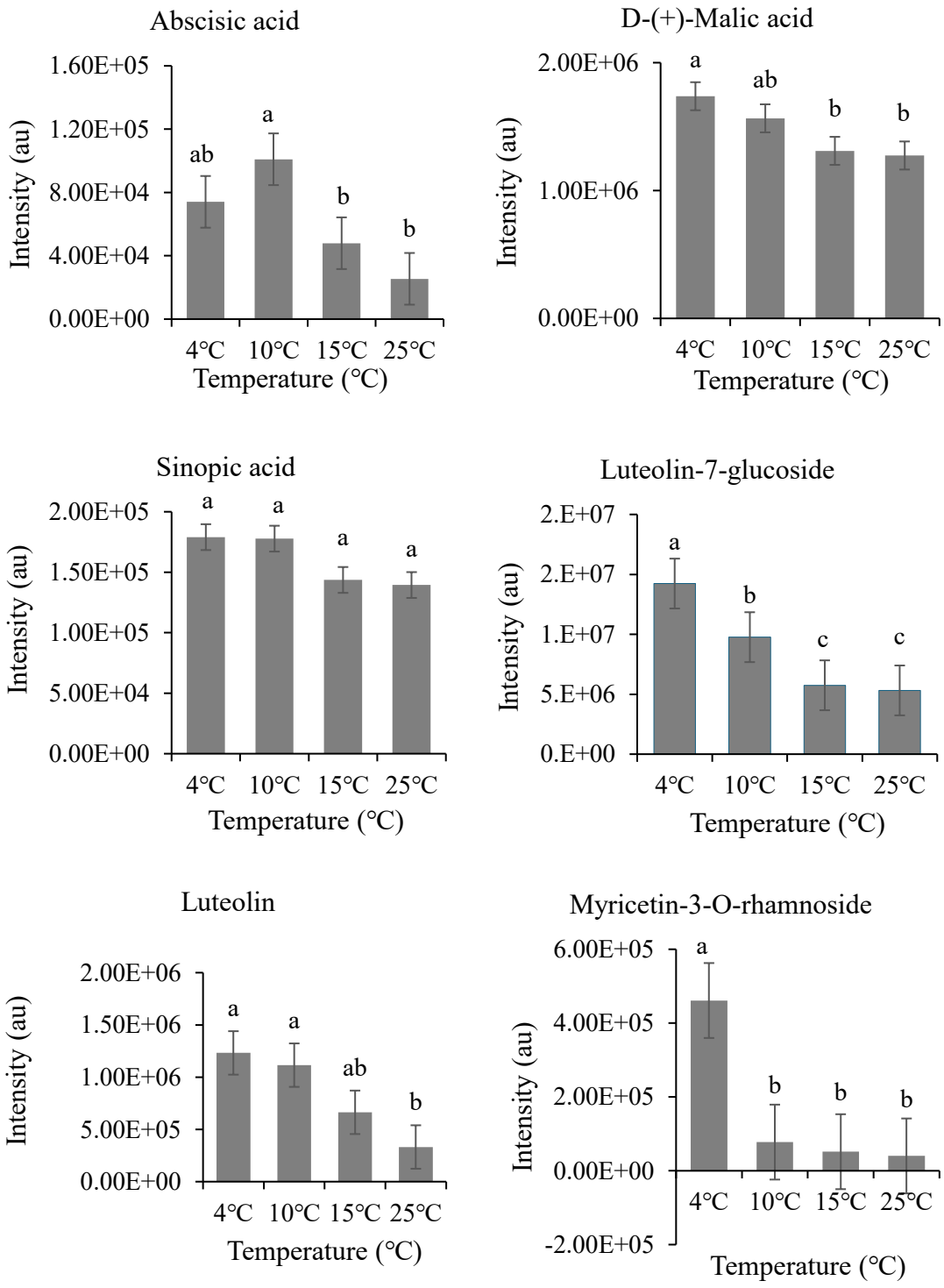
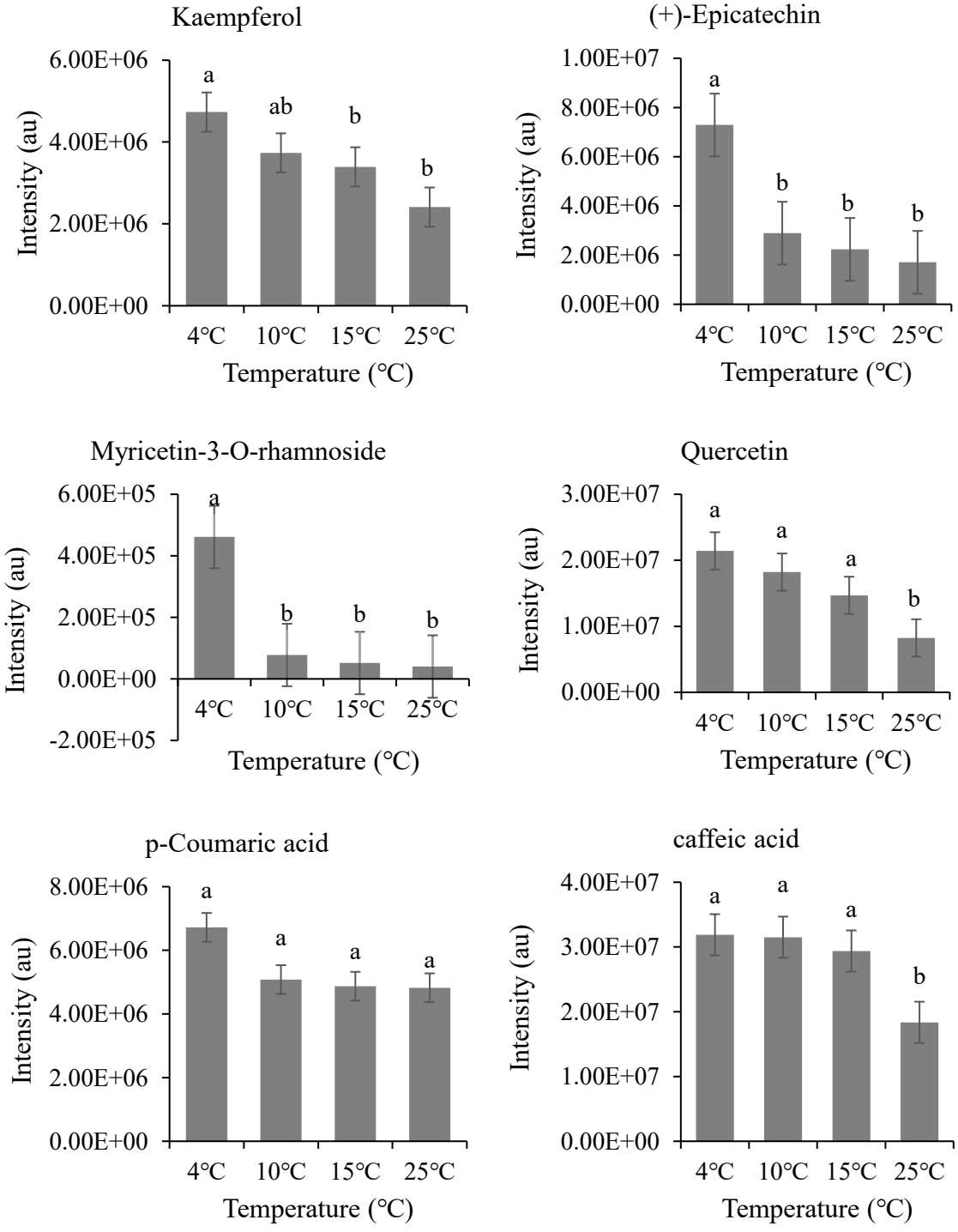
Appendix 3.2

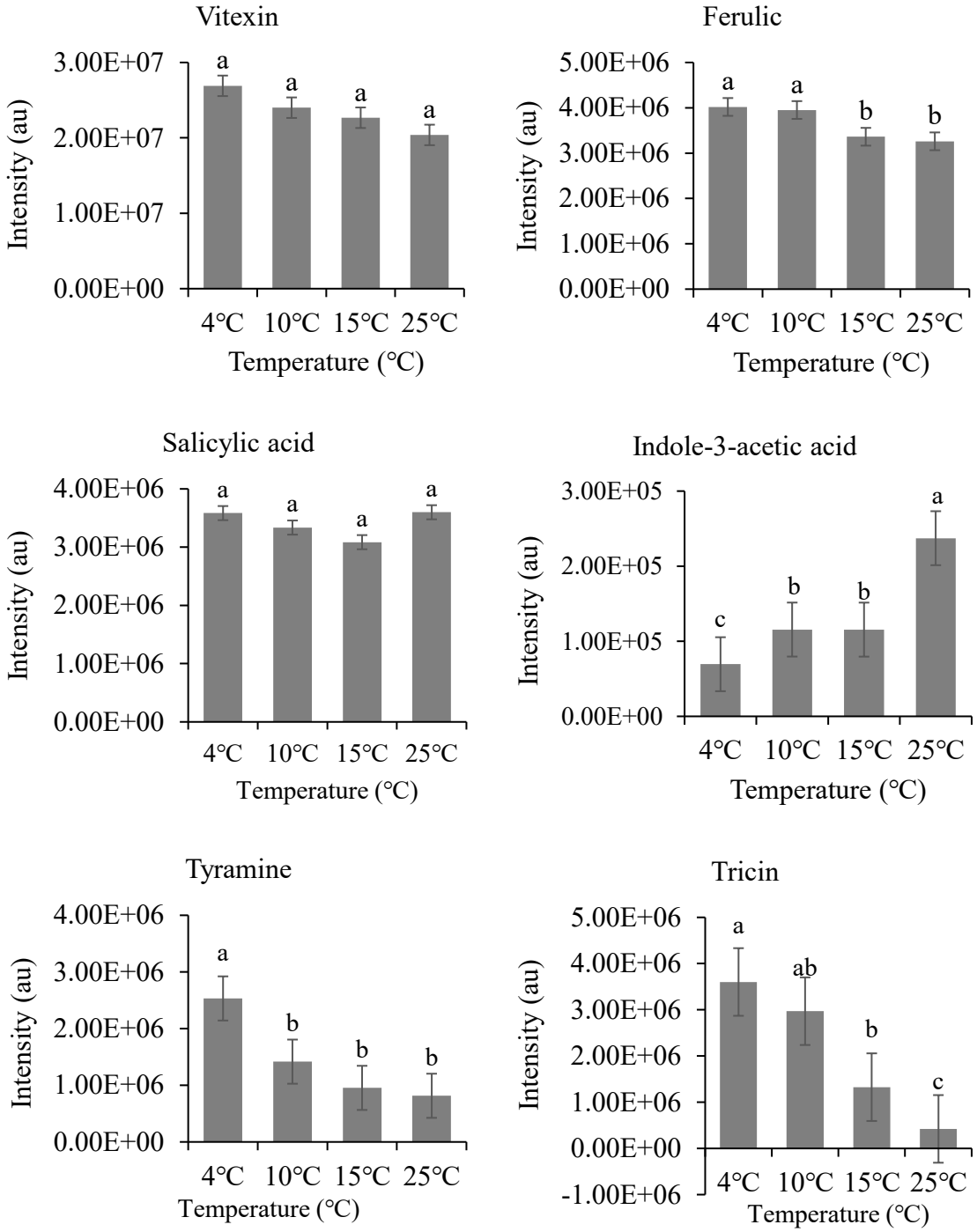

2-Ketoglutaric acid
Intensity (au)
3.00E+05
2.00E+05
1.00E+05
0.00E+00
a
b
b
b
4°C 10°C 15°C 25°C
Temperature (°C)
Tryptophan
Intensity (au)
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
a
b
c
d
4°C 10°C 15°C 25°C
Temperature (°C)
Chrysoeriol
Intensity (au)
6.00E+05
4.00E+05
2.00E+05
a
a
a
a
4°C 10°C 15°C 25°C
Temperature (°C)
Cyanidin-3-glucoside
Intensity (au)
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
a
b
b
c
4°C 10°C 15°C 25°C
Temperature (°C)
Fustin
Intensity (au)
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
b
ab
a
ab
4°C 10°C 15°C 25°C
Temperature (°C)
Catechin(+)
Intensity (au)
3.00E+06
2.00E+06
1.00E+06
0.00E+00
a
ab
b
b
4°C 10°C 15°C 25°C
Temperature (°C)



Appendix 3.3. Relative abundance of selected metabolites in moringa annotated in C18 negative mode. The compounds included 2-ketoglutaric acid, tryptophan, chrysoeriol, cyanidin-3-glucoside, fustin, catechin, and abscisic acid. Several metabolites accumulated at 4°C, reflecting cold-induced adjustments in energy metabolism, amino acids, and hormone signalling.

Appendix 3.3


Kaempferol
Intensity (au)
6.00E+06
4.00E+06
2.00E+06
0.00E+00
a
ab
b
b
4°C
10°C
15°C
25°C
Temperature (°C)
(+)-Epicatechin
Intensity (au)
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
a
b
b
b
4°C
10°C
15°C
25°C
Temperature (°C)
Myricetin-3-O-rhamnoside
Intensity (au)
6.00E+05
4.00E+05
2.00E+05
0.00E+00
-2.00E+05
a
b
b
b
4°C
10°C
15°C
25°C
Temperature (°C)
Quercetin
Intensity (au)
3.00E+07
2.00E+07
1.00E+07
0.00E+00
a
a
a
b
4°C
10°C
15°C
25°C
Temperature (°C)
p-Coumaric acid
Intensity (au)
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
a
a
a
a
4°C
10°C
15°C
25°C
Temperature (°C)
caffeic acid
Intensity (au)
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
a
a
a
b
4°C
10°C
15°C
25°C
Temperature (°C)



Appendix 3.4 Relative abundance of metabolites annotated in C18 positive ionisation mode. Flavonoids (epicatechin, quercetin, kaempferol, myricetin-3-O-rhamnoside, vitexin, and tricin) and phenolic acids (p-coumarin, caffeic, ferulic, and salicylic acid) were strongly enriched under cold stress, which demonstrated the activation of phenylpropanoid metabolism and antioxidant defences.

Chapter 7

General discussion

7.1 Main research findings

The introduction of novel crop species into new environments requires systematic evaluation of climatic suitability, invasive potential, and abiotic stress-adaptation mechanisms to local conditions to minimise the risk of unintended ecological and agronomic consequences. These aspects are particularly relevant when considering the introduction of moringa to New Zealand (NZ), a temperate country with many endemic species of flora and fauna; and different ecological and environmental conditions than moringa's native ranges.

Despite substantial evidence supporting the adaptive capacity and utilitarian value of moringa, research has largely focused on tropical and subtropical production systems, with temperate regions remaining comparatively unstudied (AgriFutures, 2024). The present study is novel in nature because it is the first to 1) assess the climate suitability and invasive risk assessment of moringa in temperate regions including NZ; 2) screen its allelopathic potential for economically important native, pasture, and weed species and profile the composition of its allelochemical compounds; and 3) investigate the physiological and metabolic adaptation mechanisms to prolonged cold stress and assess its potential to withstand prolonged cold climates for potential introduction into NZ.

The results of the climate suitability and invasive risk assessment in Chapter 4 show that moringa has the potential to be established in Northland and the surrounding microclimates in North Island, where moringa is classified as either suitable or

moderately suitable under current and projected future climates. These results are consistent with the species being adapted to warm tropical and subtropical climates, with limited incidences of frosts and moderate to high seasonal rainfall (Alshoaibi, 2021; Azam et al., 2020; Palada et al., 2019). On the basis of moringa growth suitability classifications, none of the regions in NZ were classified as highly suitable because they failed to meet the optimal climatic thresholds of a mean annual temperature of ≥ 25°C (Haile & Ayansa, 2022). These climatic limitations likely restrict moringa establishment and performance, given its adaptation to tropical and subtropical environments that favour optimal photosynthetic and growth processes (AL-Shoaibi, 2021; de Sousa Melo et al., 2020). These findings highlight the need to determine whether the development of cool-tolerant moringa cultivars is feasible, as genetic adaptation may be a prerequisite for successful establishment in temperate environments (Peter et al., 2023).

Equally critical is the low invasive risk rating derived from the weed risk assessment for currently available seed lines. Moringa has a low-risk potential for traits associated with ecological threats or invasions. However, there is a paucity of information on dispersal mechanisms, ability to form permanent soil seed banks, and periods of seed storability. These results concur with observations from other non-native cultivation contexts where moringa has not exhibited any invasive behaviour (Csurhes & Navie, 2016). The low-risk classification complements the precautionary principles underlying the Hazardous Substance and New Organisms Act (HSNO, 1996) of NZ, which supports the consideration of controlled introduction for species with low invasive potential to the environment.

Despite moringa being classified as a low-risk species under the current and future climate conditions of NZ, the species still warrants further scrutiny. The allelopathic screening presented in Chapter 5 provides complementary evidence that environmental suitability

must be assessed alongside biochemical interactions with key beneficial crop species (Iqbal et al., 2023; Pant et al., 2021). The results revealed that allelochemicals derived from moringa root exudates exert species-specific and concentration-dependent effects, with white clover exhibiting susceptibility across treatment levels. As a key nitrogen-fixing legume in NZ grazed pastoral ecosystems, white clover supports soil fertility, pasture persistence, and increased livestock productivity (Cartmill & Donaghy, 2024; Dineen et al., 2018). Such inhibition activity signals a potential risk to ecosystem functionality, particularly in landscapes or farming systems where white clover is ecologically and economically significant (Romera et al., 2017). However, its impacts are likely to depend on the mode of integration within farming systems, mainly whether moringa is intercropped with pasture or managed separately under a cut-and-carry system, as these approaches differ in their effects on plant interactions, pasture composition, and farm management.

In contrast, perennial ryegrass, another key species in NZ pasturelands, presented no detectable response under either controlled or seminatural plant–plant interaction conditions. This indicates that the allelochemical effects of moringa are not broad-spectrum but instead may influence specific components of the plant community, the results which concur with those of previous studies on selective allelopathic interactions (Cheng & Cheng, 2015). These allelopathic effects most likely occur through shifts in legume–grass equilibrium rather than through generalised suppression of dominant pasture species (Kumar et al., 2024). In NZ pasture systems, the potential allelopathic impacts of moringa are more likely to occur through alterations in the legume–grass equilibrium rather than through direct suppression of dominant grass species. Pasture productivity in these systems relies heavily on legumes such as white clover for biological nitrogen fixation and nutrient cycling (Dineen et al., 2018; Egan et

al., 2021). These selective effects on legumes could therefore modify competitive interactions, nitrogen inputs, and overall pasture function, even where grass biomass is largely unaffected.

At relatively high moringa root extract concentrations (20–100%) and under seminatural plant–plant interaction conditions, moringa root exudates significantly suppressed the growth of fathen and crabgrass, which are common invasive weed species in NZ. This suppression effect aligns with evidence that allelochemicals exert both concentration-dependent and species-specific effects through interference with germination physiology, membrane function, and nutrient acquisition (Tahir et al., 2018). However, from a land management perspective, such suppressive effects can be utilised in integrated weed management and may support future development of moringa-based bioherbicides, reducing the reliance on synthetic agrochemicals (El-Rokiek et al., 2022).

Allelochemical profiling in this study confirmed the presence of phenolic acids, flavonoids, benzenoids, and coumarins. These classes of secondary metabolites are widely recognised for their allelopathic roles, including germination inhibition, disruption of root elongation, oxidative regulation, and hormonal signalling in target plants (Macías et al., 2019). The reduction in root and shoot length could be attributed to the presence of phenylpropanoid derivatives, which are known to interfere with essential cellular processes, including reactive oxygen species (ROS), homeostasis, and auxin transport, leading to oxidative stress and reduced cell elongation (Bachheti et al., 2020). The coexistence of multiple compounds increases the likelihood of synergistic suppression effects, as reported in other allelopathic species, such as *Triticum aestivum* L. and *Calluna vulgaris* (L) Hull (Anwar et al., 2021; Effah & Clavijo McCormick, 2024).

Bioactive compounds such as umbelliferone, coumarin, 4-hydroxycoumarin, and homovanillic acid are classical examples of low-molecular-weight phenolics that are transported through pH- and transport-driven efflux mechanisms (Gani et al., 2021). These transport mechanisms are crucial for root exudation processes that facilitate the secretion of allelochemicals into the soil, where they alter microbial decomposition, nutrient recycling, or germination and growth of competing plants (Kumar et al., 2024). Although the mechanistic role of these transporters in root-mediated allelopathy remains unclear, the metabolite profiles observed in this study support their potential involvement. In this context, transporter activity can regulate the release, concentration gradients, and persistence of allelochemicals in the rhizosphere, thereby influencing the strength, range, and selectivity of allelopathic interactions (Gani et al., 2021).

The allelochemical profile in this study further revealed potential weed-suppressive benefits and aligns with the observed inhibition of fathen and crabgrass, both of which are persistent competitors in pastoral systems (Oreja et al., 2025). However, the same mechanisms that restrict weed establishment may also affect native plant regeneration, raising concerns about unintended impacts on biodiversity in mixed pastoral–native landscapes. In the present study, native mānuka seeds exhibited minimal sensitivity to moringa-derived allelochemicals under low concentrations; however, elevated allelochemical loads resulted in a measurable delay in germination. The results suggest that in high-density moringa stands where allelochemical inputs are likely to accumulate, mānuka incorporation may be constrained through germination suppression and reduced early seedling performance. These findings provide enough evidence that the phytotoxicity of allelochemicals arises from the synergistic actions of multiple chemical classes and concentration with complementary modes of action (Cheng & Cheng, 2015),

resulting in dose-dependent inhibition of germination and growth across responsive species.

Climate suitability analysis alone is not enough to guarantee the successful introduction and cultivation of new crops into novel environments (Brown et al., 2024). Since low temperatures have been described as the main limiting factor for the distribution and cultivation of moringa in temperate regions (Trigo et al., 2020), adaptation mechanism assessments were conducted to evaluate the physiological and metabolic responses of moringa to cold stress (Chapter 6). The results revealed that moringa mounts multilayered adaptation mechanisms that involve physiological downregulation, membrane destabilisation, and metabolic alteration to counter osmotic and oxidative stress, as reported in many species adapted to cold stress (He et al., 2023; Satyakam et al., 2022).

The progressive attenuation of net photosynthesis, stomatal conductance, and transpiration rates with decreasing temperature, culminating in near-complete inhibition at 4°C, demonstrates the intrinsic thermo-sensitivity of the moringa cultivar used in this study; the characteristic trait of many tropical and subtropical plant species prone to cold stress and lacking acclimation capacity (Preston & Sandve, 2013; Sandve et al., 2011). The reduction in membrane stability and corresponding increase in membrane injury at 4°C further revealed that moringa lacks constitutive cold acclimation mechanisms. These cellular disruptions are associated with lipid phase transitions and oxidative damage in cells, both of which compromise the survival of plants during prolonged and repeated chilling events (Barrero-Sicilia et al., 2017; Liu et al., 2018).

Metabolomic profiling revealed strong temperature-dependent clustering that indicated an inducible reconstitution of primary and secondary metabolites in response to cold stress (Ritonga & Chen, 2020; Terletskaya et al., 2024). The results further demonstrated

coordinated biochemical responses involving antioxidant defence and hormonal signalling, likely resulting from the enrichment of flavonoids and phenolic acids, coupled with increased accumulation of abscisic acid, which together enhance cellular protection and regulate stress-responsive pathways under cold stress (Kita et al., 2024; Ritonga & Chen, 2020). Accordingly, the metabolomic profile generated in this study may provide a reference for future research and selection efforts to identify or develop cold-tolerant moringa lines.

The inability of moringa to maintain photosynthetic functions or membrane integrity under severe chilling conditions aligns with modelling outputs (Chapter 4) that confine the currently available cultivars' suitability to Northland and surrounding microclimates, which are frost-limited zones (NIWA, 2023). Episodic cold events rather than mean seasonal temperatures are principal barriers to the field establishment and productivity of moringa, as transient frosts can cause irreversible tissue damage and mortality in non-acclimating tropical species (Pearce, 2001). This vulnerability also supports moringa's low invasive potential, as frost-prone winters in most regions in NZ act as a natural containment strategy to prevent environmental escape, naturalisation or spread beyond cultivation sites (Meurisse et al., 2023).

From an ecological and agronomic perspective, these physiological and metabolic limitations have direct implications for establishing current moringa cultivars as perennial crops in temperate regions such as NZ. Low-temperature thresholds adversely affect seedling establishment, root development, and early-season vegetative growth and reduce the likelihood of overwinter survival (Alshoaibi, 2021). Frequent exposure to temperatures below 4°C, even in the absence of frost, can inhibit growth rates, shorten the effective growing season, and ultimately limit biomass accumulation and reproductive success (Hussain et al., 2018; Soualiou et al., 2022).

In summary, this study illustrates a valuable but spatially limited opportunity for the introduction of moringa in NZ, with its establishment feasible in Northland and the surrounding microclimates in North Island, and a low likelihood of invasiveness or naturalisation due to inadequate cold tolerance mechanisms. This study also revealed that root-derived allelochemicals presented species-specific and concentration-dependent suppressive effects. Although the species mounts metabolic and physiological defence mechanisms under chilling stress, these responses fail to prevent cold stress-induced damage in moringa, which could pose a challenge for cultivation in temperate regions.

7.2 Integration of research findings and their implications for the introduction of moringa in New Zealand

The following section discusses the systematic interpretation of the results and provides implications for the introduction and cultivation of moringa into NZ farming systems under four subheadings: 1) environmental suitability and invasion risk assessment impacts; 2) ecological interactions and agronomic implications of moringa in temperate regions; 3) physiological and metabolic responses of moringa to cold stress and its implications for potential introduction in NZ; and 4) policy recommendations and pathways for moringa introduction in NZ.

7.2.1 Environmental suitability and invasive risk potential of moringa in New Zealand

Although occurrence-based species distribution models such as MaxEnt and CLIMEX are widely applied for predicting potential species distributions, the climate-matching approach adopted in this study (Chapter 4) was selected because it directly addresses agricultural suitability rather than realised species distributions. Moringa has been widely

introduced outside its native range, and many available occurrence records represent cultivated populations rather than naturalised populations, which may bias correlative distribution models (Roigé and Phillips, 2021). The B3 App instead evaluates climatic suitability using physiological thresholds relevant to crop performance, making it suitable for assessing the feasibility of introducing moringa into NZ production systems. Nevertheless, future studies could integrate climate-matching with occurrence-based modelling approaches to improve predictions under current and future climate scenarios.

Climatic conditions such as low temperatures influence the geographical distribution of most cultivated and non-cultivated plant species (Mahaut et al., 2021). A comprehensive literature review revealed that low temperature is the main limiting factor for the cultivation and distribution of moringa in most temperate regions (AL-Shoaibi, 2021; Amaglo et al., 2017; Bania et al., 2023). The confinement of moringa suitability to Northland and adjacent microclimates (Chapter 4), confirms the reliance of existing cultivars on warm temperature regimes, which aligns with global evidence of their dominance in tropical and subtropical production systems (Palada et al., 2019; Trigo et al., 2020). This pattern aligns with moringa's sub-Himalayan origin, where warm, humid tropical lowlands contrast sharply with cooler, snowfall-prone temperate hill regions (Rahim et al., 2023).

The present study revealed the significant effect of cold stress on the survival of moringa, the reduced photosynthetic activities, membrane stability and injury in this study revealed the inherent sensitivity to cold stress (Chapter 6). These results are supported by Soualiou et al. (2022), who reported that cold stress damages cellular membranes, causes membrane injury, and impairs photosynthetic activities. Most locations in NZ, including large areas of lower North Island and all of South Island, were classified as moderately suitable or not suitable for moringa establishment due to the prevalence of low winter

minima and high frost frequency (NIWA, 2023). These findings confirm that moringa introduced with the currently available cultivars would not be viable as a broad-scale crop across the national landscape but could be cautiously considered a region-specific or niche crop under controlled or semi controlled land use scenarios (MPI, 2019).

Climatic variables influence species' probability of establishment, subsequent spread, and eventual naturalisation (Bell et al., 2021; Roigé & Phillips, 2021). Hence, the invasive risk potential of moringa in NZ appears to be primarily constrained by its climatic suitability (Chapter 4)**.** The absence of any region classified within the highest suitability class (Haile & Ayansa, 2022) in the current assessment suggests that under the present climatic conditions, moringa faces substantial limitations to successful invasions. These restrictions are further supported by the species' physiological dependence on warm tropical environments (Chapter 6), which may inherently limit moringa's tolerance to prolonged periods of low temperature (Soares et al., 2023).

Consequently, the frequent frost events characteristic of the temperate climate of NZ are likely to suppress key biological processes, including seed germination, vegetative growth, and reproductive success (Muhl et al., 2011; Palada et al., 2019). These factors indicate that the current cultivars of moringa present a low risk of widespread, naturalisation and invasive behaviour within NZ, supporting the findings in Chapter 4. Nevertheless, isolated microhabitats, projected regional warming under climate change scenarios or the identification of cool-tolerant seed lines may create pockets of improved suitability, thereby easing the current constraints on persistence and spreading potential (Bania et al., 2023; Early et al., 2016).

Moringa also appears to pose a relatively low invasion risk owing to the absence of several traits commonly associated with invasive woody perennials. In contrast to species

that readily naturalise and displace native flora, moringa lacks key ecological characteristics linked to high invasiveness, including the formation of persistent soil seed banks, bird-mediated long-distance dispersal, and strong competitive dominance in early successional habitats (Cordero et al., 2023; Drenovsky et al., 2012; Gioria et al., 2021). The predicted confinement of moringa to warm microclimates in Northland further functions as an ecological containment zone that would reduce the possibility of unwanted spread beyond cultivated areas. Furthermore, the winter soil and air temperatures in most locations (classified as moderately suitable or unsuitable) regularly fall below the physiological threshold to support the germination and growth of current moringa lines (AL-Shoaibi, 2021). However, reassessment of invasive potential following the approach outlined in Chapter 4 will be needed for any cultivars developed that exhibit cold tolerance.

The combination of restricted climatic suitability and low invasive potential, currently position moringa as a species that could be assessed under a controlled introduction framework rather than triggering high-level exclusion under the Hazardous Substances and New Organisms Act (HSNO, 1996); however, further assessment is needed if cool-tolerant lines are identified. Climate suitability analysis and invasive risk assessment results indicate that moringa does not present an immediate or latent threat of invasion or naturalisation at the national scale owing to its high mortality risk under frost-prone conditions (Soares et al., 2023). These response characteristics align with NZ's precautionary biosecurity framework based on better border biosecurity, where species with low reproductive aggression and limited climate resilience may be considered for conditional introduction rather than blanket exclusion (Macneil, 2022; Murray et al., 2024).

7.2.2 Ecological interactions and agronomic implications of moringa

The impacts of introducing moringa into NZ agroecosystems are likely context dependent, with outcomes ranging from beneficial to neutral or adverse, contingent on management practices and the expression of its selective allelopathic potential (Chapter 5). The effects of moringa root extracts were both species specific and concentration dependent, with white clover, a key component of NZ pastoral systems (Cartmill & Donaghy, 2024; Egan et al., 2021), showing significant inhibitory responses. Such selective allelopathy may alter legume–grass dynamics rather than causing broad suppression of dominant pasture species if moringa is incorporated within pasture systems.

The selective allelopathic effects observed in chapter 5 suggest that moringa may facilitate changes in the vegetation composition and indirectly influence ecosystem processes by altering habitat structure, forage quality, and resource availability for soil biota, invertebrates, pollinators, and grazing livestock (Early et al., 2016; Drenovsky et al., 2012; Cordero et al., 2023). While the preceding invasive risk assessment classified moringa as a low invasive-risk species in NZ, the present physiological findings indicate that limited tolerance to severe chilling is likely to constrain its establishment beyond climatically suitable regions (Chapter 6). These findings suggest that the risk of widespread naturalisation is low under current NZ climatic conditions. Nevertheless, the introduction of moringa should follow NZ's precautionary biosecurity framework through cultivation in climatically suitable regions, routine monitoring for natural regeneration, and periodic reassessment of invasion risk under future climate change scenarios (Gioria et al., 2021; Cordero et al., 2023; Drenovsky et al., 2012).

The non-responsive nature of perennial ryegrass, another key pasture species in NZ to moringa root allelochemicals could be linked to detoxification mechanisms (Schulz & Friebe, 2023). These mechanisms involve enzymatic conjugation, vacuole compartmentalisation, and active transport mediated by ATP-binding cassette transporters that facilitate the efflux of conjugated metabolites and limit cytosolic phytotoxicity (Scavo et al., 2019; Schulz & Friebe, 2023). The high detoxification capacity of perennial ryegrass may confer a competitive advantage under the allelopathic influence of moringa. This could explain its resilience in mixed swards, where white clover suppression could be evident. However, the ability of perennial ryegrass to neutralise or compartmentalise allelochemicals through these mechanisms increases the complexity of plant–plant interactions in managed ecosystems (Makoi & Ndakidemi, 2012).

The observed suppressive effects on common weed species at relatively high extract concentrations and in seminatural plant–plant interactions indicate that moringa could outcompete weeds and potentially be a good source of bioherbicides contributing to sustainable weed management (El-Rokiek et al., 2022). The inhibitory effects observed on germination, root and shoot elongation, and root and shoot biomass could be linked to the presence of bioactive compounds, including flavonoids, phenolic acids, benzenoids, and coumarins, which are associated with allelopathic activity in plants (Cheng & Cheng, 2015; Macías et al., 2019; Scavo et al., 2019). From the perspective of integrative weed management in cropping systems, moringa-based allelochemicals could be used to reduce the use of agrochemicals, aligning well with NZ's national goals for regenerative agriculture focused on developing low-emissions, climate-resilient, and nature-positive food systems by restoring soil health, water quality, and biodiversity (Kumar et al., 2025). However, the inhibitory effects on white clover emphasise the need to weigh the beneficial effects of weed suppression against possible negative impacts on the nitrogen

fixation symbiosis of white clover, a species that forms a backbone in NZ pastoral systems (Cartmill & Donaghy, 2024; Egan et al., 2021).

The consideration of interactions with native flora further broadens the ecological context. In this case, mānuka, a species of high economic and cultural value, showed no detectable response in seminatural plant–plant interaction experiments. Nevertheless, the suppression effect of mānuka observed at elevated extract concentrations (50–100%) signifies that allelopathic effects may emerge under very high concentrations or in densely populated (residual) conditions (Kato-Noguchi & Kurniandie, 2022), this suggests that inter-plant distance is to be considered if planting mānuka and moringa together or in close vicinity.

The variable responses to allelochemicals among target species (pasture species, weeds and native species) suggests that the observed variation may arise from intrinsic detoxification capacities in certain species, whereas in others, the chelation or adsorption of allelochemicals onto soil particles may alter their bioavailability, thereby attenuating their phytotoxic effects (Scavo et al., 2019; Schulz & Friebe, 2023). This information could aid the selection of plants growing alongside moringa to optimise agroecological benefits.

The allelopathic effects observed in the present study should be interpreted in the context of the experimental approaches employed. Root extract bioassays likely represent the maximum phytotoxic potential of moringa, as grinding root tissues disrupts cellular integrity and releases intracellular metabolites that may not be immediately available under natural conditions. In contrast, intact root systems release allelochemicals gradually through root exudation, root turnover, and decomposition, resulting in lower and more

spatially heterogeneous concentrations within the rhizosphere (Macías et al., 2019; Kumar et al., 3024).

Consequently, the comparatively weaker or species-specific responses observed in the pot experiment more closely reflect allelopathic interactions likely to occur under field conditions. Although the present study focused on the effects of moringa on neighbouring pasture species, the potential for autotoxicity should also be considered. Allelochemicals released into the rhizosphere may inhibit the germination or growth of conspecific seedlings, as reported for several plant species, thereby influencing regeneration, stand establishment, and long-term productivity (Tetteh et al 2026; Wang et al., 2022). Whether moringa exhibits similar autotoxic responses remains unknown and warrants further investigation, particularly under intensive or continuous cultivation systems. Such studies would improve understanding of the ecological behaviour of moringa and support the development of sustainable management practices for its cultivation.

Although climatic suitability modelling indicated that moringa presents a low invasion risk under current NZ climatic conditions, its potential interactions with native forest ecosystems and commercial forestry warrant consideration as part of a comprehensive ecological risk assessment. The establishment of non-native tree species can alter ecosystem processes through competition for light, water, and nutrients, as well as through the production of allelochemicals that may influence seed germination, seedling recruitment, and plant community composition (El-Rokiek et al., 2022; Kato-Noguchi & Kurniandie, 2022). In addition, indirect effects on soil microbial communities, nutrient cycling, and mycorrhizal associations may influence ecosystem functioning, particularly where introduced species become naturalised (Mahaut et al., 2021; Oreja et al., 2025). However, the current strong dependence of moringa on warm, frost-free environments substantially limits its capacity to establish within NZ's indigenous forests, which are

generally characterised by cooler microclimates and winter temperatures below the physiological thresholds required for sustained growth. Likewise, although allelopathic effects were observed under controlled experimental conditions, there is currently no evidence to suggest that moringa would adversely affect commercially important forestry species, such as *Pinus radiata*, under field conditions. Nevertheless, cultivation adjacent to native forests or plantation margins should be accompanied by periodic ecological monitoring to detect any changes in vegetation composition, natural regeneration, or soil biota, particularly if moringa more tolerant to cooler conditions become available. Such a precautionary approach is consistent with NZ's biosecurity framework and would facilitate the sustainable introduction of moringa while safeguarding native biodiversity and forestry resources.

7.2.3 Physiological and metabolic responses of moringa to cold stress and its implications for potential introduction in New Zealand

The present findings indicate that moringa species possesses limited physiological and biochemical plasticity under low-temperature stress, supporting its classification as a tropical–subtropical species (CABI, 2022). The rapid reduction in photosynthetic performance and membrane stability under cold stress aligns with prior evidence from cold-sensitive species that experience disruption of cellular metabolic processes (Satyakam et al., 2022; Soualiou et al., 2022). Unlike temperate adapted species, which deploy coordinated cold acclimation responses, including the upregulation of osmoprotectant accumulation, CBF/DREB transcription factors, and membrane lipid remodelling (Barrero-Sicilia et al., 2017; Hajihashemi et al., 2018), moringa appears to lack sufficient induction of these pathways, thereby increasing its susceptibility to chilling injury at lower temperatures below the optimal range. This inherent deficiency may limit

the ability of the plants to maintain cellular homeostasis during transient but ecologically significant temperature fluctuations, especially in winter and early autumn, where temperatures fall below 4°C in many parts of the NZ.

The decrease in membrane stability at 4°C was accompanied by a sharp increase in membrane injury (electrolyte leakage), revealing membrane phase transition, lipid peroxidation, and structural membrane injury (Abd El-hady et al., 2021). Cold-induced electrolyte leakage is a primary determinant of stress injury commonly observed in noncold-acclimating species, which limits both survival and post stress recovery in most tropical and subtropical species (Steponkus, 2003). The present study provides evidence that moringa lacks the constitutive adaptation mechanisms for lipid desaturation, cryoprotection, and membrane restructuring, which are present in most cold-adapted perennial crops (Satyakam et al., 2022). The rapid onset of membrane injury further suggests that frost or suboptimal winter temperatures in most parts of NZ could act as a barrier to its establishment and reduce invasive threats in most locations (Chapter 4).

Metabolomic profiling provided further evidence of moringa's limited adaptation strategy under cold stress. The strong temperature-dependent clustering of metabolites observed in this study provides evidence for a systematic stress–response reconstitution of metabolites (He et al., 2023; Jahed et al., 2023; Y. Kong et al., 2024). Cold exposure initiates the accumulation of osmolytes, carbohydrates, amino acids, polyamines, and organic acids. These compounds are known to stabilise proteins, buffer osmotic stress, and mitigate ROS damage (Devireddy et al., 2021; He et al., 2023; Rácz et al., 2023; Terletskaya et al., 2024).

Secondary metabolism is visible and highly responsive to increased biosynthesis and metabolism of flavonoids, carbohydrates, and phenolic acids; these compounds have been

recognised as antioxidants and play membrane protective roles through scavenging free radicals, reducing oxidative stress, and stabilising cellular membranes (Ljubej et al., 2021; Salunke & Koche, 2023). The increase in abscisic acid (ABA) at 4°C demonstrates the activation of hormonal stress-signalling pathways that regulate stomatal closure and initiate oxidative defence mechanisms (Chelli-Chaabouni, 2014). Although these metabolic responses indicate some degree of biochemical and physiological plasticity under cold stress, they are insufficient to prevent membrane injury at 4°C, underscoring the cold sensitivity of the current moringa variety (PKM-1) assessed and its limited capacity for effective low-temperature acclimation (Satyakam et al., 2022; Soualiou et al., 2022).

The metabolomic profiling in this study may help to link the observed cold sensitivity to underlying biochemical responses and provides a basis for developing more cool-tolerant moringa cultivars. By identifying metabolites and pathways associated with cold stress responses, metabolites can reveal biochemical markers of tolerance that can be used to screen and select improved germplasms. This integrative approach connects climatic suitability, physiological performance, and genetic improvements, which can support more targeted breeding of moringa cultivars adapted to cooler environments (Roychowdhury et al., 2025). However, the absence of robust cold acclimation in moringa reflects evolutionary trade-offs associated with its adaptation to arid and semiarid tropical environments (Trigo et al., 2020).

Species native to tropical climates often allocate resources toward heat and drought tolerance, such as osmotic adjustment, cuticular reinforcement, and strong antioxidant systems, at the expense of mechanisms that confer cold resilience (Soualiou et al., 2022). Although some primary and secondary metabolites were upregulated in moringa during cold stress, these responses were insufficient to prevent decreases in membrane integrity

and photosynthetic efficiency. Hence, moringa cold sensitivity does not arise from a single deficient pathway but from a broader insufficiency across multiple protective systems required to sustain cellular function under low temperatures, a common characteristic trait in plants native to tropical and subtropical regions (Satyakam et al., 2022; Yadav, 2010). Consequently, the current moringa variety (PKM-1) remains highly susceptible to persistent chilling and episodic frost events, imposing significant constraints on its establishment and long-term persistence in temperate regions where seasonal temperatures frequently fall below its physiological tolerance range.

A key implication of these findings is the need to prioritise the identification of cold-tolerant moringa germplasm as a foundational step for expanding cultivation into cooler regions. Previous studies have emphasised substantial genetic variability within the moringa population, which suggests the potential for selection or breeding for improved cold stress tolerance (Alavilli et al., 2022; Balakumbahan & Boopathi, 2021; Boopathi et al., 2021; Hassanein & Al-Soqeer, 2018; Lakshmidevamma et al., 2021). Traits such as enhanced membrane stability, improved carbohydrate reserves, and altered phenological responses have been associated with cold tolerance in other crop species and may present useful targets for developing cold-tolerant moringa lines with the aim of improving the production of moringa in temperate regions (Adhikari et al., 2022; Roychowdhury et al., 2025).

The present findings highlight the importance of identifying cold-tolerant moringa germplasm as a prerequisite for expanding cultivation into temperate regions. Enhanced cold tolerance would improve establishment, overwinter survival, and biomass production under the moderate chilling conditions that characterise northern NZ, thereby increasing production stability and reducing losses associated with low-temperature stress. From an agricultural perspective, the successful introduction of cold-tolerant

moringa could diversify NZ's agricultural systems by providing a locally produced, nutrient-dense crop with applications in nutritional supplementation (Anwar et al., 2021; Palada et al., 2019; Trigo et al., 2020). The physiological and metabolomic markers identified in this study therefore provide useful selection criteria for breeding programmes aimed at improving cold adaptation while supporting the sustainable introduction of moringa into NZ production systems.

The combination of favourable climatic suitability in northern regions, limited invasive potential, and the capacity for physiological acclimation to moderate chilling suggests that moringa could become a valuable high-value multipurpose crop within diversified production systems. Its recognised applications in human nutrition, livestock supplementation, nutraceuticals, and greenhouse gas mitigation through improved ruminant feeding strategies further enhance its agricultural significance (Makkar and Becker, 1996; Leone et al., 2015; Mangar et al., 2022). However, successful commercial development will depend on selecting cold-tolerant germplasm capable of maintaining physiological performance under temperate conditions while ensuring that cultivation remains environmentally sustainable and consistent with NZ's biosecurity framework.

7.2.4 Policy recommendations and pathways for moringa introduction in New Zealand

The combined evidence from this thesis demonstrates that the feasibility of introducing moringa into the NZ farming system requires a targeted, risk-managed, and policy-aligned approach rather than broad-scale promotion or prohibition. Moringa's restricted climatic suitability, low invasive potential, species-specific and dose-dependent allelopathic effects, and limited tolerance to cold stress (Chapters 4, 5 & 6) support the feasibility of regulated introduction within defined geographic zones. In the context of

NZ farming systems, HSNO-aligned regulation of low-risk species with potential ecological interactions supports a proportional policy response that balances innovation with environmental protection (HSNO, 1996).

Conditional approval pathways enable targeted management and monitoring within pastoral or managed landscapes, ensuring that potential impacts on pasture composition, nutrient cycling, and system productivity are identified and managed without imposing unnecessary constraints on farm-level diversification (MPI, 2019; Thomas et al., 2024). The findings of this research provide evidence for such a framework by demonstrating that current moringa varieties are unlikely to escape cultivation and naturalise in NZ. However, the cultivation of moringa may alter agroecosystem species dynamics in pastoral landscapes, necessitating a precautionary stance in which management measures are applied to balance economic opportunities with environmental protection (Hulme, 2020; van Kleunen, Essl, Pergl, Brundu, et al., 2018).

The potential of moringa to contribute to crop diversification and climate adaptation strategies must be weighed against its agronomic and ecological constraints. The Ministry for Primary Industries (MPI) has identified the need for the diversification of high-value crops suited to warming North Island locations under emerging climate resilience initiatives (MPI, 2019; Thomas et al., 2024). Moringa's limited climatic niche aligns with this targeted regional development model that offers the scope for integration into Northland agricultural systems, provided that monitoring protocols and planting guidelines are established. However, allelopathic effects on white clover signal the need to develop co-cultivation approaches or exclusion from legume-dominant pastures to prevent unintended impacts on nitrogen fixation and forage quality. Similarly, the observed suppression of weed species and minimal response of mānuka under realistic conditions suggest the potential for moringa-based bioherbicide applications, which could

inform sustainable farming policies aimed at reducing synthetic herbicide use (El-Rokiek et al., 2022; Scavo et al., 2019). Henceforth, the integration of moringa into NZ pastoral systems will be a key area for further research.

The present study provides a pathway for the introduction of moringa into NZ farming systems that is grounded in a precautionary and policy-aligned approach. Initial efforts should prioritise the identification of cool-tolerant moringa lines suited to regions classified as moderately suitable by enabling targeted selection and breeding within controlled research environments (Challinor et al., 2014; Hulme, 2020). Any subsequent expansion should proceed gradually and under the regulatory oversight of the Environment Protection Authority (EPA), in accordance with the HSNO Act 1996, which requires assessment of environmental and biosecurity risks prior to release (EPA, 2011; HSNO, 1996).

This initiative-taking policy pathway involves staged introduction through pilot trials rather than commercial scale development. Initial cultivation could be conducted in frost-limited zones, which include most locations on North Island (NIWA, 2023), with evaluations of plant–soil–community dynamics, growth recovery post-winter stress, and management feasibility. This could concur with the best practices for novel crop evaluation under NZ's biosecurity and agricultural innovation systems (MPI, 2019), where demonstration plots could be used to generate local performance data for regulatory purposes (Hulme, 2020). Additionally, Māori land-based enterprises, regional councils, and the MPI could play leading roles in codeveloping guidance on location-specific suitability, cultural acceptability, and ecological safeguarding. The integration of moringa into agroforestry, silvopastoral, or protected cultivation systems could further mitigate ecological risks while capitalising on its economic and biocontrol value.

7.3 Conclusion and future perspectives

This study provides an integrated assessment of the potential introduction of moringa into NZ through climate modelling, invasive risk evaluation, allelopathic potential, and cold stress-related physiological and metabolic responses. The current results confirm that the environmental suitability of moringa is limited to Northland and the surrounding areas on North Island, which are classified as suitable locations for the growth and cultivation of moringa. The plant was further classified as a low invasive risk species under both current and future climatic scenarios due to its inability to form a permanent soil seed bank and acclimatise to cold stress. For widespread introduction, cultivars of moringa with greater tolerance to cold temperatures are needed.

Allelopathic studies revealed species-specific and concentration-dependent effects, with white clover, a key nitrogen-fixing legume in pastoral systems, showing significant growth inhibition, whereas perennial ryegrass and mānuka was not affected. At higher concentrations and managed plant–plant interactions in pot trials, moringa root exudates suppressed the germination and growth of common weed species. Metabolomic profiling confirmed the presence of bioactive compounds, including phenolic acids, flavonoids, benzenoids, and monoterpenes, which provided evidence for allelopathic effects in response species. These findings may indicate the potential use of moringa extracts as bioherbicides and the need for further research to determine the impact of moringa on pasture plants.

Cold stress physiology and metabolomic profiling revealed that moringa possesses metabolic plasticity but has limited tolerance to cold stress, with sharp decreases in photosynthesis, membrane stability, and WUE at 8°C and near-complete inhibition at 4°C. Although the species accumulates osmolytes and activates antioxidant and hormonal

defence mechanisms under chilling stress, these mechanisms fail to prevent cellular injury. While research findings have demonstrated that moringa could be introduced safely under controlled and region-specific conditions in this study, its agronomic potential remains constrained by climate and ecological interactions. This work focused on a single variety of moringa (Variety PKM-1), which was developed in the warm tropical environment of Tamil Nadu. Assessment of seed lines grown from more cold-hardy moringa varieties, such as STX-1 and STX-2, could provide information on climate suitability and potential introduction in temperate regions, including NZ (Peter et al., 2023).

Overall, this thesis demonstrates that evaluating the introduction of crops growing in tropical regions into temperate environments requires an integrated approach that combines ecological modelling with physiological, biochemical, and metabolomic analyses. By linking climatic suitability with the mechanisms governing cold tolerance and ecological interactions, this study provides a robust scientific basis for the sustainable introduction of moringa into NZ. More broadly, the framework developed in this thesis may be applicable to the evaluation of other tropical and subtropical crop species being considered for cultivation in temperate agricultural systems under changing climatic conditions.

Future studies should prioritise the identification of molecular markers associated with cold responsiveness, long-term field trials in marginal climates, and modelling studies that assess how shifting temperature patterns may influence the feasibility of moringa production. Research should further explore the persistence and mechanisms of moringa allelochemicals by focusing on their influence on pasture legumes and soil microbial processes. Selection, genotype screening, and breeding to increase cold resilience should be emphasised to expand its agricultural potential, while studies on compound isolation

may support the development of moringa-based bioherbicides for sustainable weed control. Once introduced to NZ, interactions between moringa and surrounding herbivores and microorganisms should also be studied, to explore pest and disease resistance and other relevant ecological interactions. Policy guidelines should focus on a controlled introduction framework involving the MPI, regional councils, Māori land enterprises, and environmental agencies to ensure ecological safety, cultural compatibility, and sustainable land use goals.

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