Phenotypic evaluation of *Trifolium repens* × *Trifolium uniflorum* F_1 interspecific hybrids as predictors of BC_1 hybrid progeny

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Abstract

Interspecific hybrids between white clover (*Trifolium repens*) and its close relatives are being created to address the lack of variation within white clover for traits relating to persistence and drought tolerance. This study addresses two concepts related to developing hybrid breeding strategies using *Trifolium repens* x *Trifolium uniflorum* hybrids. A first sandframe experiment investigated whether some of the first generation hybrid plants (F_1) with common parents were better than others as future parents. A second experiment assessed whether the performance of the first back cross (BC_1) progenies could be predicted from the parental F_1 phenotypes. The foliage, fertility, roots and dry weight production of four families of F_1 hybrids were evaluated following a period of growth in sand. From each family, the F_1 hybrids with the highest and lowest dry weight production were selected and back crossed to two contrasting white clover cultivars. The resulting BC_1 hybrid phenotypes were evaluated to ascertain whether any F_1 hybrids were markedly better as future parents in hybrid breeding programmes, and whether the F_1 phenotype could be used to predict that of the BC_1 progeny.

Differences in expression of more than half of the traits evaluated were found both between F_1 families, and among genotypes within F_1 families. Evaluation of the subsequent BC_1 generation identified large amounts of variation in expression of most traits both within and among hybrid families. However correlations between trait expression of the F_1 parent and the corresponding BC_1 progeny were weak to non-existent for most traits evaluated. The absence of correlations indicated that the performance of an F_1 hybrid genotype is not able to be used as a predictor of the BC_1 progeny phenotypes, and that selection out of the F_1 generation is futile in the formation of interspecific hybrid breeding populations.

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Abbreviations

°C	Degrees Celsius
BC_1	Backcross 1 hybrid
cm	Centimetres
cv	Cultivar
F1	First filial generation
FDW	Foliar dry weight
FLO	Florets per inflorescence
g	Grams
IL	Internode length
LA	Leaf area
MA	Minimum anchoring
mm	Millimetres
MS	Maximum plant spread
NRD	Nodal root diameter
PL	Peduncle length
PS	Pollen stainability
RD	Root depth
RDW	Root dry weight
SD	Stolon density
SDi	Stolon diameter

sq	Square transformed
sqrt	Square-root transformed

Chapter 1

1 Introduction

Overview

White clover (*Trifolium repens* L.) is a widely used forage legume in temperate pasture systems, predominantly grown with perennial ryegrass (*Lolium perenne* L.) to produce a highly nutritive forage for grazing by livestock. As intensive pasture based farming expands into more marginal environments in an attempt to keep up with food production for an ever increasing global population, there comes an increased pressure from pests, diseases, drought and low fertility. Under these conditions, white clover often fails to perform and persist. The white clover species alone lacks the genetic diversity required to breed cultivars with traits adapted to environmental stresses such as low fertility and drought. Wide hybridisation has been utilised in many crops of economic importance to improve cultivated varieties. Similarly, hybridisation of white clover with its wild *Trifolium* relatives facilitates the introgression of genes for adaptive traits to enable improved performance in suboptimum environments. *Trifolium uniflorum* L., native to the Mediterranean region and adapted to hot and dry climates, possesses traits such as woody taproots, thick leaves and short internodes. Successfully introgressed into white clover, these traits could potentially achieve this. The creation of a successful hybrid cultivar for use in agriculture is not only dependent on the creation of hybrids, but also the development of efficient breeding strategies in order to reap their genetic potential.

1.1 White clover

White clover (*T. repens*), an allotetraploid (2n=4x=32), is a temperate pasture species of economic importance in animal grazing systems due to its high nutritional quality, production, palatability, and its ability to fix atmospheric nitrogen in the soil (Williams, 1987a; Caradus *et al.*, 1996). It thrives best on damp soils and in grassy swards, and is a very diverse species, with adaptations to a range of environmental niches (Williams & Nichols, 2011). A highly heterozygous, outcrossing species, white clover can reproduce both sexually, with plants capable of producing thousands of seeds per season, and asexually via the production of vegetative horizontal stems, or stolons. The stolons produce nodal roots, which anchor them to the soil surface (Williams, 2014), and form independent plants as the base of the stolon (furthest from the growing tip) continually senesces. The stoloniferous growth and phenotypic plasticity of white clover make it an ideal companion legume in most grass swards and enable it to withstand severe defoliation (Woodfield & Caradus, 1994). Despite general plasticity in shoot morphology, white clover varieties can be categorised by leaf size. Small leaf type clover varieties generally have higher stolon densities and can endure the hard grazing characteristic of set stocked grazing systems (Brock & Hay, 1996). Large leaf types have a comparably low stolon density, and are more commonly utilised in rotational grazing systems.

While white clover thrives in moist environments, productivity drastically declines when moisture and nutrients are limited. Thomas (1984) attributed the poor performance of white clover in a sward under drought and nutrient stress in part to its fibrous and relatively shallow root system, making it less able to compete with larger ryegrass root systems for soil water reserves. White clover root development goes through two distinct phases. First is the taprooted phase, lasting up to 18 months following germination (Widdup & Barrett, 2011) where the seminal root system (taproot) forms. Second is the clonal phase, commencing upon the death of the taproot, and characterised by the fragmentation of the stolons, and the dependence of each clonal stolon fragment upon its own nodal root systems (Brock *et al.*, 2000). The thin, shallow nodal roots have limited ability to access soil moisture, and become vulnerable in dry periods and in infertile soils, in turn increasing white clover's vulnerability to pests and diseases (Williams, 2014). The seeds of white clover are quite small when compared with other legumes, and are slow to establish, leading to poor establishment in competition with forage grasses (Nichols, 2012, p. 10).

White clover cultivar development has been occurring since the 1930s, and a study of 110 cultivars published by Woodfield and Caradus (1994) found that there was a 6% rate of genetic gain for dry weight production per decade. Despite being a highly heterozygous outcrossing species, white clover lacks the genetic variation for traits that would improve its adaptation to a wider range of environments, especially semi-arid soils of low fertility (Williams *et al.*, 2007). Although it is the most used temperate legume in grazed pastures, it could be used more widely if the gene pool was enhanced by the introgression of wild relatives (Williams & Nichols, 2011).

1.2 The genus *Trifolium*

The genus *Trifolium* is made up of 250-300 species, more than 20 of which are cultivated as forages (Zohary & Heller, 1984; Williams, 1987b; Ellison *et al.*, 2006; Williams & Nichols, 2011). Williams and Nichols (2011) estimate that there are almost 50 other species of interest, for either direct use in agriculture, or as sources of genes for the incorporation of new traits in cultivated species. All species are herbaceous perennials or annuals, often prostrate, with distinctive leaves usually composed of three leaflets (Ellison *et al.*, 2006). Ellison *et al.* (2006) analysed both nuclear and chloroplast DNA from 218 species of *Trifolium*, and as a result, redefined relationships between species, and classified a new Section *Trifoliastrum*, containing what Williams *et al.* (2006) designated "the white clover complex". The eight species and white clover within this complex appear closely related, and are therefore potential sources of genetic variation through interspecific hybridisation (Williams *et al.*, 2006; Nichols, 2012). Originating from a wide range of geographic locations, these related species offer a wealth of variety in their adaptations to environments, resistance to pests and diseases, and in their plant morphology.

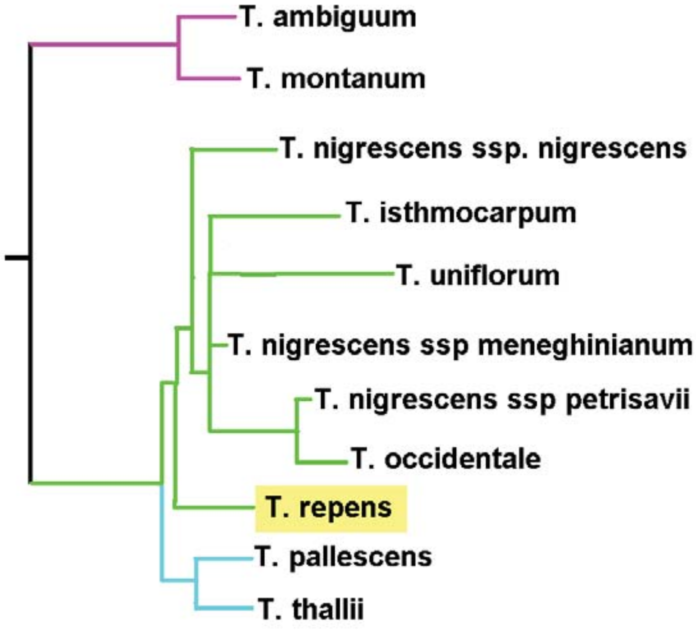


Figure 1.1: The "white clover complex" from within the Section *Trifoliastrum*, designated by Williams *et al.* (2006).

1.3 Wide hybridisation

As intensification of farming increases, plant breeders seek to develop cultivars that are more productive, more nutritious, and better adapted to environments without the need for increased external inputs, such as the application of water and nutrients. Breeders can exploit the extended heritable variation that results from interspecific hybridisation (Marshall *et al.*, 1995) and improved rates of genetic gain can be achieved when new traits that are not known in either parent are expressed in hybrids (Tanksley & McCouch, 1997). Harlan and de Wet (1971) describe three gene pools from which breeders can source genetic diversity for cultivar development. The primary gene pool includes individuals of the same species, where mating occurs easily, and progeny are viable and fertile. When genes for traits of interest cannot be found within a species, breeders can look to related species for further variability. The secondary gene pool includes members of closely related species that will cross with the crop, and produce progeny, however they tend to be sterile, and often weak. The tertiary gene pool also contains related species, however, hybrid progeny tend to be abnormal, lethal or completely sterile.

While wide hybridisation offers many promising advances in plant breeding, problems including, but not limited to, the cross incompatibility between the wild related species and cultivated crop species, F_1 hybrid sterility, reduced recombination between chromosomes of the two species (Zamir, 2001), and the phenomenon of linkage drag – the unplanned introgression of undesirable or deleterious genes linked to the target genes (Acquaah, 2009) – can stand in the way of progress. Despite the grim prognosis for hybrid survival, there are tools to overcome the hybridisation barriers. Failure of hybrids post-fertilisation is commonly associated with endosperm abortion and the consequent starvation of the hybrid embryo (Williams & De Lautour, 1980). Hybrids have been produced by dissecting embryos from young ovules shortly after pollination then growing the on media containing nutrients and hormonal substances (Williams & De Lautour, 1980), a method known as embryo rescue. Endosperm failure as a result of crossing two species with different ploidy levels can be overcome by inducing polyploidy (Jansky, 2006) via colchicine treatment, or by using a bridge cross with a species at the lower ploidy level, followed by chromosome doubling, then subsequent hybridisation with the cultivated species (Dwivedi *et al.*, 2008). Sterility can also be overcome by chromosome doubling using colchicine (Acquaah, 2009).

Interspecific hybrids have been used successfully to improve many crops of economic importance. For example, a wild relative of tomato (*Lycopersicon hirsutum* Dunal) that produces small, inedible fruit that remain green upon ripening has been hybridised with a processing tomato cultivar (*Lycopersicon esculentum* L.) and backcrossed to create a hybrid that outperformed the original elite variety by 48, 22 and 33% for yield, soluble solids content, and fruit colour, respectively (Tanksley & McCouch, 1997). Traits for resistance to viral and bacterial diseases, and insect pests in rice (*Oryza sativa* L.) have been successfully introgressed into cultivated rice varieties from wild relatives such as *Oryza longistaminata* A. Chev & Roehr (bacterial blight resistance) and *Oryza officinalis* Wall. ex Watt (brown planthopper resistance) (Brar & Khush, 1997) to name just a few.

1.4 Wide hybridisation with *Trifolium repens*

It is widely accepted that allotetraploid *T. repens* originated from the interspecific hybridisation of two species. However, the identity of these species has been long debated, with varying combinations of *Trifolium nigrescens* Viv., *Trifolium occidentale*

Coombe, *T. uniflorum*, and *Trifolium isthmocarpum* Brot. all proposed as candidates, based on the characteristics of the species, their cross compatibility with white clover, chromosome pairing in the hybrids (Brewbaker & Keim, 1953; Gibson & Beinhart, 1969; Chen & Gibson, 1972; Michaelson-Yeates, 1986), and more recently isozyme analysis (Badr *et al.*, 2002). Based on DNA sequence phylogenetics, Ellison *et al.* (2006) concluded that *T. occidentale* and *Trifolium pallescens* Schreb. were the parents of white clover. Williams *et al.* (2012) provided experimental evidence in support of the hypothesis that *T. pallescens* hybridised with *T. occidentale* (both diploids) to produce tetraploid *T. repens*, through DNA sequencing, molecular cytogenetics, interspecific hybridisation and breeding experiments.

Williams (2014) has given emphasis to species showing potential relevance to white clover breeding, by placing the species from within the "white clover complex" into gene pools. White clover alone forms the primary gene pool, as it does not naturally cross with any other species to form hybrid populations. The secondary gene pool consists of 2x *T. occidentale* (and the artificial 4x form), and *T. nigrescens*, ssp. *nigrescens*, ssp. *petrisavii* (Clem.) and ssp. *meneghinianum* (Clem.). The tertiary gene pool contains *T. uniflorum*, *T. isthmocarpum*, and 4x *Trifolium ambiguum* M. Beib. The author also includes a quaternary gene pool, of species not yet hybridised with white clover, but able to be crossed with another species in the complex. This quaternary gene pool includes 2x and 6x *T. ambiguum*, *Trifolium thalii* Vill., and *T. pallescens*. Most of the species in the white clover complex have been crossed with white clover, either directly or via a bridging species, and hold a range of valuable traits (Williams, 2014; Nichols *et al.*, 2016a).

To date, only one commercial interspecific hybrid clover cultivar is available. Bred by researchers at the Institute of Biological, Environmental and Rural Sciences (IBERS) at

Aberystwyth University in the United Kingdom for improved drought resistance, AberLasting is a cross between *T. ambiguum* and white clover (Nichols *et al.*, 2016a). Hybrids between white clover and both *T. occidentale* and *T. uniflorum* look to hold the most promise in New Zealand (Nichols *et al.*, 2016a).

1.4.1 Hybridisation with *T. ambiguum*

Caucasian clover, also known as Kura clover (*T. ambiguum*) is a long-lived, rhizomatous perennial legume found in Romania, Turkey, Crimea, Iran, Iraq, and the Caucasus (Zohary & Heller, 1984; Williams & Nichols, 2011). It is able to grow to very high altitudes and is adapted to a wide range of habitats, including river banks, forest fringes and scree slopes (Williams *et al.*, 2011). Different levels of ploidy naturally exist within the species (Elliot *et al.*, 1998), with diploid populations generally adapted to the highest altitudes, hexaploid at the lowest, and tetraploids in between (Williams & Nichols, 2011; Williams *et al.*, 2011). Traits of interest for introgression into the white clover gene pool include rhizome formation and deeper rooting habit, both of which can improve drought tolerance, and virus resistance (Williams & Nichols, 2011). Of all the species in the "white clover complex", *T. ambiguum* is the most distant relative of white clover (Ellison *et al.*, 2006), and as the different ploidy populations of *T. ambiguum* sit in the tertiary ($4x$) and quaternary ($2x$ and $6x$) gene pools (Williams, 2014), it does not freely cross with white clover. Very small numbers of fertile hybrids have been obtained to date by embryo rescue (Williams & Verry, 1981) and ovule culture (Meredith *et al.*, 1995). The research group at IBERS have succeeded in creating back cross hybrids (BC_3) with rhizome expression, that otherwise perform similarly to white clover agronomically (Abberton *et al.*, 1998; Marshall *et al.*, 2003), and BC_2 hybrids with better drought tolerance than white clover (Marshall *et al.*, 2001).

1.4.2 Hybridisation with *T. occidentale*

T. occidentale is a self-fertile, diploid stoloniferous perennial species adapted to sandy, dry, salty soils. It grows on beaches and sea cliffs on the gulf-stream coasts of Western Europe, Portugal, Spain, France, Wales, and Ireland, usually within 50–100m of the sea (Coombe, 1961; Williams *et al.*, 2009; Williams & Nichols, 2011; Williams, 2014). A putative ancestor of white clover (Ellison *et al.*, 2006; Williams *et al.*, 2012), *T. occidentale* appears similar to small-leaved white clover types, but with thicker leaflets that have a shiny, waxy under-surface, and the presence of hairs on the peduncles and petioles (Williams & Nichols, 2011). Gibson and Beinhart (1969) created the first *T. repens* × *T. occidentale* hybrids using both 2x *T. occidentale*, which resulted in a small number of sterile hybrids, and colchicine doubled 4x *T. occidentale*, which produced greater numbers of F_1 hybrids of varying fertility. Hussain and Williams (2013) produced *T. repens* × 4x *T. occidentale* F_1 hybrids, which were backcrossed with white clover to create BC_1F_1 hybrids. In the drought study undertaken by the authors using these plants, the hybrids showed a greater relative root growth response as the soil dried than either parent species – a positive result from unselected hybrid material in support of interspecific hybridisation as a route to improving white clover.

1.4.3 *T. occidentale* as a bridging species

Gibson *et al.* (1971) created multi-species hybrids from crosses between the *T. uniflorum*-*T. occidentale* hybrid and *T. repens*, and *T. uniflorum* and the *T. occidentale*-*T. repens* hybrid. These crosses frequently resulted in multiple species hybrids, and the authors suggested that first crossing *T. repens* or *T. uniflorum* with 4x *T. occidentale* reduced the barrier to combining *T. repens* and *T. uniflorum*. Williams *et al.* (2011) crossed 2x *T. ambiguum* with 2x *T. occidental*e to give 2x (*T. ambiguum* × *T. occidentale*), and a colchicine-doubled 4x form was produced. It was found that both the 4x form and open pollinated progeny plants from the *T. ambiguum* × *T. occidentale* hybrid were inter-fertile with white clover, suggesting that *T. occidentale* could be used as a genetic bridge to create hybrids incorporating 2x *T. ambiguum* into white clover (Williams *et al.*, 2011; Williams, 2014). Success in obtaining tri-species hybrids enables the simultaneous transfer of genomes from the two related species, into white clover.

1.5 *Trifolium uniflorum*

1.5.1 Morphology

T. uniflorum (2n=32) is a perennial, wild species native to the Mediterranean region, found in Greece, Turkey, southern France, southern Italy and Libya (Zohary & Heller, 1984; Williams *et al.*, 2011). It is generally self-incompatible (Pandey, 1957), however Gibson and Chen (1971) reported some self-compatibility in three seed lots studied (Williams & Nichols, 2011). It has thick woody taproots, small thick leaves, short internode lengths, and relatively large seed when compared with other *Trifolium* species (Pandey & Petterson, 1978; Williams, 2014). Florets occur singly or in small clusters of twos or threes, on short peduncles. *T. uniflorum* is highly variable in morphology, and has been divided into seven varieties by Vierhapper (1919) differing mainly in floral morphology (Zohary & Heller, 1984; Williams & Nichols, 2011). *T. uniflorum* may have some resistance to the root damaging New Zealand grass grub (*Costelytra zealandica* White) (Dymock *et al.*, 1989) and also to fungal leaf spot diseases and some viruses (Gibson *et al.*, 1971). An evaluation of genetic diversity in a number of *Trifolium* species and accessions by Badr *et al.* (2002) found *T. uniflorum* to exhibit the highest amount of diversity and differentiation among accessions.

1.5.2 Nodulation

In laboratory experiments, Howieson *et al.* (2005) found that *T. uniflorum* formed effective or partially effective nodules with three strains of *Rhizobium leguminosarum* bv. *trifolii* from *Trifolium subterraneum* L., C2483g, TA1 and WSM409, and one strain from *Trifolium medium* L., CC4335. *T. repens* also formed partially effective nodules with these strains. The two strains, WSM1313 and WSM1689, of *R. leguminosarum* bv. *trifolii* evaluated from *T. uniflorum* were ineffective on *T. repens* (Howieson *et al.*, 2005; Williams & Nichols, 2011).

1.5.3 Hybridisation between *T. repens* and *T. uniflorum*

The first viable interspecific hybrid between *T. repens* and *T. uniflorum* was reported by Pandey (1957), and despite both parents being self-incompatible, the hybrid plant was self-fertile, and cross compatible with both parents. The hybrid morphology was intermediate to the two parent species in floret size, peduncle length, and the number of florets per inflorescence.

Following on from the success in hybrid generation of Pandey (1957), a small number of other researchers (Evans, 1962; Gibson *et al.*, 1971; Pandey *et al.*, 1987) achieved hybridisation between *T. repens* and *T. uniflorum*. Evans (1962) sought to increase the frequency of hybrids in particular species using two methods; a) grafting the parents and hybridizing those genotypes that were graft compatible, and b) excising young hybrid embryos and culturing them on artificial media. It was shown that graft compatible genotypes set a significantly greater number of embryos than graft incompatible genotypes. Embryo rescue prior to abortion increased the production of hybrid seedlings, with 25% of the embryos excised at 15 days post pollination and 50% of embryos excised at 25 days following pollination forming seedlings. Despite the creation of fertile hybrids, with potential characteristics for improving white clover, no hybrid breeding populations were created (Hussain *et al.*, 2012).

1.5.4 Hybrid Characteristics

Hybrids created by Gibson *et al.* (1971) were intermediate to the parents for length of internodes, and shape of stipules, with leaf markings on the F_1s corresponding to those of the *T. uniflorum* pollen parent. The authors found cytological evidence for hybridity, in the presence of three satellite chromosomes in somatic cells of the F_1 progeny. Pandey *et al.* (1987) produced reciprocal hybrids using *T. repens* and *T. uniflorum*, and found that using *T. repens* as the maternal parent resulted in a higher success rate of gaining mature hybrid plants than when *T. uniflorum* was the maternal plant. F_2 hybrids, and backcrosses of the F_1 to both *T. repens* and *T. uniflorum* were also created. The F_2 hybrids were diverse in morphology, but all intermediate between the parent species for most vegetative and floral traits. The F_1 backcrosses to *T. repens* were vegetatively more like *T. repens*, but retained larger florets more akin to *T. uniflorum*.

The F_1 backcrosses to *T. uniflorum* were more like *T. uniflorum* vegetatively, and the most vigorous plants showed a stronger, deeper root system than *T. repens*, with some plants showing an occasional deep tap-root-like structure from stolon nodes, a trait not seen in either parent species. Nichols *et al.* (2014b) found that, in general, BC_1 hybrids had vegetative traits that were intermediate between the parents, with a more prostrate growth habit compared with white clover. The proportion of shoot N derived from fixation, or shoot %N content was not affected by hybridisation.

1.5.5 Pest and Disease Resistance

In an evaluation of eight *Trifolium* species and seven interspecific hybrids of *Trifolium* related to white clover for resistance to the southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood (Pederson & Windham, 1989), the hybrid of *T. repens* × *T. uniflorum* along with one other hybrid had the most variation for the numbers of galls per root system, and among the *T. repens* × *T. uniflorum* hybrids with the least galling due to *M. incognita*, only one was fertile, and of any use for introgressing resistance to *M. incognita* into *T. repens*. One *T. repens* × *T. uniflorum* hybrid was able to maintain a high proportion of fibrous roots, despite a high number of galls, suggesting an expression of tolerance to *M. incognita*.

Despite the apparent tolerance of *T. uniflorum* to the New Zealand grass grub, the *T. repens* × *T. uniflorum* hybrids studied by Dymock *et al.* (1989) did not show any significant difference in larval weight gains to those on *T. repens.*

The low susceptibility of *T. uniflorum* to virus infection and fungal disease did not affect the disease scores of the BC_1 and BC_2 hybrids, which had a much higher susceptibility, similar in range to white clover (Nichols *et al.*, 2014b).

1.5.6 Effects of hybridisation on drought tolerance

Recent field studies have evaluated the effect of hybridisation with *T. uniflorum* on the drought resistance of *T. repens* (Nichols *et al.*, 2013; Nichols *et al.*, 2014a; Widdup *et al.*, 2014). Of the white clover, BC_1 hybrids, and BC_2 hybrids studied, dry matter production of the BC_1 hybrids was significantly less affected by drought stress (49% reduction in production) than the BC_2 hybrids and white clover (68% and 69% reduction respectively). Changes in leaf area, internode length, petiole length and senescence in drought conditions also supported greater drought resistance in the BC_1 generation. It was concluded that *T. repens* × *T. uniflorum* BC_1 hybrids have potential for improved productivity and survival in dry conditions compared with white clover. The authors acknowledged that there may be even larger effects on performance under circumstances different to the experimental conditions. Following on from the morphological drought studies, Nichols *et al.* (2015b) identified physiological and biochemical characteristics that may have contributed to the increased drought resistance of *T. repens* × *T. uniflorum* BC_1 hybrids. Both kaempferol glycosides and hydroxycinnamic acid compounds were present in higher levels in the BC_1 generation than white clover or the BC_2 hybrids, suggesting that they may play a role in drought protection, and that there is possibly scope to select for these characteristics in first generation hybrids when breeding for drought resistance.

1.5.7 Effects of hybridisation on nutrient use

White clover is typically adapted to moist, fertile environments. It responds with high growth to added phosphorus (P), but grows poorly when soil-P is low. Nichols *et al.* (2014c) investigated the effect of hybridisation on the growth and macronutrient composition of white clover compared with BC_1 hybrids of *T. repens* × *T. uniflorum* and found that the BC_1 hybrids were able to maintain growth at lower nutrient concentrations, with both shoot and root dry weights of BC_1 hybrids greater than those of white clover. Further investigation by Nichols *et al.* (2014d) found that although internal P concentrations did not differ between the clovers, it was the hybrid's greater ability to acquire soil P through the production of frequently branching roots and growth of root tips into fresh soil that resulted in its greater growth in low P soils. Further root studies (Nichols *et al.*, 2015a; Nichols *et al.*, 2016b) confirmed that hybridisation with *T. uniflorum* had an effect on the overall root architecture, and the survival of clover tap roots, with BC_1 hybrids having a higher tap root survival and lower tap root deterioration than white clover.

1.5.8 Breeding

Hybridisation between *T. repens* and *T. uniflorum* has much potential as a tool for creating a wider, more variable gene pool for clover breeding (Pandey & Petterson, 1978). From this we have improved scope to lift the performance of white clover, particularly the root system and its ability to tolerate pests and diseases. A study by Hussain *et al.* (2012) to generate new hybrids between *T. repens* and *T. uniflorum* and to create BC_1 progeny from backcrosses of the F_1 hybrids to *T. repens*, resulted in BC_1 progeny that were superior in the expression of key traits to the F_1 parents. From this, it was concluded that key traits can be improved through successful introgression followed by backcrossing, with the view that future backcrosses derived from F_1 interspecific hybrids could provide breeding pools with novel genetic variation for use in white clover improvement.

Most studies have focussed on the mean performance of populations of hybrids, which usually lies between the performances of the parent species. However, Nichols *et al.*

(2014b) reported that some hybrid families and individual hybrid plants are superior in improving characteristics of their specific white clover parent line, suggesting the possibility of developing elite breeding populations by recurrent selection of the superior hybrids.

Published studies characterising the phenotypes of hybrids thus far indicate that there is large potential for interspecific hybrids of *T. repens* × *T. uniflorum* as pasture legumes in environments with low soil moisture and or fertility. The future of these hybrids in agriculture now depends on the development of efficient breeding strategies to harness their genetic potential.

1.6 Objectives and hypotheses of this study

The objectives of this study were to investigate the variation among F_1 *T. repens* × *T. uniflorum* hybrids, and to then evaluate the BC_1 progeny performance.

There were two principal hypotheses for this study. The first was that some F_1 *T. repens* x *T. uniflorum* hybrids would be better parents in a breeding programme than others. The second was that the performance of the BC_1 hybrids (*T. repens* x F_1 hybrids) could be predicted from the F_1 phenotype.

Chapter 2

2 Materials and Methods

2.1 Plant Material

2.1.1 F_1 experiment

Twenty-six *Trifolium repens* x *T. uniflorum* F_1 (first filial) hybrid plants were chosen from the *Trifolium* interspecific hybrid collection generated by Isabelle Williams (Germplasm Development, AgResearch Grasslands, Palmerston North) via embryo rescue. The F_1 hybrids were grouped in to four families based on parentage, with each family of plants having either one, or both, parents in common (Table 2.1). Three *T. repens* cv. Kopu II genotypes (Kopu II-2, K-R-904 and K-R-907) and two *T. uniflorum* genotypes (AZ4383-18-OP-3 and AZ4382 T66-5-OP-3) were selected as parental controls.

Table 2.1: F_1 clover entries used in the 2012 sandframe experiment, with parental details. Entry numbers correspond to the experimental design (Figure 2.2). AZxxxx = Accession number, Margot Forde Forage Germplasm Centre (Palmerston North, New Zealand), OP = open pollinated.

Entry #	entry name	*T. repens* parent (female)	*T. uniflorum* parent (male)	Group
1	900-3	Kopu II-2	AZ4383-11	1
2	900-4	Kopu II-2	AZ4383-11	1
3	900-5	Kopu II-2	AZ4437-2	1
4	K283-13	Kopu II-2	AZ4383-1	1
5	K283-19	Kopu II-2	AZ4383-1	1
6	K283-12	Kopu II-2	AZ4383-1	1
7	K283-18	Kopu II-2	AZ4383-1	1
8	POW-134	Kopu II-2	AZ4382 T66-6-OP-2	2
9	POW-136	Kopu II-2	AZ4382 T66-6-OP-2	2
10	POW-137	Kopu II-2	AZ4382 T66-6-OP-2	2

11	POW-139	Kopu II-2	AZ4382 T66-6-OP-2	2
12	POW-140	Kopu II-2	AZ4382 T66-6-OP-2	2
13	POW-141	Kopu II-2	AZ4382 T66-6-OP-2	2
14	POW-2	K-R-907	AZ4383-18-OP-3	3
15	POW-3	K-R-907	AZ4383-18-OP-3	3
16	POW-4	K-R-907	AZ4383-18-OP-3	3
17	POW-20	K-R-907	AZ4383-18-OP-3	3
18	POW-21	K-R-907	AZ4383-18-OP-3	3
19	POW-16	K-R-904	AZ4383-18-OP-3	3
20	POW-18	K-R-904	AZ4383-18-OP-3	3
21	POW-19	K-R-904	AZ4383-18-OP-3	3
22	POW-8	K-R-907	AZ4382 -T66-5-OP-3	4
23	POW-10	K-R-907	AZ4382 -T66-5-OP-3	4
24	POW-11	K-R-907	AZ4382 -T66-5-OP-3	4
25	POW-14	K-R-907	AZ4382 -T66-5-OP-3	4
26	POW-15	K-R-907	AZ4382 -T66-5-OP-3	4
27	Kopu II-2			*T. repens* Control
28	K-R-904			*T. repens* Control
29	K-R-907			*T. repens* Control
30	AZ4383-18-OP-3			*T. uniflorum* Control
31	AZ4382 T66-5-OP-3			*T. uniflorum* Control

In early October 2011, multiple clonal cuttings consisting of the stolon tip subtended by two to three centimetres of stolon and at least two nodes were taken from each of the 26 mature F_1 hybrid genotypes, three *T. repens* cv. Kopu II genotypes, and two *T. uniflorum* genotypes. The cut end of each stolon cutting was dipped in powdered indole-3-butyric acid rooting hormone, planted into potting mix, and grown under natural lighting and day length in a glasshouse at AgResearch Grasslands, Palmerston North (40°22’52.3”S, 175°36’43.0”E) with a temperature range of 15-28°C until established. Insect pests, such as thrips, were treated with Confidor® as required, and slug bait was applied approximately once a month. After six weeks in the glasshouse, the plants were shifted outside to acclimatise for two weeks, then transplanted into a one metre deep sandframe for evaluation in early December 2011. Once in the sandframe, the trial was watered daily with a sprinkler on a timer, and 150mL of Yates Thrive® Soluble All Purpose Plant Food (NPK of 27:5.5:9 + trace elements) was applied to each plant twice a week up until the plants were harvested in September 2012. Sandframes were used in the experiments to minimise variation in soil quality and nutrient availability across the trial, and increase the ease of recovering the entire plants at harvest.

2.1.2 BC_1 experiment

BC_1 families were created from 26th November to 14th December 2012 by hand crossing the best and worst performing entry from each F_1 family based on dry matter (DM) yield in the F_1 sandframe trial with one white clover genotype from each of cv. Tahora and cv. Trophy representing different leaf morphologies (small and large leaved respectively) to create 16 BC_1 families.

Hand Crossing

The inflorescence due to be pollinated was prepared as described by Williams (1954); removing all open florets, and emasculating the remaining florets by removing the corolla, staminal tube and anthers using forceps, leaving the carpel exposed. A spatula was used to collect ripe pollen from the anthers of the male parent, and placed on the exposed stigma of the prepared maternal floret. The plants were kept in a greenhouse free of pollinating insects during flowering to ensure that no unplanned pollination occurred and only the target crosses were made. A number of unpollinated inflorescences on the maternal plants were used as controls and left until maturity to ensure there was no self-pollination occurring, confirmed by the lack of seed production. Upon maturity, between six and eight weeks after pollination, the seed was harvested and dressed.

Seed was scarified with sandpaper and germinated on wet filter paper in Petri dishes in June, 2014. Germinated seed were transferred to trays of seed raising mix once the radicles were visible (approximately 0.5-1mm long). Seedlings were grown under natural lighting and day length in a glasshouse at AgResearch Grasslands, Palmerston North (40°22'52.3"S, 175°36'43.0"E) until established, then in early August shifted outside into the nursery to subject plants to temperatures cool enough for vernalisation to occur. On the 1st of September, 2014, cuttings were taken from each BC_1 genotype and propagated in the same fashion as the F_1s to allow for development of the root system prior to planting. Cuttings were used so that nodal root morphology could be observed in the absence of a tap root, simulating the performance of a plant that has passed the tap root growth phase (>18 months old).

Table 2.2: BC_1 clover entries used in 2014 sandframe experiment and their parental details. Entry numbers correspond to the experimental design (Figure 2.3). Parental controls in italics.

Entry No.	Entry name	*T. repens* parent (female)	F_1 hybrid parent (male)	F_1 parentage
1	Trophy R4-1/900-3	Trophy R4-1	900-3	Kopu II-2 x AZ4383-11
2	Trophy R4-1/900-3	Trophy R4-1	900-3	Kopu II-2 x AZ4383-11
3	Trophy R4-1/900-3	Trophy R4-1	900-3	Kopu II-2 x AZ4383-11
4	Trophy R4-1/900-4	Trophy R4-1	900-4	Kopu II-2 x AZ4383-11
5	Trophy R4-1/900-4	Trophy R4-1	900-4	Kopu II-2 x AZ4383-11
6	Trophy R4-1/900-4	Trophy R4-1	900-4	Kopu II-2 x AZ4383-11
7	Trophy R4-1/POW-139	Trophy R4-1	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
8	Trophy R4-1/POW-139	Trophy R4-1	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
9	Trophy R4-1/POW-139	Trophy R4-1	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
10	Trophy R4-1/POW-140	Trophy R4-1	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
11	Trophy R4-1/POW-140	Trophy R4-1	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
12	Trophy R4-1/POW-140	Trophy R4-1	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
13	Trophy R4-1/POW-4	Trophy R4-1	POW-4	K-R-907 x AZ4383-18-OP-3
14	Trophy R4-1/POW-4	Trophy R4-1	POW-4	K-R-907 x AZ4383-18-OP-3
15	Trophy R4-1/POW-4	Trophy R4-1	POW-4	K-R-907 x AZ4383-18-OP-3
16	Trophy R4-1/POW-2	Trophy R4-1	POW-2	K-R-907 x AZ4383-18-OP-3
17	Trophy R4-1/POW-2	Trophy R4-1	POW-2	K-R-907 x AZ4383-18-OP-3

18	Trophy R4-1/POW-2	Trophy R4-1	POW-2	K-R-907 x AZ4383-18-OP-3
19	Trophy R4-1/POW-10	Trophy R4-1	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
20	Trophy R4-1/POW-10	Trophy R4-1	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
21	Trophy R4-1/POW-10	Trophy R4-1	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
22	Trophy R4-1/POW-8	Trophy R4-1	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
23	Trophy R4-1/POW-8	Trophy R4-1	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
24	Trophy R4-1/POW-8	Trophy R4-1	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
25	Tahora-21/900-3	Tahora-21	900-3	Kopu II-2 x AZ4383-11
26	Tahora-21/900-3	Tahora-21	900-3	Kopu II-2 x AZ4383-11
27	Tahora-21/900-3	Tahora-21	900-3	Kopu II-2 x AZ4383-11
28	Tahora-21/900-4	Tahora-21	900-4	Kopu II-2 x AZ4383-11
29	Tahora-21/900-4	Tahora-21	900-4	Kopu II-2 x AZ4383-11
30	Tahora-21/900-4	Tahora-21	900-4	Kopu II-2 x AZ4383-11
31	Tahora-21/POW-139	Tahora-21	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
32	Tahora-21/POW-139	Tahora-21	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
33	Tahora-21/POW-139	Tahora-21	POW-139	Kopu II-2 x AZ4382 T66-6-OP-2
34	Tahora-21/POW-140	Tahora-21	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
35	Tahora-21/POW-140	Tahora-21	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
36	Tahora-21/POW-140	Tahora-21	POW-140	Kopu II-2 x AZ4382 T66-6-OP-2
37	Tahora-21/POW-4	Tahora-21	POW-4	K-R-907 x AZ4383-18-OP-3
38	Tahora-21/POW-4	Tahora-21	POW-4	K-R-907 x AZ4383-18-OP-3
39	Tahora-21/POW-4	Tahora-21	POW-4	K-R-907 x AZ4383-18-OP-3
40	Tahora-21/POW-2	Tahora-21	POW-2	K-R-907 x AZ4383-18-OP-3
41	Tahora-21/POW-2	Tahora-21	POW-2	K-R-907 x AZ4383-18-OP-3
42	Tahora-21/POW-2	Tahora-21	POW-2	K-R-907 x AZ4383-18-OP-3
43	Tahora-21/POW-10	Tahora-21	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
44	Tahora-21/POW-10	Tahora-21	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
45	Tahora-21/POW-10	Tahora-21	POW-10	K-R-907 x AZ4382 -T66-5-OP-3
46	Tahora-21/POW-8	Tahora-21	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
47	Tahora-21/POW-8	Tahora-21	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
48	Tahora-21/POW-8	Tahora-21	POW-8	K-R-907 x AZ4382 -T66-5-OP-3
49	*Trophy R4-1*			
50	*Trophy R4-1*			
51	*Tahora-21*			
52	*Tahora-21*			
53	*900-3*			Kopu II-2 x AZ4383-11
54	*900-4*			Kopu II-2 x AZ4383-11
55	*POW-139*			Kopu II-2 x AZ4382 T66-6-OP-2
56	*POW-140*			Kopu II-2 x AZ4382 T66-6-OP-2
57	*POW-4*			K-R-907 x AZ4383-18-OP-3

58	*POW-2*	K-R-907 x AZ4383-18-OP-3
59	*POW-10*	K-R-907 x AZ4382 -T66-5-OP-3
60	*POW-8*	K-R-907 x AZ4382 -T66-5-OP-3

The BC$_1$ hybrids, the white clover parents, and the F$_1$ parents were planted out into a sandframe on 13 October 2014 and grown for four months before being harvested. Once in the sandframe, the trial was watered with a sprinkler daily on a timer, and 150mL of Yates Thrive® Soluble All Purpose Plant Food (NPK of 27:5.5:9 + trace elements) was applied to each plant twice a week.

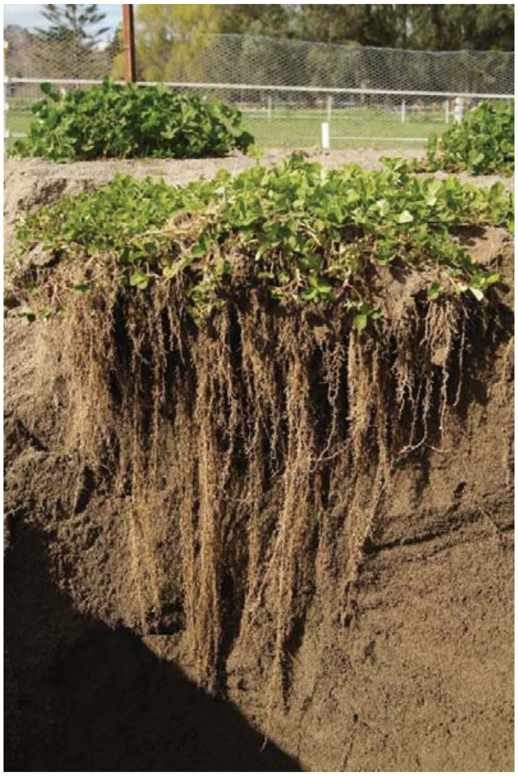
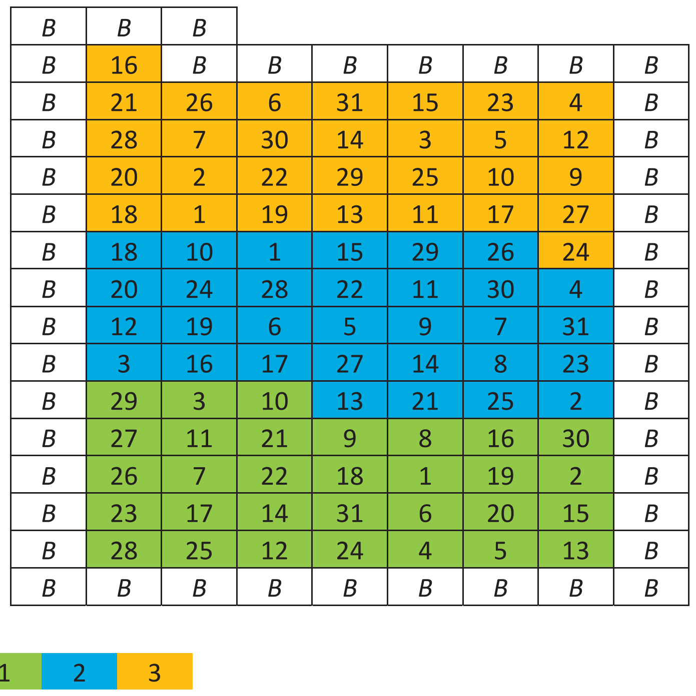


Figure 2.1: Excavation of an intact plant from the F$_1$ Experiment

2.2 Experimental Design

2.2.1 F$_1$ experiment

Each hybrid and parent genotype were replicated in the trial three times (except for POW-134 where only two cuttings established) in a row-column design (Figure 2.2). Plants were spaced 0.75m apart, and experimental entries were surrounded by a border row of plants to reduce edge effects. The plants were grown for nine months in the sandframe described above. Stolon density, plant spread, and pollen stainability were evaluated during the growing period, while the remaining traits were evaluated following excavation and destructive harvest. The plants were harvested by excavating the complete plant, with roots intact (see Figure 2.1), starting with a deep trench adjacent to the plant, and carefully removing the sand by brushing it away from around the roots.



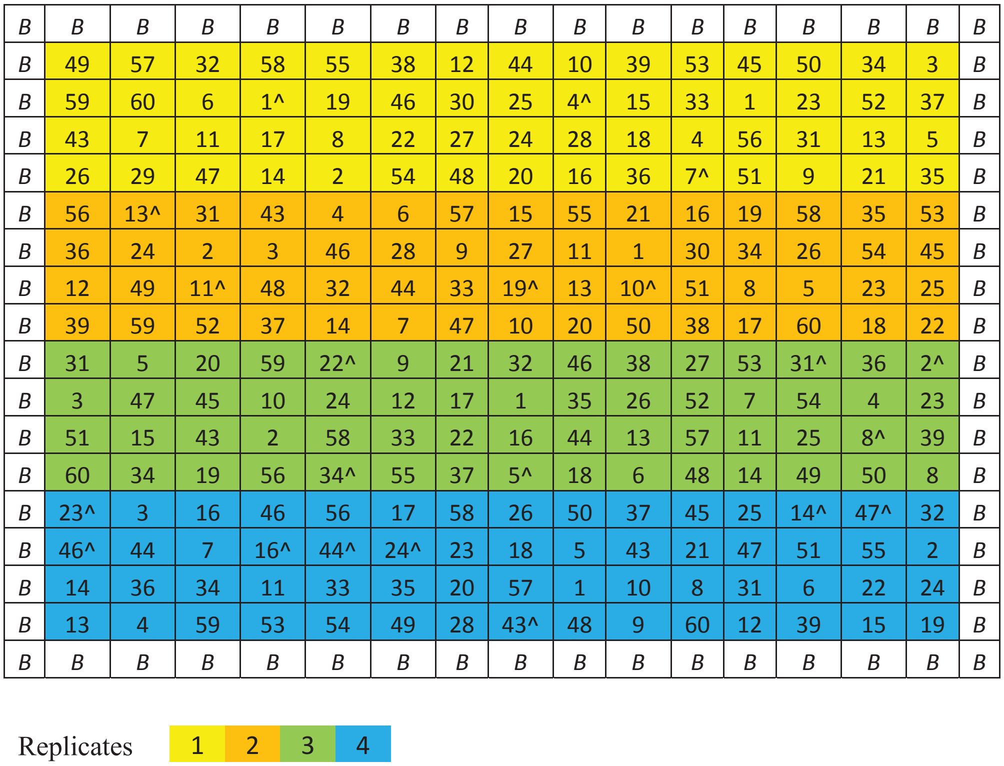
B	B	B						
B	16	B	B	B	B	B	B	B
B	21	26	6	31	15	23	4	B
B	28	7	30	14	3	5	12	B
B	20	2	22	29	25	10	9	B
B	18	1	19	13	11	17	27	B
B	18	10	1	15	29	26	24	B
B	20	24	28	22	11	30	4	B
B	12	19	6	5	9	7	31	B
B	3	16	17	27	14	8	23	B
B	29	3	10	13	21	25	2	B
B	27	11	21	9	8	16	30	B
B	26	7	22	18	1	19	2	B
B	23	17	14	31	6	20	15	B
B	28	25	12	24	4	5	13	B
B	B	B	B	B	B	B	B	B

Replicates 1 2 3

Figure 2.2: F$_1$ 2012 Sandframe trial layout. Numbers 1-31 represent experimental entries as outlined in Table 2.1, B indicates a border plant.

2.2.2 BC_1 experiment

Four replicates of sixty entries were evaluated in a row-column factorial design. Replicates contained three different genotypes from each BC_1 family, one plant of each F_1 parent and two plants of each white clover parent as controls, for a total of 240 experimental plants (detailed in Table 2.2). Plants were spaced 0.5m apart, and experimental entries were surrounded by a border row of plants to reduce edge effects. Where insufficient seed of experimental entries germinated, spare plants from other entries were used (show as ^ in Figure 2.3).



B	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*
B	49	57	32	58	55	38	12	44	10	39	53	45	50	34	3	*B*
B	59	60	6	1^	19	46	30	25	4^	15	33	1	23	52	37	*B*
B	43	7	11	17	8	22	27	24	28	18	4	56	31	13	5	*B*
B	26	29	47	14	2	54	48	20	16	36	7^	51	9	21	35	*B*
B	56	13^	31	43	4	6	57	15	55	21	16	19	58	35	53	*B*
B	36	24	2	3	46	28	9	27	11	1	30	34	26	54	45	*B*
B	12	49	11^	48	32	44	33	19^	13	10^	51	8	5	23	25	*B*
B	39	59	52	37	14	7	47	10	20	50	38	17	60	18	22	*B*
B	31	5	20	59	22^	9	21	32	46	38	27	53	31^	36	2^	*B*
B	3	47	45	10	24	12	17	1	35	26	52	7	54	4	23	*B*
B	51	15	43	2	58	33	22	16	44	13	57	11	25	8^	39	*B*
B	60	34	19	56	34^	55	37	5^	18	6	48	14	49	50	8	*B*
B	23^	3	16	46	56	17	58	26	50	37	45	25	14^	47^	32	*B*
B	46^	44	7	16^	44^	24^	23	18	5	43	21	47	51	55	2	*B*
B	14	36	34	11	33	35	20	57	1	10	8	31	6	22	24	*B*
B	13	4	59	53	54	49	28	43^	48	9	60	12	39	15	19	*B*
B	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*	*B*

Replicates 1 2 3 4

Figure 2.3: 2014 BC_1 sandframe experimental layout. Numbers 1-60 represent experimental entries as outlined in Table 2.2; ^ indicates replacements for missing plants; *B* represents a border plant.

2.3 Phenotypic Data Collection

2.3.1 Evaluation of Fertility traits

Male fertility was estimated by extracting mature pollen onto a microscope slide, staining with 2% acetocarmine, and counting the number of full-sized, fully stained grains in a minimum sample of 300 grains at 100x magnification (Williams *et al.*, 2012). Florets were counted on three inflorescences per entry to obtain a mean value for florets per inflorescence. Peduncle length was obtained by measuring the peduncle of three mature flowers of the plant with Vernier callipers.

2.3.2 Evaluation of root characteristics

Root depth values, for the 2012 F_1 experiment only, were obtained by measuring the distance between the soil level at the centre of the plant to the tip of the longest root. Minimum stolon anchoring was calculated from a subsample of three stolons per entry as a percentage of the overall stolon length (half plant spread) using the distance from the stolon growing tip to the closest nodal root. Maximum nodal root diameter values were obtained by measuring with callipers the diameter of the largest nodal root at the point where it connected to the stolon.

2.3.3 Evaluation of stolon and foliage characteristics

Stolon density values were obtained by visually scoring each plant on a scale of 1 to 5, where a score of 1 indicated a low stolon density and 5 a high stolon density. Leaflet area was determined by multiplying the length and width of the middle leaflet on the second fully expanded trifoliate, sampled from three different stolons per entry and reported as the mean of these three measurements. Stolon diameter values were obtained by measuring with callipers the stolon thickness at the second fully expanded trifoliate, on the same three stolons used to determine leaflet size. Mean internode length was determined by measuring a representative portion of stolon and counting nodes within that portion, on three separate stolons.

2.3.4 Evaluation of dry matter/agronomic performance characteristics

Root dry mass (F_1 experiment only), and above ground dry mass were obtained by destructive harvest; cutting the roots off the stolons, drying at 80°C for 48 hours and weighing. Maximum plant spread was determined by measuring the diameter of each plant prior to excavation and destructive harvest.

2.4 Data Analysis

2.4.1 Analysis of Variation

The data analysis of the F_1 experiment was conducted using ANOVA in Genstat 18 (Genstat, 2015) to calculate the variation both within families of hybrids, and between families of hybrids. Analysis of the BC_1 experiment was conducted using ANOVA also. The analyses generated final mean phenotypic values for each trait.

2.4.2 Pattern Analysis

Family × trait matrices of means from the ANOVA analysis of the BC_1 experiment were generated for all trial entries (Table A5.1), the Tahora BC_1 families and their parents (Table A5.2), and the Trophy BC_1 families and their parents (Table A5.3). These matrices were individually analysed using pattern analysis – a combination of cluster analysis and principal component analysis (Gabriel, 1971; Kroonenberg, 1994; Watson *et al.*, 1995; Jahufer *et al.*, 2008). The objective of conducting pattern analysis was to provide from the large data set a graphical summary of the phenotypic variation present among the BC_1 families, and the relationships among traits in the form of biplots.

2.4.3 Correlations

Pearson correlation coefficients were calculated in Minitab 17.21 to determine the presence of correlations in performance between offspring and parent for each trait.

Chapter 3

3 Results

3.1 Experiment One (Sand frame 2012): Assessment of variation within and between families of F_1 hybrids

3.1.1 Foliar Traits

Highly significant ($P<0.001$) differences in leaflet area were observed within Families 1 (Table A1.1) and 3 (Table A1.3), and significant ($P<0.05$) differences were observed in Family 2 (Table A1.2). POW-140 (Family 2) had the smallest leaflet area (72 mm^2) and POW-19 (Family 3) the largest leaflet area (341 mm^2) (Table A1.3). There were significant differences between some of the hybrid families (Table A1.5), all were significantly ($P<0.001$) smaller than the *T. repens* controls, and significantly ($P<0.001$) larger than *T. uniflorum* for leaflet area.

For internode length, all F_1 hybrid families showed significant (Family 1, $P<0.01$; Families 2-4, $P<0.001$) differences between genotypes (Tables A1.1-A1.4). Internode lengths in Family 3 had the greatest range (2.9–12.5 mm), while hybrids in Family 1 varied the least (3.1–6.2 mm). Family 1 had significantly ($P<0.001$) shorter mean internode lengths than the three other F_1 families (Table A1.5). All families had significantly smaller internode lengths than *T. repens,* and significantly larger means than *T. uniflorum,* on average *T. repens* internode lengths ($\bar{x}$=12 mm) were more than double those of the F_1 hybrid internode lengths ($\bar{x}$=4.8–6.6mm).

Differences in stolon diameter values between genotypes from hybrid families 1, 2, and 3 were highly significant ($P<0.001$), and all had very similar ranges (Tables A1.1-A1.3).

Genotypes from Family 4 showed very little variation, with mean stolon diameters ranging from 1.4–1.5 mm (Table A1.4). Family 3 had significantly ($P<0.001$) larger mean stolon diameters than Families 2 and 4. Each hybrid family had significantly ($P<0.001$) smaller stolon diameters than *T. repens* (Table A1.5).

There were significant ($P<0.05$) differences in stolon density within families 2, 3, and 4 (Tables A1.2, A1.3 & A1.4). There was a highly significant ($P<0.001$) difference observed between Family 4 and Families 1 and 3 (Table A1.5). All of the hybrid families had significantly higher mean stolon densities than *T. uniflorum* (Table A1.5). While still intermediate between the two parents, stolon density was the only trait studied where the hybrid groups were closer in performance to *T. repens* than *T. uniflorum*.

For maximum plant spread, significant ($P<0.001$) differences between genotypes were observed within F_1 hybrid families 2 and 3 (Table A1.2 and Table A1.3 respectively). Family 2 had the largest range of means, where the smallest plant (POW-140) measured on average 5 cm in diameter, and the largest plant (POW-139) had a diameter of 38 cm (Table A1.2). The three largest hybrid genotypes in the trial were from three different families. Family 4 had a significantly ($P<0.001$) larger mean maximum plant spread than the other families (Table A1.5). All F_1 hybrid families were significantly ($P<0.001$) smaller than *T. repens*, and significantly larger than *T. uniflorum* (Table A1.5).

For foliar dry mass, significant ($P=0.016$) differences were only observed between genotypes in hybrid family 3 (Table A1.3). Foliar dry mass of all families were significantly ($P<0.001$) smaller than *T. repens* (Table A1.5). The hybrid groups were intermediate between the two parents, but closer in above ground mass to *T. uniflorum* (Table A1.5).

3.1.2 Fertility Traits

For pollen stainability, highly significant ($P<0.001$) differences were observed between the F_1 hybrids within Families 1, 2, and 4 (Tables A1.1, A1.2 & A1.4 respectively). Significant ($P=0.014$) differences were seen between the F_1 hybrids within Family 3 (Table A1.3), however this may not be a true representation of the variance as only 50% of the genotypes in this family were in flower at the time of sampling. There were highly significant ($P<0.001$) differences in pollen stainability between Family 1 and each of the other families, and between each F_1 family and the *T. uniflorum* control plants (Table A1.5). Individually, none of the F_1 hybrids, which ranged from 15–85% stainability, were as fertile as the *T. uniflorum* control AZ4382-T66-5-OP-3 (91%). No data were collected from the *T. repens* controls due to the absence of flowers at the time of sampling.

For mean number of florets per inflorescence, the results showed significant ($P<0.001$ for Families 1, 2 & 4; $P=0.025$ for Family 3) differences between genotypes in all families (Tables A1.1-A1.4). There were significant differences between some of the families of F_1 hybrids, and there was a highly significant ($P<0.001$) difference in the number of florets per inflorescence between each of the families ($\bar{x}$=7–10) and both *T. repens* ($\bar{x}$=85) and *T. uniflorum* ($\bar{x}$=2) controls (Table A1.5).

For mean peduncle length, there were significant differences ($P<0.01$) between the genotypes in each of the hybrid families (Tables A1.1-A1.4). Family 3 showed the largest variation in peduncle length, ranging from 3.2–35 mm (Table A1.3). The mean peduncle length of family 1 was significantly lower ($P<0.001$) than those of families 2, 3 and 4, and significant differences were observed between each family and both controls (Table A1.5). The *T. uniflorum* controls ($\bar{x}$=4.2 mm) had shorter peduncle lengths than all of the hybrid families ($\bar{x}$=9–17mm) (Table A1.5). The *T. repens* control plants had peduncle lengths that were on average five times longer than those of the hybrids (Table A1.5).

3.1.3 Root traits

For mean stolon anchoring, there were significant ($P<0.001$) differences between genotypes of Families 2 and 3 (Table A1.2 and Table A1.3, respectively). Anchoring within Family 2 (Table A1.2) ranged between no stolon rooting at all (POW-140), to roots occurring 94% down the stolon length. There were no significant ($P<0.001$) differences between the F_1 family means, however there were significant ($P<0.001$) differences between each of the Families and the *T. uniflorum* controls (Table A1.5).

For mean maximum nodal root diameter, there were significant differences observed in Families1, 2 and 4, ($P=0.034$, $P=0.012$ and $P<0.001$ respectively) (Tables A1.1, A1.2 & A1.4). Family 1 had a significantly ($P=0.005$) smaller mean nodal root diameter than families 3 and 4 (Table A1.5). There were no significant ($P=0.005$) differences between the *T. repens* controls and Family 3 and 4 (Table A1.5). Individually, when compared with the controls, the maximum nodal root diameter of at least one hybrid genotype from each of the four families equalled, or was larger than the maximum nodal root diameter of the largest control (*T. repens*).

Root depth varied significantly ($P=0.006$ & $P<0.001$) between genotypes in Families 2 and 4 only (Table A1.2 & Table A1.4). Within these families, the minimum and maximum mean root depths differed by up to approximately 30 cm. Family 2 had significantly ($P<0.001$) shallower roots than the other hybrid families, as did each hybrid family compared to the *T. repens* controls, however there were no significant differences between any of the F_1 families and the *T. uniflorum* controls (Table A1.5).

There were significant ($P<0.05$) differences in root dry mass observed between genotypes in hybrid families 1, 2 and 3 (Tables A1.1, A1.2 & A1.3). POW-11 ($\bar{x}$=35 g) had the largest mean root dry mass of the hybrids (Table A1.4), and KopuII-2 ($\bar{x}$=61 g) was the control (*T. repens*) with the highest mean root dry mass. Family 4 had a significantly ($P<0.001$) greater mean root mass than the other F_1 families (Table A1.5). Root mass of all families were significantly ($P<0.001$) smaller than *T. repens* (Table A1.5). The hybrid families were intermediate between the parents, but more similar in root mass to *T. uniflorum*.

3.2 Experiment Two (Sand frame 2014-15): Evaluation of BC_1 hybrid phenotypes

Two analyses were conducted for each trait within this experiment. The first compared each BC_1 hybrid family with both combinations of its parents – two white clovers and an F_1 hybrid (*T. repens* x *T. uniflorum*), for example, POW-4 (F_1), Trophy and Tahora (white clovers), Trophy/POW-4 and Tahora/POW-4 (BC_1s) (Appendix 4). The second analysis made comparisons within two groups, based on white clover parentage - i.e. Trophy and the Trophy BC_1 hybrid families (Appendix 3, Table A3.1); or Tahora and the Tahora BC_1 families (Appendix 2, Table A2.1). Data were transformed where appropriate to meet the assumptions of ANOVA.

3.2.1 Foliar Traits

All of the BC_1 hybrid families besides Trophy/POW-4 had a significantly higher mean stolon density than their respective white clover parents ($P<0.001$). All of the BC_1 hybrid families besides Trophy/POW-4 had a higher mean stolon density than their respective F_1 parents (ns for POW-4, POW-8 & POW-139 hybrids). There were significant ($P=0.012$) differences between the Tahora BC_1 hybrid family means (Table A2.1), and highly significant ($P<0.001$) differences between the Trophy BC_1 family means (Table A3.1).

In all cases, the BC_1 mean Maximum Spread was not significantly different to the mean max spread of the respective white clover parent (Tables A2.1 & A3.1). Each BC_1 hybrid family had a greater mean max spread than the corresponding F_1 parent (significance ranging from $P=0.007$ to $P<0.001$) (Appendix 4). There were no significant differences in means between the Tahora BC_1 families (P=0.171) (Table A2.1), or Trophy BC_1 families (P=0.364) (Table A3.1).

Each Tahora BC_1 hybrid family had a significantly (P<0.001) greater mean dry weight than the Tahora white clover parent (Table A2.1). The Tahora BC_1 hybrid families had significantly larger (P<0.001) dry weights than their respective F_1 parents in all cases except Tahora/POW-4, which were not significantly different (Appendix 4). The mean dry weight of the Tahora white clover was not significantly different from any of the F_1 dry weights apart from 900-3, which was significantly smaller (P<0.001) than Tahora (Appendix 4).

While all Trophy BC_1 hybrid families had a greater mean foliar dry weight than the Trophy white clover parent, the difference was only significant (p<0.001) in the cases of Trophy/900-4, Trophy/POW-139 and Trophy/POW-10 (Table A3.1). All Trophy BC_1 hybrid families had a significantly greater dry weight than their respective F_1 parents (P=0.006 for Trophy/POW-4, all other hybrids P<0.001) (Appendix 4).

The mean leaflet areas of all Trophy BC_1 families (except Trophy/POW-4) were significantly greater (p<0.001) than those of the respective F_1 parents (Appendix 4). Trophy/POW-2 was the only Trophy BC_1 hybrid with a greater (ns) leaflet area than its white clover parent (Table A3.1). Most Trophy BC_1s were intermediate between the two parents for mean leaflet area (Appendix 4). For the Trophy/POW-8, Trophy/POW-139, Trophy/POW-140 and Trophy/900-4 families, the BC_1 hybrid mean leaflet area was significantly smaller (P<0.001) than the LA of the Trophy parent, and the mean leaflet area of the Trophy/POW-4, Trophy/POW-10 and Trophy/900-3 BC_1 families were not significantly different from Trophy(Appendix 4). All of the Tahora BC_1 hybrids had significantly larger (P<0.001) mean leaflet areas than the Tahora white clover parent (Table A2.1). Only Tahora/POW-10, Tahora/900-3, and Tahora/900-4 had significantly larger ($P<0.001$) mean leaflet areas than their respective F_1 parents (Appendix 4). Tahora/POW-4 had a significantly smaller ($P<0.001$) mean leaflet area than POW-4 (Table A4.4). The remaining Tahora BC_1 families had mean leaflet areas that were not significantly different to those of their respective F_1 parents (Appendix 4). Each Tahora BC_1 family had a significantly greater ($P<0.001$) mean stolon diameter than the Tahora white clover parent (Table A2.1). Tahora/POW-4 and Tahora/POW-8 were the only Tahora BC_1 hybrids with a significantly lower ($P<0.001$) mean stolon diameter than their respective F_1 parents; the remaining BC_1 families were not significantly different (Appendix 4). All Trophy BC_1 hybrid families had mean stolon diameters that were significantly greater than ($P<0.001$), or not significantly different to, both of their respective parents (Appendix 4). Specifically, five of the Trophy BC_1 hybrids had significantly greater ($P<0.001$) stolon diameters than their respective F_1 parents (Appendix 4).

The BC_1 hybrid families all had mean internode lengths that were intermediate between the two parents, and significantly longer ($P<0.001$) than the internode lengths of their respective F_1 parents (Appendix 4). Trophy/POW-139 was the only Trophy BC_1 family that had a mean internode length that wasn't significantly less ($P<0.001$) than that of the Trophy white clover parent (Table A3.1). 900-4 was the only F_1 that produced BC_1 families that differed significantly ($P<0.001$) in mean internode length depending on the white clover parent (Table A4.2).

3.2.2 Fertility Traits

For peduncle lengths, all Trophy BC_1 hybrids were intermediate and significantly different ($P<0.001$) from the mean of both parents; greater than the F_1, less than Trophy

(Appendix 4). All Tahora hybrids except Tahora/POW-4 were intermediate between the two parents, but only Tahora hybrids with POW-8, POW-10 and 900-4 were significantly different ($P<0.001$) to both parents (Appendix 4). No hybrid had a significantly greater peduncle length than white clover (although Tahora/POW-4 was numerically greater, but not significantly) (Table A2.1). There were significant differences ($P<0.001$) between both the Tahora (Table A2.1) and Trophy (Table A3.1) BC_1 families for mean peduncle length.

The BC_1 hybrid mean florets per inflorescence were all intermediate and significantly different ($P<0.001$) to both respective parents (Appendix 4), with the exception of Tahora/POW-4 (Table A2.1), which was the only BC_1 hybrid with the mean floret number not significantly different to the white clover parent ($P<0.001$).

Most of the BC_1 families were intermediate between the two parents for mean pollen stainability, and were not significantly different from both parents (Appendix 4). The Trophy/900-3 (Table A4.1), Trophy/900-4 (Table A4.2), Tahora/POW-139 (Table A4.7), Tahora/POW-140 (Table A4.8) and Tahora/900-3 (Table A4.1) hybrid families were the exception and each had significantly ($P<0.001$) lower mean pollen stainability than both parents. There were significant ($P<0.001$ – $P=0.016$) differences between Trophy BC_1 families for mean pollen stainability (Table A3.1). Tahora/POW-4 was the only BC_1 family significantly ($P>0.001$) different from any of the other Tahora BC_1 families, and was not significantly different from Tahora for mean pollen stainability (Table A2.1).

3.2.3 Root traits

Within the Tahora group (Table A2.1), all of the BC_1 families except Tahora/POW-4 had a significantly (P=0.003) larger mean maximum nodal root diameter than the Tahora white clover parent. All of the Trophy BC_1 families had numerically larger mean nodal root diameters than Trophy, but not significantly (P=0.45).

With the exception of BC_1 hybrids from POW-4 which were intermediate to both parents for nodal root diameter, all of the BC_1 hybrid families had greater nodal root diameters than their respective F_1 parent (ns for Trophy/POW-139, Tahora/POW-139 &Tahora/900-3).

The BC_1 hybrids all had mean minimum stolon anchoring distances (the distance from the stolon growing tip to the first nodal root) that were intermediate between, or less than the minimum anchoring of both parents (Appendix 4). The differences were non-significant for all BC_1 families except Trophy/900-4, which had a mean minimum anchoring that was significantly (P=0.003) less than the Trophy parent (Table A4.2).

3.3 Correlations between F_1 hybrids and their BC_1 progeny

3.3.1 Foliar traits

There were significant ($P \leq 0.1$) positive correlations between the F_1 parents in experiment one and experiment two for leaflet area (r=0.65) (Figure A6.8), stolon density (r=0.61) (Figure A6.2), and foliar dry weight (r=0.62) (Figure A6.4).

There was a highly significant correlation between the F_1 parents in experiment two, and the Trophy BC_1 families (r=0.87, p=0.005) for internode length (Figure A6.10), and also between the F_1 parents in experiment two, and the Tahora BC_1 families (r=-0.79, p=0.03) for foliar dry weight (Figure A6.4).

While further weak to moderate correlations were found between the F_1 parents and the BC_1 progenies for the foliar traits, none were significant (Appendix 6).

3.3.2 Fertility Traits

There was a positive correlation between the F_1 parents in experiment one, and the same plants in experiment two for the number of florets per inflorescence (r=0.87, p=0.005) (Figure 3.1). There was no significant correlation across experiments for pollen stainability (Figure A6.1) or peduncle length (Figure A6.7) of the F_1 plants.

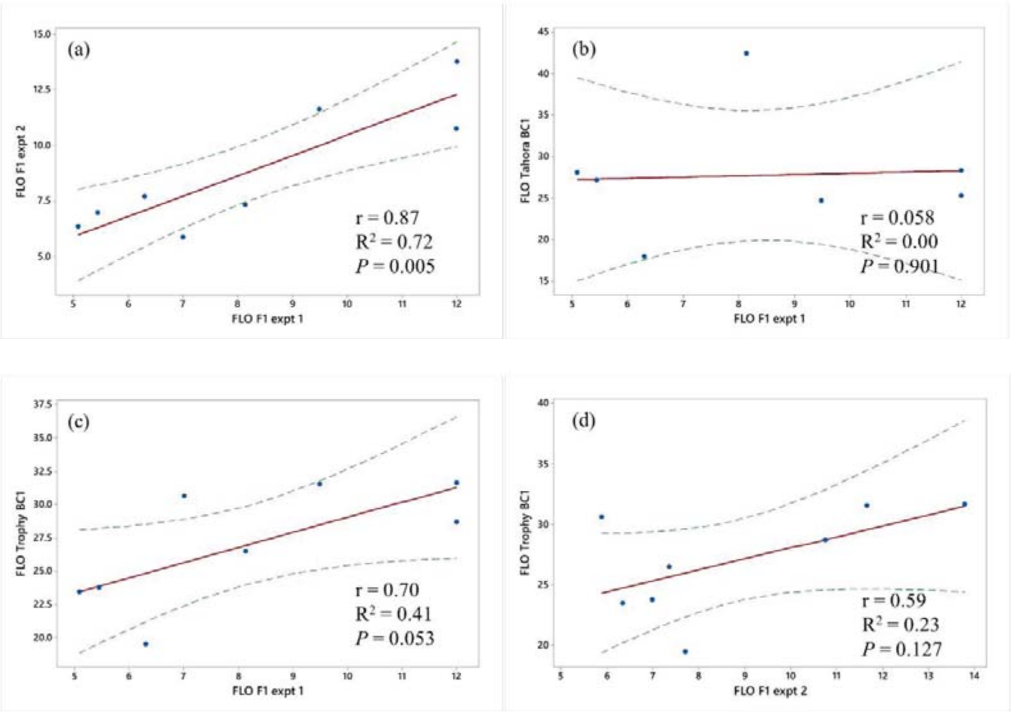


Figure 3.1: Correlations of florets per inflorescence (FLO) between (a) F1 hybrids in experiment 2 and F1 hybrids in experiment 1, (b) Tahora BC1 families and F1 hybrid parents in experiment 1, (c) Trophy BC1 families and F1 hybrid parents in experiment 1, and (d) Trophy BC1 families and F1 hybrid parents in experiment 2.

There were positive correlations when the Trophy BC$_1$ families were regressed against both the F$_1$ parents from experiment one (r= 0.70, p=0.05) and the F$_1$ parents from experiment two (r=0.59, ns) for the number of florets per inflorescence (Figure 3.1). There were no significant correlations between the F$_1$ parents and the Trophy and Tahora BC$_1$ families for pollen stainability (Figure A6.1) or peduncle length (Figure A6.7).

3.3.3 Root traits

There was a positive but non-significant correlation for maximum nodal root diameter (r=0.42) (Figure A6.5) and a non-significant negative correlation for minimum anchoring (r=-0.41) (Figure A6.6) in the regressions between the F_1 parents in experiment two and the same plants in experiment one.

The same patterns were observed between the Tahora BC_1 offspring and the F_1 parents from experiment one for both maximum nodal root diameter (r=0.50, ns) (Figure A6.5) and minimum anchoring (r=-0.32, ns) (Figure A6.6). There were no correlations between the Trophy BC_1 families and the F_1 parents from either experiment for either of the root traits.

3.4 Pattern Analysis

Principle component analysis (PCA) of the family × trait adjusted mean matrices for the 15 BC_1 families and their parents enabled concurrent assessment of the association among all traits evaluated, and the relationships between families. Correlation structure among traits is indicated by the directional vectors of the biplots. The biplots from the PCA showed similar positive associations among the traits FLO, PL, IL, MS, FDW and SD for both the Tahora hybrids (Figure 3.2) and Trophy hybrids (Figure 3.3). The point at which all of the directional vectors meet indicates the trait mean of the population; families positioned near this point have trait values close to the trait means.

Table 3.1: Tahora cluster analysis group means for each morphological trait based on the three clusters generated from cluster analysis of 8 Tahora BC$_1$ *T. repens* x *T. uniflorum* hybrids, a *T. repens* cv. Tahora genotype, and 8 F$_1$ *T. repens* x *T. uniflorum* hybrids grown in sand.

Group no	No. in group	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL
1	8	55.70	3.10	39.03	14.33	5.01	11.31	35.96	29.91	79.84	2.42	11.97
2	2	68.75	2.75	20.00	7.08	6.75	17.25	16.18	10.65	103.98	2.68	5.39
3	6	50.88	1.92	15.08	4.25	4.19	11.00	10.61	8.22	64.71	2.53	3.79

Cluster analysis of the Tahora BC$_1$ families and their parents based on all the evaluated traits separated the families into three groups (Table 3.1). Families that are located close together have similar expression of all the traits. Group 1 was comprised of the Tahora white clover genotype and all seven Tahora BC$_1$ hybrids. They had higher than average FLO, PL, IL, MS, SD, and DW, and smaller than average SDi. Group 2 had two members – POW-139 and POW-4 – and had trait expression closer to the population mean for PL, IL, MS, SD, NRD, PS, MA and DW than the F$_1$ hybrids in Group 3. Group 3 contained the remaining F$_1$ parents (900-3, 900-4, POW-10, POW-140, POW-2, POW-8). Group 3 had below population mean values for FLO, PL, IL, MS, SD, DW, LA and NRD. The cluster analysis (Figure 3.2) showed on the whole that the BC$_1$ hybrids were intermediate to both parents, but more like the Tahora white clover parent in trait expression.

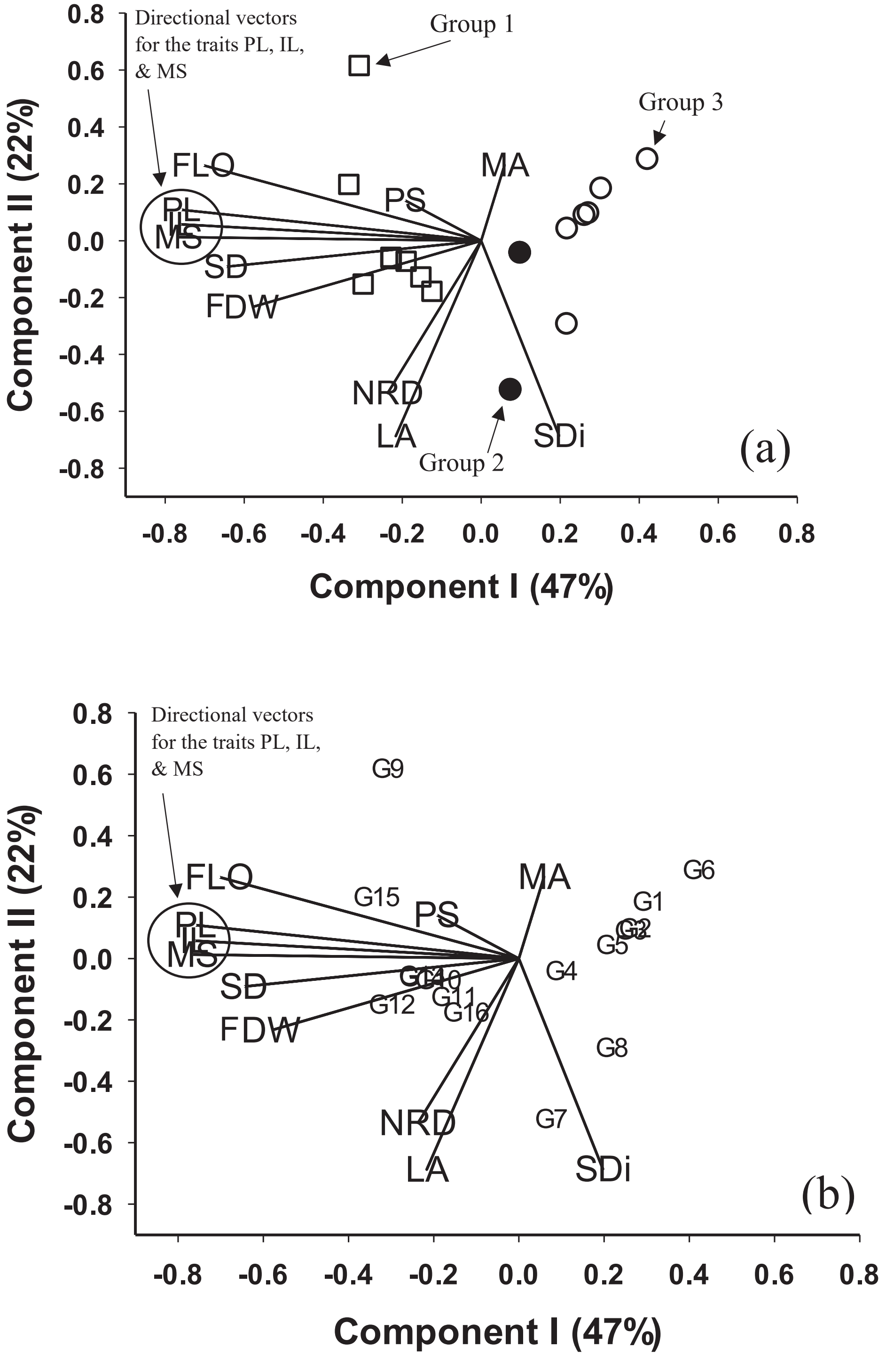


Figure 3.2: Tahora BC$_1$ biplot generated using adjusted means for morphological traits (Table A5.2) from 7 Tahora BC$_1$ *T. repens* x *T. uniflorum* hybrids (G10-16 in (b)), a *T. repens* cv. Tahora genotype (G9), and 8 F$_1$ *T. repens* x *T. uniflorum* hybrids (G1-8) grown in sand. The different symbols indicate genotype Groups 1 to 3 generated from cluster analysis (a), and the different numbers indicate individual hybrid families (b). The vectors represent the morphological traits.

Cluster analysis of the Trophy BC_1 families and their parents based on the traits evaluated separated the families into three distinct groups (Table 3.2). Group 1 comprised of Trophy white clover and all eight Trophy BC_1 families, and displayed above population mean expression of FLO, PL, NRD, LA, IL, MS, DW, SD and SDi. The F_1 parents were again separated into two groups – Group 2 contained POW-139 and POW-4, and the remaining F_1s were in Group 3. Group 2 had above population mean expression of PS, NRD, MA, and LA, and displayed near population mean expression of SD and SDi. A high MA value was undesirable as it meant that anchoring by nodal roots was further from the stolon growing tip. Group 3 had below population mean expression of PS, SD, MS, DW, NRD, PL, FLO, LA, SDi and IL (Figure 3.3).

Table 3.2: Trophy cluster analysis group means for each morphological trait based on the three clusters generated from cluster analysis of 8 Trophy BC_1 *T. repens* x *T. uniflorum* hybrids, a *T. repens* cv. Trophy genotype, and 8 F_1 *T. repens* x *T. uniflorum* hybrids grown in sand.

Group no	No. in group	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL
1	9	50.89	3.05	34.83	24.92	6.48	9.52	38.58	31.77	126.64	2.94	11.99
2	2	68.75	2.75	20.00	7.08	6.75	17.25	16.18	10.65	103.98	2.68	5.39
3	6	50.88	1.92	15.08	4.25	4.19	11.00	10.61	8.22	64.71	2.53	3.79

Principal component analysis of all the trial entries (15 BC_1 families, two white clovers, and eight F_1 hybrids) × trait mean matrix (Table A5.1) allowed assessment of the associations among traits. MS, PL, and IL were highly positively correlated as indicated by vectors in similar directions (Figure 3.4). There were varying degrees of positive correlations between FLO, MS, PL, IL, SD, FDW, NRD, LA and SDi. MA was negatively correlated (vector angle greater than 90°) with all traits except PS.

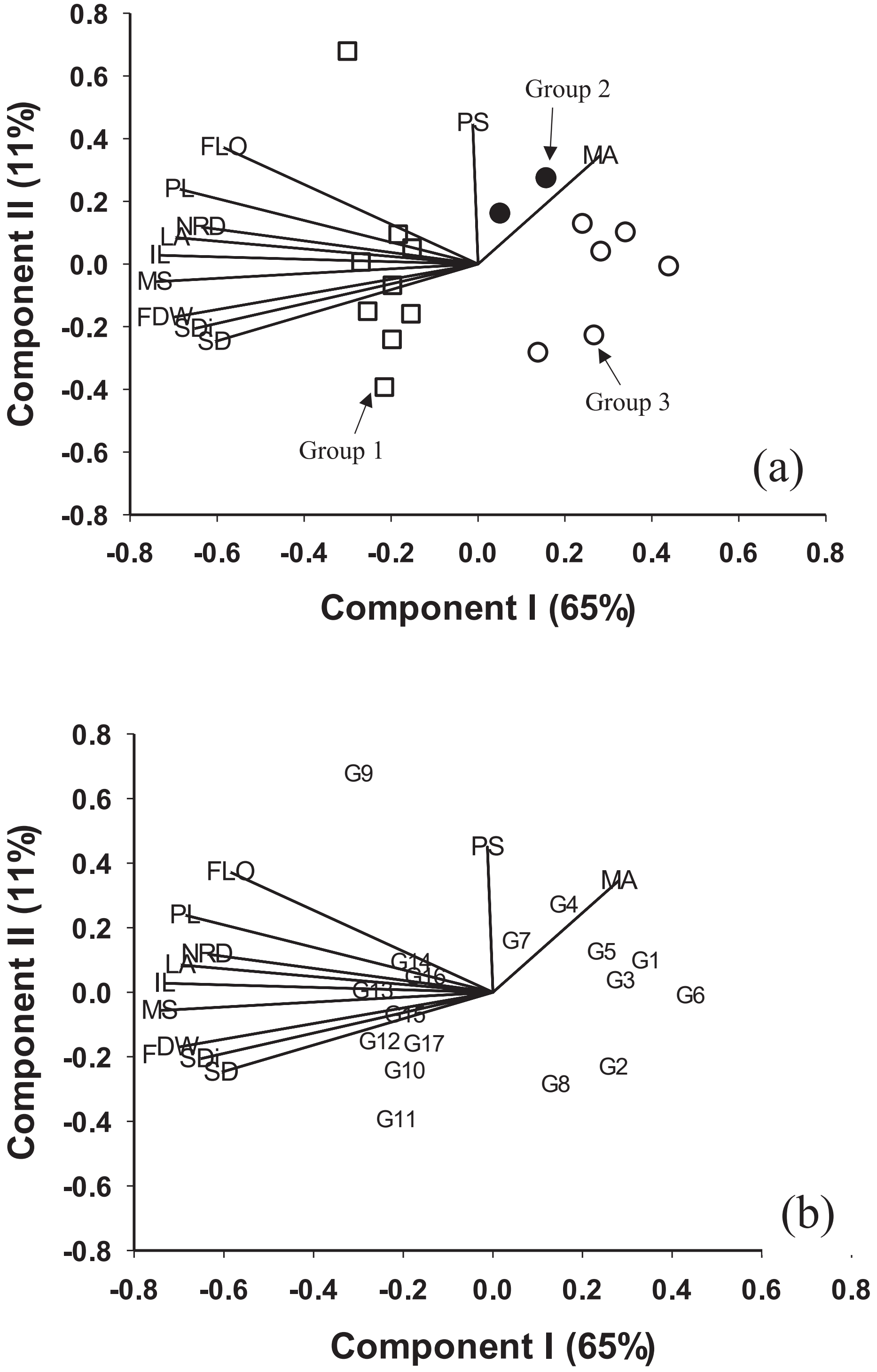


Figure 3.3: Trophy BC$_1$ biplot generated using adjusted means for morphological traits (Table A5.3) from 8 Trophy BC$_1$ *T. repens* x *T. uniflorum* hybrids (G10-17 in (b)), a *T. repens* cv. Trophy genotype (G9), and 8 F$_1$ *T. repens* x *T. uniflorum* hybrids (G1-8) grown in sand. The different symbols indicate genotype Groups 1 to 3 generated from cluster analysis (a), and the different numbers indicate individual hybrid families (b). The vectors represent the morphological traits.

Cluster analysis of all trial entries again generated three distinct groups (Table 3.3). Group 1 consisted of the eight F_1 parents (Figure 3.4), with expression of FLO, MS, PL, IL, SD, DW, NRD, and LA below the population means. Group 2 comprised of Tahora, and the 7 Tahora BC_1 hybrid families, with most members positioned close to the population mean for all traits. Group 3 consisted of Trophy, and the 8 Trophy BC_1 hybrid families, with above population mean expression for most traits. As per the previous cluster analyses, the BC_1 hybrid families were intermediate in trait expression to the white clover and F_1s.

Table 3.3: Cluster analysis group means of all 2014 BC_1 clover experiment entries for each trait based on the three groups generated from all Experiment 2 genotypes grown in sand.

Group no	No. in group	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL
1	8	55.34	2.13	16.31	4.96	4.83	12.56	12.00	8.83	74.53	2.57	4.19
2	8	55.70	3.10	39.03	14.33	5.01	11.31	35.96	29.91	79.84	2.42	11.97
3	9	50.89	3.05	34.83	24.92	6.48	9.52	38.58	31.77	126.64	2.94	11.99

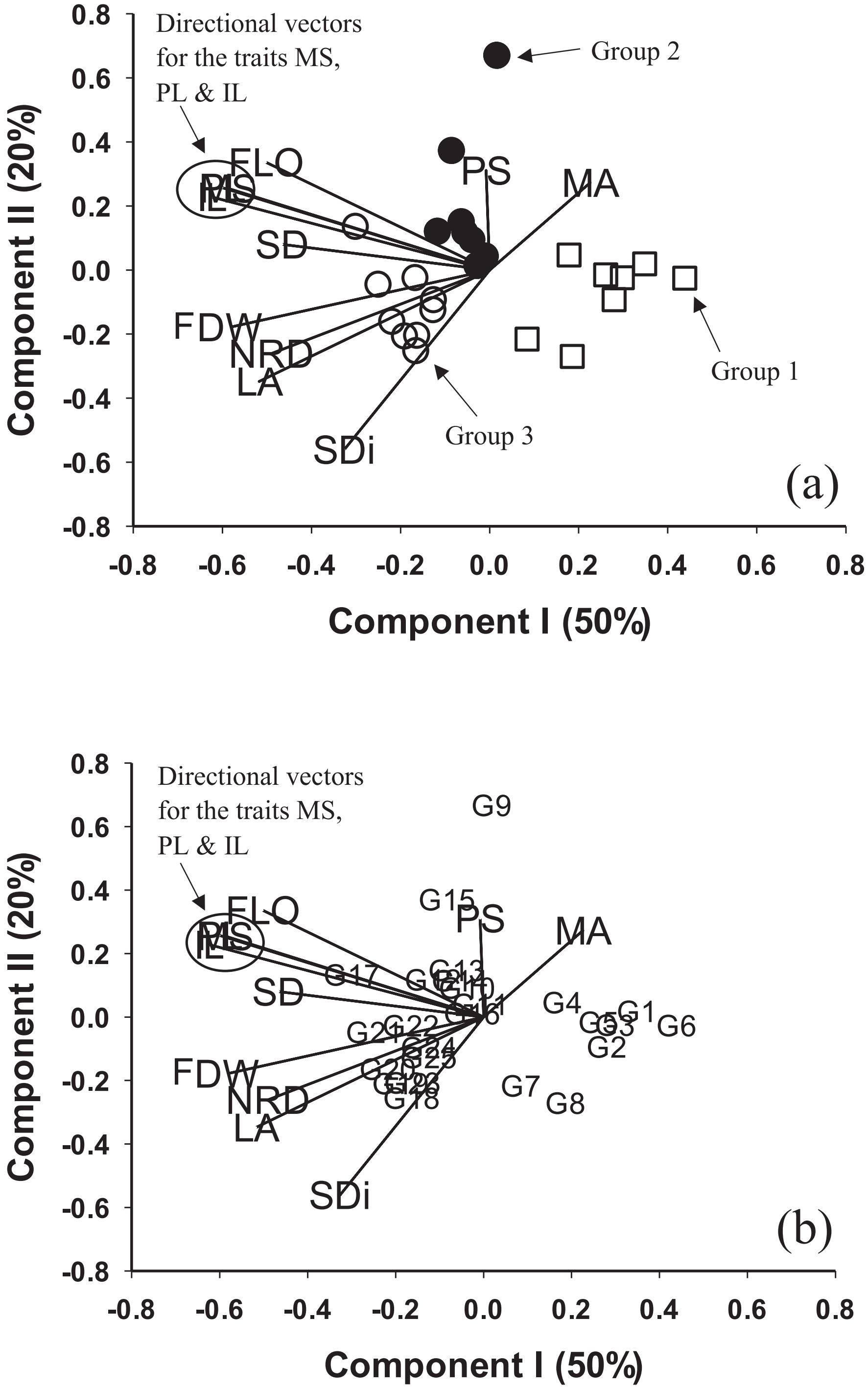


Figure 3.4: Biplot of all entries in the 2014 BC$_1$ sandframe experiment, generated using adjusted means for morphological traits from all experimental entries (Table A5.1), comprised of 8 F$_1$ *T. repens* x *T. uniflorum* hybrids (G1-8 in (b)), one each of *T. repens* cv. Trophy (G17) and Tahora (G9) genotypes, 7 Tahora BC$_1$ *T. repens* x *T. uniflorum* hybrids (G10-16), and 8 Trophy BC$_1$ *T. repens* x *T. uniflorum* hybrids (G18-25) grown in sand. The different symbols indicate genotype Groups 1 to 3 generated from cluster analysis (a), and the different numbers indicate individual hybrid families (b). The vectors represent the morphological traits.

Chapter 4

4 Discussion

The principal hypotheses for this study were that some of the F_1 *T. repens* x *T. uniflorum* hybrids would be better parents in a breeding programme than others, and that the performance of the BC_1 hybrids (*T. repens* x F_1 hybrids) could be predicted from the F_1 phenotype.

The variation among F_1 hybrid genotypes within four families was investigated to determine whether or not F_1s with the same parents behaved similarly. By selecting from these families and creating BC_1 hybrids, it was possible to ascertain by means of correlation whether the F_1 phenotype was a predictor of the performance of its BC_1 progeny.

4.1 Evaluation of phenotypic variation among F_1 hybrid families and genotypes

It is widely accepted that the species *T. repens* does not contain enough genetic variation for traits such as shorter internode lengths and high nodal root diameter associated with drought tolerance and persistence in adverse conditions (Williams *et al.*, 2007). Individual stolons have relatively short lifespans, and the spread and establishment of stolons by frequent branching is vital for persistence. Multiple stolon branching is generally related to shorter, or more frequent internodes (Williams, 1987a). Populations created by means of interspecific hybridisation between *T. repens* and *T. uniflorum* are expected to contribute increased variability for these traits as a result of high heterozygosity present in F_1 hybrids, and as expected, there was significant variation between genotypes within each family for over half of the evaluated traits, indicating that F_1s with the same parents do not always behave similarly.

Differences between F_1 families were generally only significant between the extreme values for most trait means, with the exception of florets per inflorescence, leaf area, and stolon density, all of which are highly heritable traits (Woodfield & Caradus, 1990; Annicchiarico *et al.*, 1999). It was expected that variation for trait expression among genotypes within F_1 hybrid families would be similar across all four families, and the top performing F_1s would not belong to just one family.

The results show that in general, the F_1 hybrids were very poor in vigour, due to the many negative alleles inherited from *T. uniflorum*, as well as genomic incompatibility known to cause a reduction in plant function and productivity in new interspecific hybrids (Zamir, 2001; Mason & Batley, 2015).

F_1 genotypes were selected based on foliar dry weight to be backcrossed to *T. repens*. This trait was decided upon as a selection criterion despite differences between hybrid genotypes being insignificant within three out of four families, as current white clover breeding practice is to select on dry matter yield (Woodfield & Caradus, 1994). The hybrids with the highest and lowest yield were selected, with the difference between bottom and top in families being between two and 30 fold.

With one defining feature of *T. uniflorum* being large, woody nodal tap roots (Chen & Gibson, 1971; Williams & Nichols, 2011), and a trait of interest for introgression into *T. repens*, it was expected that the F_1 hybrids would have larger nodal root diameters and deeper roots than the white clover parent. For both traits, this was not the case. Both *T. uniflorum* and the F_1 hybrid families had significantly smaller mean nodal root diameters and root depths than *T. repens*. Blaikie and Mason (1990) found a strong correlation between root and shoot growth in white clover, which could explain why in the case of the F_1 hybrids, on average, the expression of root traits was also poor. Another contributing factor to the poor expression of root traits may have been the fact that only 5-8 genotypes were used to represent each hybrid family, a sample size not representative of a highly variable, widely segregating population, given that individual hybrid genotypes with larger nodal root diameters than *T. repens* were observed. The lack of environmental pressure on the plants due to regular watering and nutrient application from the soil surface, as opposed to the natural environment in which *T. uniflorum* is found where surface moisture is limited could also have had an effect on root structure. The process of excavating the root systems in their entirety involved digging around each plant, and removing the roots from the one metre deep sandpit. While utmost care was taken to achieve this, there is a possibility that some roots were broken off during the excavation process, impacting on the root depth measurements, and subsequently the level of variation observed. Root morphology plays a key role in plant persistence; deeper root systems and nodal taproots increase water and nutrient capture in less favourable conditions, and the ability of a plant to anchor its stolons by producing roots from the nodes close to the growing tip improves the likelihood of plant fragments surviving if the stolon is broken off due to animal treading, insect damage, or decay (Brock & Hay, 2001).

4.2 BC_1s

Extreme heterozygosity of F_1 interspecific hybrids results in the tremendous diversity of types that appear in the F_2 and later generations, with many segregants likely to have entirely new characteristics, different from those of either parent, that could not have been predicted from a study of the morphology or physiology of the parental species (Allard, 1960). Many valuable exotic alleles have been discovered by crossing wild related species with elite breeding lines (Tanksley & McCouch, 1997), but with the positive alleles come many negative alleles, which take generations of back crossing in order to break linkage drag and remove. The presence of good genes is not always obvious based on hybrid phenotype alone. It is therefore quite remarkable in the case of the BC_1 hybrids at such an early stage in backcrossing that there are some hybrids performing so well, with some exhibiting transgressive segregation. Transgressive segregation is described as the presence of phenotypes in segregating hybrid populations that exceed parental phenotypic values in either a negative or positive direction. It seems to be ubiquitous in plant hybrids, affecting characters of adaptive significance, and expanding ecological tolerances (Lewontin & Birch, 1966; de Vicente & Tanksley, 1993; Rieseberg *et al.*, 1999).

4.2.1 BC_1 Fertility traits

As expected, mean florets per inflorescence and peduncle length of the Trophy and Tahora BC_1 hybrids were intermediate between the two parents. In a study on hybrid seed production traits, Naeem *et al.* (2017) found the BC_1 hybrids to be intermediate to *T. uniflorum* and *T. repens* for these traits, however, F_1 hybrids weren't included in the comparisons. For potential cultivars to be commercially viable, plants must yield acceptable amounts of seed that can be mechanically harvested. A high number of florets per inflorescence is desirable, as this trait governs, in part, the seed yield potential of a plant. The positive correlation between florets per inflorescence of the F_1 parents and their BC_1 progeny in this study indicated that the BC_1 floret numbers could be predicted from the F_1 phenotype, and selection for this trait worthwhile. Naeem

(2013) showed that this trait had the highest heritability of all the floral traits studied, indicating high responsiveness to selection, and experimental data showed that the number of florets per inflorescence of the BC_3 generation was not different from that of white clover. Thus, florets per inflorescence could easily be improved in the hybrids via recurrent selection and back crossing to white clover. Peduncle length affects various stages of seed production – short peduncles that do not extend above the leaf canopy, such as those on *T. uniflorum* and the F_1 hybrids, are unfavourable for pollinator access, and seed does not mature properly in the shade of the canopy, often rotting or sprouting in humid conditions beneath the leaf canopy (Thomas, 1981; Marshall, 1995). Peduncle lengths that place the inflorescence above the leaf canopy are necessary for ease of harvesting commercially by machinery. In a recent study by Naeem *et al.* (2017), peduncle length responded to positive selection, with the hybrid mean peduncle length equivalent to that of white clover after the third generation of back crossing. A more appropriate measure of how accessible the hybrid flowers were to pollinators and how harvestable seed is from these hybrids would have been a comparison of petiole (leaf stalk) length (no data collected in this study) and peduncle length.

Partial or complete hybrid sterility is a frequent difficulty with interspecific hybridisation (Fehr, 1987), so it was expected that pollen stainability, a measure of male sterility, would be variable between and within hybrid families. This was proven to be the case, whereby many of the BC_1s were intermediate to both parents for pollen stainability, and others were negatively transgressively segregated, ie, had lower pollen stainability than both parents, and notably the only trait measured that had any negatively transgressive expression.

4.2.2 BC_1 root traits

Data from the BC_1 populations reported here suggests that transgressive segregation may be occurring for maximum nodal root diameter. This occurred in both the Tahora and Trophy BC_1 families with the BC_1 hybrids having larger mean maximum nodal root diameters than both parents, although not significantly in the latter. Previous studies conducted in sand culture by Hussain *et al.* (2012) and Nichols *et al.* (2014a) also reported the same result for this trait in the BC_1 population compared with the white clover parent. As mentioned previously, nodal root diameter is a trait of interest for introgression from *T. uniflorum* for improving the persistence of white clover. Root diameter is associated with improved moisture stress tolerance and improved nutrient uptake, and tap-root like nodal roots are more likely to penetrate to greater depths, potentially also increasing access to deep soil water (Caradus & Woodfield, 1998; Nichols *et al.*, 2016b). Selections for root morphological characteristics by Woodfield and Caradus (1990) and later by Caradus and Woodfield (1998) in white clover appeared to be successful, supporting the notion that recurrent selection for larger nodal roots in the BC_1 population would be beneficial in decreasing the vulnerability of white clover in dry periods and infertile soils. It is likely that BC_1 hybrids with thicker nodal roots would be better adapted than conventional white clover cultivars to pastoral environments in dryland and/or drought conditions, and more likely to persist following seminal tap root loss at around 18 months when the plant becomes dependent on the nodal roots of a clonal stolon fragment for survival (Brock *et al.*, 2000). The BC_1s with the thickest nodal root diameters were not the offspring of any particular F_1, and nodal root diameter could not be confidently predicted from the F_1 parent phenotype; no statistically significant correlations were observed. Stronger correlations may have existed with greater plant numbers, but BC_1 seed quantities and growing space were limited in this study.

It was not feasible to collect root depth and root dry weight data from the BC_1 experiment due to the number of plants in the experiment, and the time taken to process samples for these traits in a destructive harvest; it would have spread the harvest over a much longer period of time and added undue sampling error to data for the other traits.

4.2.3 BC_1 foliar traits

Foliar and stolon morphologies of the BC_1 hybrids were expected to be intermediate between the two parental morphologies, however this was only the case for internode length, stolon diameter for Tahora hybrids, and leaf area for Trophy hybrids. Unexpectedly, maximum spread, foliar dry weight, stolon density, leaf area (Tahora hybrids) and stolon diameter (Trophy hybrids) were all equal to, or greater than both parents. While these results are a positive outcome in the sense of breeding advances, they were not predictable from the F_1 hybrid phenotypes and, like previously discussed traits, were not from any particular F_1.

Stolon characteristics play a large part in determining how well plant fragments will persist if part of the plant were to be damaged due to factors such as animal treading, insect damage, or decay. There is potential at each node for the formation of a new stolon or inflorescence, as well as root growth. For this reason, a plant exhibiting frequent nodes, and high stolon density is preferable. Stolon density has been identified as an important morphological trait for use in breeding white clover with better persistence under short term drought conditions, and a high stolon density improves persistence through maintenance of growing points (Brock & Hay, 1996; Widdup &

Barrett, 2011; Nichols *et al.*, 2014b). Stolon diameter is an important factor in persistence, and can dictate how well plants recover from defoliation. A larger stolon diameter provides more resource in the stolon to aid in recovery after grazing, and aids survival in high density swards where getting leaves up through the pasture canopy is essential to survival.

White clover cultivars are generally categorised by leaf size, a highly heritable trait (Woodfield & Caradus, 1990), ranging from small leaved, highly stoloniferous types that endure hard grazing such as in a set stocked system (Brock & Hay, 1996) to large leaved, high yielding, but less stoloniferous types commonly utilised in rotational grazing systems (Abberton *et al.*, 1998). A likely explanation for the transgressive expression of leaf area in the Tahora BC_1 hybrids, despite Tahora white clover being a small leaf type cultivar, is the use of the large leaved cultivar Kopu II as the white clover parent in the formation of F_1 hybrids. The fact that Trophy is a large leaved cultivar, and no transgressive leaf size was seen in the Trophy BC_1 hybrids, supports this theory.

All BC_1 families had maximum spreads that were not significantly different from those of the corresponding white clover parents. Despite the similarity in spread, the Tahora BC_1 families had significantly larger foliar dry weights than Tahora white clover, which had sparsely leaved, long, thin stolons. This result is most likely attributable to the much greater stolon diameter and leaf cover in the BC_1 hybrids.

4.2.4 Associations among individuals and traits

Pattern analysis gave a graphical representation of the variation present across all genotypes and traits, simultaneously. Given the genomic makeup of the BC_1 hybrid population (75% *T. repens*, 25% *T. uniflorum*), it was expected that the hybrid population's associations among traits would be similar to those known for white clover. The cluster analysis of the genotype × trait mean data matrix in the present study confirmed this, in both the Tahora and Trophy BC_1 hybrids. For both sets of hybrids, the BC_1s were grouped with the white clover parent with above average expression of florets per inflorescence, peduncle length, internode length, maximum spread and dry weight, while the F_1 parents were arranged in two groups of genotypes, below average for these traits. The cluster analysis of all trial entries produced three groups; two groups each made up of a white clover parent and their corresponding BC_1 hybrid progeny, and one group comprising all of the F_1 hybrid parents (Figure 3.4). This highlighted the impact of the white clover plant selected as a backcross parent, as the two white clover plants used in this study had very different morphologies, further recognised in the cluster analysis.

4.3 Correlation between F_1 and BC_1 trait expression

The linear regression, or alternatively correlation, of the offspring performance on that of the parent can be used as a method of estimating heritability (Fehr, 1987). While some statistically significant correlations between F_1 parents and BC_1 offspring were observed, there were fewer than expected. Traits historically reported to have high heritabilities such as leaflet size and stolon density (Woodfield & Caradus, 1990; Annicchiarico *et al.*, 1999) showed little or no correlation between the performance of the F_1 parents and the BC_1 offspring. The high genetic diversity within a population of interspecific hybrids such as these, meant that the small number of seeds available to evaluate were not representative of the population. Statistical power was sacrificed as a result of relatively low replication, and as such, heritability was not able to be calculated due to small sample size.

The strong correlation between the F_1 plants in experiment one and in experiment two for florets per inflorescence was not unexpected as this trait is highly heritable and therefore the expression of this trait is largely unaffected by environment (Acquaah, 2009). This model relationship demonstrates that if correlations were present for other traits between parents and offspring then this system would be able to detect them.

The correlations between parent and offspring gave an indication of whether or not the BC_1 performance could be predicted by the F_1. For most traits, the BC_1 phenotype was not able to be predicted from the F_1 parent. The statistical power of correlations would likely have been improved to some extent by increasing replication of BC_1 families in the trial for traits widely reported as having high heritability, making some selection justified, but probably without any long term gains in terms of building variation within hybrid populations.

In barley breeding programmes incorporating valuable donor alleles from non-adapted wild material into inbred crops, Falk (2010) recommended a recurrent breeding system that encourages rapid cycling and increased opportunities for recombination over time, resulting in a larger number of positive alleles in the coupling phase along a chromosome, regardless of the population size, thus giving rise to more lines with an increased frequency of positive alleles, and ultimately achieving an accumulation of these alleles over time. The results of the current study show that the same principles should be applied to an outcrossing hybrid population, using an elite line of white clover and the non-adapted *T. uniflorum*.

4.4 Concluding remarks

The results of this study have shown through the weak to non-existent correlations between F_1 and BC_1 trait expression that making selections out of the F_1 generation was not beneficial in the generation of breeding populations for cultivar development of the outcrossing interspecific hybrid *T. repens* × *T. uniflorum*, and that a breeding programme incorporating alleles from the non-adapted donor species should involve further cycles of back crossing and recombination beyond the F_1 generation, without selection. The use of two contrasting elite parent types in this study highlighted the importance of back crossing to the same or similar elite lines (not necessarily the same genotype), in order to maintain the majority of the elite white clover parent genome, with the positive donor alleles added. The large leaved Trophy variety was similar in foliar morphology to Kopu II (the white clover parent in the F_1 hybrids) and, as shown in the cluster analysis, there was very little shift of the Trophy BC_1s from Trophy for these traits, especially stolon diameter, leaf area and foliar dry weight. By using the same white clover type for each generation of backcrossing, the white clover foliar morphology was maintained in the BC_1 hybrids. By contrast, the Tahora BC_1s were derived using different white clover types, (large leaved Kopu II in the formation of the F_1 hybrids, and then small leaved Tahora to create the BC_1s). Consequently, the hybrids did not maintain the foliar characteristics of either white clover parent type. The use of inconsistent white clover types in successive generations would require further generations of back crossing in order to restore the desired white clover foliar traits.

Overall, the results of the present study have shown that even when using small numbers of genotypes, variation existed within the BC_1 hybrid population for almost all traits, producing a wide range of morphologies. It is likely that valuable alleles for traits associated with tolerance to drought and persistence in environments not normally favourable for white clover production can be successfully incorporated into white clover breeding pools from the wild related species *T. uniflorum*, however selection should not begin until after the F_1 generation, and future breeding design will dictate just how successful this will be.

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Appendices

Appendix 1 F1 mean morphological data

Table A1.1: Mean morphological data of Family 1 F_1 hybrid genotypes (± SEM). Means that have been log transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter, within columns, show no significant differences in variability at the 5% level.

F1 hybrid	PS	sqrt FLO	sqrt PL	log RDW	RD	max NRD	LA	sqrt MA	SD	sqrt MS	FDW	log Sdi	log IL
900-3	77 ± 4.5^{a}	2.3 ± 0.08^{c}	3.2 ± 0.18^{ab}	1.92 ± 0.268^{ab}	47.0 ± 4.75^{b}	4.0 ± 0.50^{b}	135 ± 11^{bc}	4.3 ± 0.50^{ab}	3.0 ± 0.55^{ab}	5.6 ± 0.34^{a}	16.2 ± 4.27^{ab}	0.70 ± 0.049^{c}	1.60 ± 0.113^{c}
		(5.1)	(10.4)	(6.81)				(18.7)		(31.5)		(2.01)	(4.97)
900-4	61 ± 4.5^{bc}	2.5 ± 0.08^{b}	2.8 ± 0.18^{bc}	0.96 ± 0.268^{c}	55.3 ± 4.75^{ab}	3.6 ± 0.50^{b}	121 ± 116^{c}	2.8 ± 0.50^{b}	2.0 ± 0.55^{bc}	4.5 ± 0.34^{b}	6.3 ± 4.27^{ab}	0.90 ± 0.049^{a}	1.13 ± 0.113^{a}
		(6.3)	(7.6)	(2.60)				(8.1)		(20.7)		(2.47)	(3.08)
900-5	69 ± 4.5^{ab}	2.1 ± 0.08^{c}	3.5 ± 0.18^{a}	2.06 ± 0.268^{ab}	54.3 ± 4.75^{ab}	4.1 ± 0.50^{b}	180 ± 11^{a}	3.2 ± 0.50^{b}	3.3 ± 0.55^{ab}	5.3 ± 0.34^{ab}	18.4 ± 4.27^{a}	0.75 ± 0.049^{bc}	1.66 ± 0.113^{bc}
		(4.8)	(12.5)	(7.84)				(10.4)		(28.1)		(2.13)	(5.23)
K283-13	15 ± 4.5^{e}	3.0 ± 0.08^{a}	2.5 ± 0.18^{c}	1.21 ± 0.268^{bc}	65.7 ± 4.75^{a}	3.8 ± 0.50^{b}	125 ± 11^{c}	4.8 ± 0.50^{a}	1.3 ± 0.55^{c}	4.5 ± 0.34^{b}	3.5 ± 4.27^{b}	0.64 ± 0.049^{c}	1.75 ± 0.113^{c}
		(8.8)	(6.5)	(3.34)				(22.9)		(20.2)		(1.89)	(5.74)
K283-19	63 ± 4.5^{bc}	3.1 ± 0.08^{a}	3.5 ± 0.18^{a}	2.36 ± 0.268^{a}	59.0 ± 4.75^{ab}	4.5 ± 0.50^{b}	177 ± 11^{a}	3.1 ± 0.50^{b}	3.7 ± 0.55^{a}	5.4 ± 0.34^{ab}	12.4 ± 4.27^{ab}	0.87 ± 0.049^{ab}	1.75 ± 0.113^{ab}
		(9.5)	(12.5)	(10.54)				(9.8)		(29.0)		(2.38)	(5.74)
K283-12	52 ± 4.5^{c}	3.0 ± 0.08^{a}	2.9 ± 0.18^{bc}	1.38 ± 0.268^{bc}	55 ± 4.75^{ab}	6.2 ± 0.50^{a}	118 ± 11^{c}	4.1 ± 0.50^{ab}	3.0 ± 0.55^{ab}	4.9 ± 0.34^{ab}	6.0 ± 4.27^{ab}	0.62 ± 0.049^{c}	1.33 ± 0.113^{c}
		(8.8)	(8.4)	(3.96)				(16.4)		(24.3)		(1.87)	(3.78)
K283-18	29 ± 4.5^{d}	3.1 ± 0.08^{a}	3.2 ± 0.18^{ab}	1.99 ± 0.268^{ab}	57.5 ± 4.75^{ab}	5.0 ± 0.50^{ab}	156 ± 11^{ab}	4.1 ± 0.50^{ab}	2.7 ± 0.55^{abc}	5.0 ± 0.34^{ab}	10.3 ± 4.27^{ab}	0.94 ± 0.049^{a}	1.83 ± 0.113^{a}
		(9.3)	(10.5)	(7.33)				(16.6)		(24.7)		(2.55)	(6.21)
P	*<0.001*	*<0.001*	*0.001*	*0.046*	*0.287*	*0.034*	*<0.001*	*0.127*	*0.113*	*0.227*	*0.251*	*<0.001*	*<0.001*
range	15-77	4.8-9.5	2.8-3.5	2.6-10.54	47.0-65.7	3.6-6.2	118-180	8.1-22.9	1.3-3.7	20.2-31.5	3.5-18.4	1.87-2.55	3.08-6.21
mean	52	7.5	9.8	6.1	56.3	4.5	143	3.8	2.7	25.5	10.4	2.19	4.97

PS, pollen stainability (%); FLO, florets per inflorescence; PL, peduncle length (mm); RDW, root dry weight (g); RD, root depth (cm); NRD, maximum nodal root diameter (mm); LA, leaf area (mm^2); MA, minimum anchoring (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); SDi, stolon diameter (mm); IL, internode length (mm).

Table A1.2: Mean morphological data of Family 2 F$_1$ hybrid genotypes (± SEM). Means that have been log transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter, within columns, show no significant differences in variability at the 5% level.

Family	PS	FLO	PL	RDW	RD	NRD	log LA	MA	SD	MS	FDW	Sdi	log IL
POW-134	*	11.2±0.58^{ab}	21.1±1.69^{a}	10.31±2.056^{ab}	51.5±2.95^{a}	6.2±0.62^{a}	4.9±0.15^{a} (130)	22.9±8.46^{b}	3.5±0.40^{ab}	27.0±3.93^{ab}	16.7±5.90^{a}	2.20±0.087^{b}	1.90±0.102^{ab} (6.66)
POW-136	64±2.2c	10.6±0.48^{bc}	12.3±1.38^{c}	10.53±1.679^{ab}	49.7±2.41^{a}	4.5±0.50^{ab}	4.7±0.12^{abc} (107)	10.6±6.91^{b}	2.7±0.32^{bc}	30.5±3.21^{a}	12.7±4.82^{a}	2.16±0.071^{b}	1.83±0.083^{ab} (6.23)
POW-137	62±2.2c	6.4±0.476^{e}	17.5±1.38^{ab}	4.45±1.679^{bc}	40.0±2.41^{b}	4.1±0.50^{bc}	4.5±0.12^{acd} (91)	23.0±6.91^{b}	2.0±0.32^{c}	17.0±3.21^{b}	7.9±4.82^{a}	2.00±0.071^{bc}	1.74±0.083^{b} (5.70)
POW-139	78±2.2b	12.0±0.48^{a}	19.3±1.38^{a}	12.72±1.679^{a}	44.0±2.41^{ab}	5.3±0.50^{ab}	4.6±0.12^{abc} (103)	6.7±6.91^{b}	4.3±0.32^{a}	38.0±3.21^{a}	15.7±4.82^{a}	1.78±0.071^{d}	2.04±0.083^{a} (7.73)
POW-140	62±2.2c	9.5±0.48^{c}	16.8±1.38^{ab}	0.69±1.679^{c}	31.0±2.41^{c}	2.5±0.50^{c}	4.3±0.12^{d} (72)	100.0±6.91^{a}	3.1±0.32^{b}	5.0±3.21^{c}	0.4±4.82^{a}	1.90±0.071^{cd}	0.92±0.083^{d} (2.52)
POW-141	85±2.2a	7.9±0.48^{d}	15.0±1.38^{bc}	14.76±1.679^{a}	47.3±2.41^{ab}	5.7±0.50^{ab}	4.9±0.12^{ab} (129)	7.9±6.91^{b}	3.3±0.32^{ab}	30.7±3.21^{a}	18.5±4.82^{a}	2.59±0.071^{a}	1.41±0.083^{c} (4.09)
P	*<0.001*	*<0.001*	*0.002*	*0.022*	*0.006*	*0.012*	*0.014*	*<0.001*	*0.009*	*<0.001*	*0.275*	*<0.001*	*<0.001*
range	62-85	6.4-12.0	12.3-21.1	0.69-14.76	31.0-51.5	2.5-6.2	72-130	3.7-100	2.0-4.3	5.0-38	0.4-18.5	1.78-2.59	2.52-7.73
mean	70	95.6	17	8.91	43.9	4.7	105	28.5	3.2	24.7	12	2.1	5.48

PS, pollen stainability (%); FLO, florets per inflorescence; PL, peduncle length (mm); RDW, root dry weight (g); RD, root depth (cm); NRD, maximum nodal root diameter (mm); LA, leaf area (mm^2); MA, minimum anchoring (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); SDi, stolon diameter (mm); IL, internode length (mm), * indicates missing data.

Table A1.3: Mean morphological data of Family 3 F_1 hybrid genotypes (± SEM). Means that have been log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter, within columns, show no significant differences in variability at the 5% level.

Family	PS	FLO	log PL	log RDW	RD	NRD	sqrt LA	log MA	sqrt SD	MS	FDW	Sdi	sqrt IL
POW-2	*	7.0±0.40bc	3.0±0.08b	1.36±0.282c	43.3±4.69a	5.3±1.06a	11.9±0.80cd	2.7±0.36b	1.5±0.15bc	13.5±2.74c	4.3±3.79c	1.92±0.087d	1.73±0.148d
			(20.4)	(3.90)			(140)	(14.2)	(2.3)				(2.98)
POW-3	*	7.8±0.40abc	2.6±0.08c	1.81±0.282bc	59.3±4.69b	5.5±1.06a	13.3±0.80bc	2.6±0.36b	1.4±0.15bc	22.6±2.74b	9.6±3.79bc	2.38±0.087b	2.43±0.148bc
			(13.9)	(6.09)			(178)	(13.9)	(1.9)				(5.92)
POW-4	*	8.1±0.40ab	2.5±0.08cd	2.89±0.282a	63.3±4.69b	5.3±1.06a	15.1±0.80b	1.5±0.36c	2.0±0.15a	40.3±2.74a	31.8±3.79a	2.29±0.087b	2.69±0.148b
			(12.6)	(18.01)			(228)	(4.4)	(4.0)				(7.23)
POW-20	72±2.5ab	8.0±0.40ab	1.2±0.08e	1.20±0.282c	54.0±4.69ab	3.3±1.06a	10.7±0.80d	1.0±0.36c	1.7±0.15ab	26.0±2.74b	3.6±3.79c	2.27±0.087bc	2.42±0.148bc
			(3.2)	(3.33)			(114)	(2.7)	(3.0)				(5.87)
POW-21	66±2.5b	6.8±0.40c	2.3±0.08d	1.67±0.282bc	57.0±4.69ab	6.6±1.06a	12.1±0.80cd	3.1±0.36ab	1.6±0.15abc	22.0±2.74bc	9.7±3.79bc	2.69±0.087a	2.42±0.148bc
			(10.4)	(5.29)			(146)	(23.2)	(2.5)				(5.85)
POW-16	*	7.8±0.40abc	2.6±0.08c	1.33±0.282c	62.0±4.69b	4.0±1.06a	10.3±0.80d	1.2±0.36c	1.7±0.15ab	26.0±2.74b	4.7±3.79c	1.89±0.087d	2.15±0.148c
			(13.3)	(3.78)			(105)	(3.3)	(3.0)				(4.62)
POW-18	68.±2.5b	8.7±0.40a	2.9±0.08b	1.55±0.282bc	56.7±4.69ab	5.9±1.06a	14.0±0.80bc	4.2±0.36a	1.1±0.15c	21.0±2.74bc	9.2±3.79bc	2.04±0.087cd	3.40±0.148a
			(18.0)	(4.73)			(195)	(69.3)	(1.3)				(11.55)
POW-19	78±2.5a	8.3±0.40a	3.5±0.08a	2.48±0.282ab	66.3±4.69b	7.0±1.06a	18.5±0.80a	4.0±0.36a	1.4±0.15bc	35.0±2.74a	22.1±3.79ab	2.79±0.087a	3.53±0.148a
			(33.9)	(11.95)			(341)	(55.9)	(1.9)				(12.45)
P	0.014	0.025	<0.001	0.043	0.109	0.312	<0.001	<0.001	0.049	<0.001	0.016	<0.001	<0.001
range	66-78	6.8-8.7	3.2-33.9	3.33-18.01	43.3-66.3	3.3-7.0	105-341	2.74-69.27	1.3-4.0	13.5-40.3	3.6-31.8	1.89-2.79	2.98-12.45
mean	71	7.8	15.7	7.13	57.7	5.4	181	23.4	2.5	25.8	11.9	2.28	7.06

PS, pollen stainability (%); FLO, florets per inflorescence; PL, peduncle length (mm); RDW, root dry weight (g); RD, root depth (cm); NRD, maximum nodal root diameter (mm); LA, leaf area (mm^2); MA, minimum anchoring (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); SDi, stolon diameter (mm); IL, internode length (mm), * indicates missing data.

Table A1.4: Mean morphological data of Family 4 F_1 hybrid genotypes (± SEM). Means that have been log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter, within columns, show no significant differences in variability at the 5% level.

Family	PS	FLO	PL	RDW	RD	log NRD	sqrt LA	log MS	SD	MS	FDW	Sdi	sqrt IL
POW-8	50.7±2.90c	12.0±0.49a	25.3±1.95a	8.62±4.910b	43.0±2.99b	1.5±0.05b	12.6±1.02a	3.0±0.46a	5.0±0.30a	27.8±4.83a	15.5±5.78a	1.47±0.046a	3.05±0.124a
						(4.4)	(158)	(19.9)					(9.29)
POW-10	69.7±2.90b	5.4±0.49c	18.2±1.95b	25.09±4.910ab	66.3±2.99a	1.6±0.05b	11.3±1.02a	2.0±0.46a	4.7±0.30a	35.3±4.83a	24.9±5.78a	1.38±0.046a	2.63±0.124b
						(5.1)	(129)	(7.0)					(6.92)
POW-11	68.3±2.90b	8.2±0.49b	14.7±1.95b	35.17±4.910a	71.0±2.99a	1.6±0.05b	13.1±1.02a	1.9±0.46a	3.7±0.30b	41.0±4.83a	23.0±5.78a	1.49±0.046a	2.41±0.124bc
						(4.8)	(171)	(6.7)					(5.81)
POW-14	76.9±2.90ab	8.2±0.49b	18.9±1.95b	17.95±4.910ab	62.3±2.99a	1.9±0.05a	13.4±1.02a	2.4±0.46a	3.5±0.30bc	37.0±4.83a	16.4±5.78a	1.46±0.046a	2.30±0.124bc
						(6.8)	(181)	(10.5)					(5.27)
POW-15	83.0±2.90a	7.4±0.49b	16.6±1.95b	8.26±4.910b	62.7±2.99a	1.9±0.05a	12.7±1.02a	2.5±0.46a	2.7±0.30c	39.3±4.83a	16.3±5.78a	1.40±0.046a	2.12±0.124c
						(6.6)	(160)	(11.7)					(4.48)
P	*<0.001*	*<0.001*	*0.007*	*0.065*	*<0.001*	*<0.001*	*0.661*	*0.484*	*0.003*	*0.407*	*0.697*	*0.36*	*<0.001*
range	50.7-83.0	5.4-12.0	14.7-25.3	8.26-35.17	43.0-71.0	4.4-6.8	129-181	6.7-19.9	2.7-5.0	27.8-41.0	15.5-24.9	1.38-1.49	4.48-9.29
mean	70	8.3	18.7	19.02	61.1	5.5	160	11.2	3.9	36.1	19.2	1.44	6.35

PS, pollen stainability (%); FLO, florets per inflorescence; PL, peduncle length (mm); RDW, root dry weight (g); RD, root depth (cm); NRD, maximum nodal root diameter (mm); LA, leaf area (mm^2); MA, minimum anchoring (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); SDi, stolon diameter (mm); IL, internode length (mm).

Table A1.5 **Mean morphological trait data for F_1 hybrid families and controls (± SEM). Means that have been log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter, within columns, show no significant differences in variability at the 5% level.**

Family	PS	sqrt FLO	log PL	sqrt RDW	RD	log NRD	log LA	log MA	SD	sqrt MS	sqrt FDW	log SDi	log IL
1	60 ± 2.0^{c}	2.7 ± 0.05^{d}	2.2 ± 0.05^{c}	2.49 ± 0.208^{cd}	56.2 ± 2.01^{b}	1.5 ± 0.05^{c}	4.9 ± 0.05^{c}	2.6 ± 0.18^{bc}	2.7 ± 0.21^{bc}	5.0 ± 0.21^{c}	3.1 ± 0.28^{c}	0.77 ± 0.021^{bc}	1.57 ± 0.050^{c}
		(7.18)	(9.3)	(6.21)		(4.3)	(138)	(13.3)		(25.3)	(9.3)	(2.15)	(4.82)
2	72 ± 3.0^{b}	3.1 ± 0.07^{b}	2.8 ± 0.08^{b}	3.06 ± 0.313^{c}	45.6 ± 3.02^{c}	1.6 ± 0.08^{bc}	4.7 ± 0.08^{d}	2.3 ± 0.27^{bc}	3.2 ± 0.32^{ab}	5.2 ± 0.32^{c}	3.5 ± 0.42^{bc}	0.75 ± 0.032^{c}	1.74 ± 0.076^{b}
		(9.73)	(15.9)	(9.34)		(4.8)	(108)	(10.4)		(26.7)	(12.1)	(2.11)	(5.67)
3	72 ± 2.0^{b}	2.8 ± 0.05^{c}	2.8 ± 0.05^{b}	2.68 ± 0.208^{cd}	57.7 ± 2.01^{b}	1.7 ± 0.05^{ab}	5.2 ± 0.05^{b}	2.8 ± 0.18^{b}	2.4 ± 0.21^{c}	5.0 ± 0.21^{c}	3.3 ± 0.3^{c}	0.82 ± 0.021^{b}	1.89 ± 0.050^{b}
		(7.92)	(16.2)	(7.20)		(5.4)	(182)	(16.8)		(25.1)	(11.2)	(2.27)	(6.61)
4	74 ± 3.0^{b}	2.8 ± 0.07^{cd}	2.9 ± 0.08^{b}	3.94 ± 0.313^{b}	60.4 ± 3.02^{b}	1.7 ± 0.08^{ab}	5.0 ± 0.08^{c}	2.4 ± 0.27^{bc}	3.9 ± 0.32^{a}	5.9 ± 0.32^{b}	4.2 ± 0.42^{b}	0.72 ± 0.032^{c}	1.77 ± 0.076^{b}
		(7.79)	(17.4)	(15.49)		(5.5)	(149)	(10.6)		(35.3)	(18.0)	(2.05)	(5.86)
Tr	*	9.2 ± 0.09^{a}	4.5 ± 0.10^{a}	6.41 ± 0.417^{a}	79.3 ± 4.02^{a}	1.8 ± 0.10^{a}	6.3 ± 0.10^{a}	1.9 ± 0.36^{c}	3.9 ± 0.42^{a}	7.5 ± 0.43^{a}	7.9 ± 0.55^{a}	1.14 ± 0.042^{a}	2.47 ± 0.101^{a}
		(84.71)	(90.9)	(41.06)		(6.0)	(522)	(6.6)		(56.0)	(62.2)	(3.13)	(11.87)
Tu	91 ± 4.0^{a}	1.5 ± 0.09^{e}	1.4 ± 0.10^{d}	1.77 ± 0.417^{d}	53.5 ± 4.02^{bc}	1.5 ± 0.10^{bc}	4.2 ± 0.10^{e}	4.3 ± 0.36^{a}	1.2 ± 0.42^{d}	4.05 ± 0.43^{d}	2.6 ± 0.55^{c}	0.74 ± 0.042^{bc}	1.18 ± 0.101^{d}
		(2.28)	(4.2)	(3.13)		(4.5)	(67)	(74.0)		(15.8)	(6.9)	(2.09)	(3.24)
P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.005*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); FLO, florets per inflorescence; PL, peduncle length (mm); RDW, root dry weight (g); RD, root depth (cm); NRD, maximum nodal root diameter (mm); LA, leaf area (mm^2); MA, minimum anchoring (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); SDi, stolon diameter (mm); IL, internode length (mm).

Appendix 2 Tahora BC$_1$ family trait means.

Table A2.1: Tahora BC$_1$ hybrid family morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	MS	log FDW	log NRD	log MA	log PL	sqrt FLO	log LA	SDi	IL
Tahora-21	89 ± 7.8^{a}	3.5 ± 0.31^{a}	38.7±4.13a	1.80 ± 0.243^{d} (6.0)	1.38 ± 0.104^{c} (4.0)	2.9 ± 0.31^{a} (18.3)	3.7 ± 0.10^{ab} (41.7)	6.7 ± 0.21^{a} (45)	3.91 ± 0.087^{d} (49.9)	1.84 ± 0.09^{e}	13.5 ± 0.81^{a}
Tahora-21/900-3	43 ± 6.4^{b}	2.0 ± 0.37^{b}	40.2±3.37a	3.11 ± 0.198^{ab} (19.4)	1.64 ± 0.085^{ab} (5.2)	2.4 ± 0.25^{a} (10.9)	3.6 ± 0.08^{bc} (37.5)	5.3 ± 0.17^{b} (28)	4.41 ± 0.071^{abc} (82.2)	2.56 ± 0.074^{bc}	12.0 ± 0.7^{a}
Tahora-21/900-4	47 ± 7.8^{b}	3.5 ± 0.37^{a}	34.8±4.13a	3.54 ± 0.243^{a} (20.3)	1.75 ± 0.104^{ab} (4.0)	2.5 ± 0.31^{a} (12.8)	3.3 ± 0.10^{d} (27.6)	4.2 ± 0.21^{c} (18)	4.47 ± 0.087^{abc} (87.7)	2.73 ± 0.09^{ab}	10.3 ± 0.81^{a}
Tahora-21/POW-10	53 ± 4.9^{b}	3.5 ± 0.24^{a}	42.3±2.61a	3.09 ± 0.153^{ab} (18.9)	1.83 ± 0.066^{a} (5.7)	2.3 ± 0.2^{a} (10.0)	3.5 ± 0.06^{bcd} (33.6)	5.2 ± 0.13^{b} (27)	4.50 ± 0.055^{ab} (89.7)	2.49 ± 0.057^{c}	12.4 ± 0.51^{a}
Tahora-21/POW-139	47 ± 4.9^{b}	3.4 ± 0.24^{a}	39.0±2.61a	3.01 ± 0.153^{abc} (12.6)	1.84 ± 0.066^{a} (6.1)	2.5 ± 0.2^{a} (12.0)	3.2 ± 0.06^{bcd} (33.6)	5.0 ± 0.13^{b} (25)	4.35 ± 0.055^{bc} (^{77}.6)	2.43 ± 0.057^{c}	12.5 ± 0.51^{a}
Tahora-21/POW-140	50 ± 4.9^{b}	3.0 ± 0.24^{a}	38.7±2.61a	2.94 ± 0.153^{bc} (18.9)	1.81 ± 0.066^{ab} (5.2)	2.2 ± 0.2^{a} (9.0)	3.6 ± 0.06^{bcd} (34.7)	5.0 ± 0.13^{b} (25)	4.41 ± 0.055^{abc} (82.0)	2.42 ± 0.057^{c}	11.7 ± 0.51^{a}
Tahora-21/POW-4	70 ± 6.4^{a}	2.8 ± 0.31^{ab}	45.1±3.37a	2.53 ± 0.198^{c} (12.6)	1.62 ± 0.085^{bc} (5.0)	2.2 ± 0.25^{a} (9.4)	3.8 ± 0.08^{a} (46.7)	6.5 ± 0.17^{a} (42)	4.31 ± 0.071^{c} (74.5)	2.15 ± 0.074^{d}	12.2 ± 0.66^{a}
Tahora-21/POW-8	47 ± 4.9^{b}	3.1 ± 0.24^{a}	33.4±2.61a	2.96 ± 0.153^{abc} (6.0)	1.80 ± 0.066^{ab} (5.0)	2.1 ± 0.2^{a} (8.0)	3.5 ± 0.06^{cd} (32.3)	5.3 ± 0.13^{b} (28)	4.56 ± 0.055^{a} (95.2)	2.77 ± 0.057^{a}	11.1 ± 0.51^{a}
P	*<0.001*	*0.012*	*0.171*	*<0.001*	*0.003*	*0.43*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.061*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Appendix 3 Trophy BC$_1$ family trait means.

Table A3.1: Trophy BC$_1$ hybrid family morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	MS	sqrt FDW	log NRD	log MA	sqrt PL	sqrt FLO	log LA	SDi	IL
Trophy R4-1	62 ± 6.1^{ab}	2.0 ± 0.27^{d}	36.3 ± 2.92^{a}	4.2 ± 0.43^{d} (17.6)	1.7 ± 0.09^{a} (5.43)	2.4 ± 0.21^{a} (11.2)	8.0 ± 0.27^{a} (64)	8.4 ± 0.15^{a} (70)	5.0 ± 0.08^{ab} (143)	2.81 ± 0.076^{a}	14.6 ± 0.78^{a}
Trophy R4-1/900-3	14 ± 4.4^{c}	3.1 ± 0.2^{bc}	33.2 ± 2.13^{a}	4.7 ± 0.32^{cd} (22.2)	1.9 ± 0.07^{a} (6.50)	2.3 ± 0.15^{a} (10.3)	6.1 ± 0.19^{bcd} (37)	4.8 ± 0.11^{d} (23)	4.9 ± 0.06^{abc} (136)	3.03 ± 0.055^{a}	11.0 ± 0.57^{de}
Trophy R4-1/900-4	7 ± 4.4^{c}	3.4 ± 0.2^{ab}	38.1 ± 2.13^{a}	5.3 ± 0.32^{abc} (28.3)	1.9 ± 0.07^{a} (6.83)	1.7 ± 0.15^{a} (5.5)	6.0 ± 0.19^{cde} (35)	4.4 ± 0.11^{e} (20)	4.7 ± 0.06^{cd} (115)	2.91 ± 0.055^{a}	13.0 ± 0.57^{abc}
Trophy R4-1/POW-10	64 ± 4.4^{ab}	3.7 ± 0.2^{a}	35.7 ± 2.13^{a}	5.8 ± 0.32^{a} (33.8)	1.9 ± 0.07^{a} (6.57)	2.2 ± 0.15^{a} (9.1)	5.7 ± 0.19^{def} (32)	4.9 ± 0.11^{d} (24)	4.8 ± 0.06^{bcd} (125)	3.07 ± 0.055^{a}	11.6 ± 0.57^{cd}
Trophy R4-1/POW-139	63 ± 4.4^{ab}	3.4 ± 0.2^{ab}	37.6 ± 2.13^{a}	5.7 ± 0.32^{ab} (32.1)	1.9 ± 0.07^{a} (6.40)	2.3 ± 0.15^{a} (9.5)	6.7 ± 0.19^{b} (44)	5.6 ± 0.11^{b} (32)	4.8 ± 0.06^{bcd} (119)	2.92 ± 0.055^{a}	13.3 ± 0.57^{ab}
Trophy R4-1/POW-140	67 ± 4.4^{a}	3.0 ± 0.2^{bc}	34.5 ± 2.13^{a}	4.9 ± 0.32^{abcd} (24.2)	1.9 ± 0.07^{a} (6.70)	2.2 ± 0.15^{a} (8.7)	6.3 ± 0.19^{bc} (39)	5.6 ± 0.11^{b} (32)	4.7 ± 0.06^{d} (113)	2.83 ± 0.055^{a}	12.0 ± 0.57^{bcd}
Trophy R4-1/POW-2	53 ± 4.4^{b}	3.2 ± 0.2^{ab}	31.1 ± 2.13^{a}	4.8 ± 0.32^{bcd} (22.8)	2 ± 0.07^{a} (7.29)	2.2 ± 0.15^{a} (9.4)	5.5 ± 0.19^{ef} (30)	5.5 ± 0.11^{b} (31)	5.0 ± 0.06^{a} (149)	3.01 ± 0.055^{a}	9.8 ± 0.57^{e}
Trophy R4-1/POW-4	67 ± 4.4^{a}	2.6 ± 0.2^{cd}	34.8 ± 2.13^{a}	4.6 ± 0.32^{cd} (20.9)	1.9 ± 0.07^{a} (6.44)	2.5 ± 0.15^{a} (12.0)	5.9 ± 0.19^{cdef} (35)	5.1 ± 0.11^{cd} (27)	4.8 ± 0.06^{bcd} (121)	2.93 ± 0.055^{a}	11.1 ± 0.57^{de}
Trophy R4-1/POW-8	60 ± 4.4^{ab}	3.1 ± 0.2^{abc}	32.3 ± 2.13^{a}	4.7 ± 0.32^{cd} (22.4)	1.8 ± 0.07^{a} (6.20)	2.3 ± 0.15^{a} (10.0)	5.4 ± 0.19^{f} (29)	5.4 ± 0.11^{bc} (29)	4.8 ± 0.06^{bcd} (118)	2.94 ± 0.055^{a}	11.4 ± 0.57^{cde}
P	*<0.001*	*<0.001*	*0.364*	*0.029*	*0.45*	*0.072*	*<0.001*	*<0.001*	*0.019*	*0.052*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Appendix 4 BC_1 hybrid and parent morphological trait means

Table A4.1: 900-3 BC_1 hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	sqrt MS	log FDW	log NRD	log MA	log PL	sqrt FLO	log LA	SDi	log IL
900-3	70 ± 10.6^{ab}	2.0 ± 0.37^{b}	3.6 ± 0.36^{b}	1.0 ± 0.31^{c}	1.33 ± 0.143^{c}	2.4 ± 0.37^{a}	2.3 ± 0.11^{c}	2.5 ± 0.26^{e}	4.2 ± 0.10^{c}	2.36 ± 0.108^{c}	0.91 ± 0.091^{d}
			(13.0)	(2.8)	(3.77)	(11.0)	(9.9)	(6.4)	(64.1)		(2.49)
Tahora-21	89 ± 8.2^{a}	2.0 ± 0.29^{b}	6.2 ± 0.27^{a}	1.8 ± 0.31^{b}	1.38 ± 0.109^{c}	2.9 ± 0.28^{a}	3.7 ± 0.08^{b}	6.7 ± 0.20^{b}	3.9 ± 0.08^{c}	1.84 ± 0.083^{d}	2.59 ± 0.070^{ab}
			(38.0)	(6.0)	(3.97)	(18.4)	(41.7)	(45.0)	(49.9)		(13.29)
Tahora-21/900-3	43 ± 8.2^{c}	3.5 ± 0.29^{a}	6.3 ± 0.27^{a}	3.1 ± 0.16^{a}	1.64 ± 0.109^{bc}	2.4 ± 0.28^{a}	3.6 ± 0.08^{b}	5.3 ± 0.20^{c}	4.4 ± 0.08^{b}	2.56 ± 0.083^{c}	2.42 ± 0.070^{bc}
			(39.6)	(22.4)	(5.15)	(10.9)	(37.5)	(28.1)	(82.2)		(11.21)
Trophy R4-1	62 ± 8.2^{bc}	2.0 ± 0.29^{b}	6.0 ± 0.27^{a}	2.8 ± 0.16^{a}	1.69 ± 0.109^{ab}	2.4 ± 0.28^{a}	4.1 ± 0.08^{a}	8.4 ± 0.20^{a}	5.0 ± 0.08^{a}	2.81 ± 0.083^{b}	2.65 ± 0.070^{a}
			(36.1)	(15.7)	(5.44)	(11.1)	(63.1)	(70.0)	(143.3)		(14.20)
Trophy R4-1/900-3	14 ± 5.7^{d}	3.1 ± 0.20^{a}	5.7 ± 0.19^{a}	3.1 ± 0.16^{a}	1.87 ± 0.076^{a}	2.3 ± 0.20^{a}	3.6 ± 0.06^{b}	4.9 ± 0.14^{d}	4.9 ± 0.05^{a}	3.04 ± 0.058^{a}	2.34 ± 0.049^{c}
			(32.7)	(21.5)	(6.50)	(10.3)	(36.5)	(23.5)	(135.8)		(10.38)
p	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.002*	*0.533*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.2: 900-4 BC_1 hybrid and parent morphological trait means(±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	MS	log FDW	log NRD	log MA	log PL	sqrt FLO	log LA	SDi	IL
900-4	37 ± 7.3^{c}	1.8 ± 0.38^{b}	19.8 ± 4.15^{b}	1.4 ± 0.30^{c}	1.55 ± 0.123^{bc}	1.8 ± 0.34^{bc}	2.2 ± 0.12^{d}	2.8 ± 0.19^{d}	3.8 ± 0.11^{d}	2.48 ± 0.109^{b}	5.85 ± 0.881^{d}
				(3.9)	(4.69)	(6.2)	(8.7)	(7.7)	(45.0)		
Tahora-21	89 ± 7.3^{a}	2.0 ± 0.38^{b}	38.7 ± 4.15^{a}	1.8 ± 0.30^{c}	1.38 ± 0.123^{c}	2.9 ± 0.34^{a}	3.7 ± 0.12^{b}	6.7 ± 0.19^{b}	3.9 ± 0.11^{d}	1.84 ± 0.109^{c}	13.54 ± 0.881^{ab}
				(6.0)	(3.97)	(18.3)	(41.7)	(45.0)	(49.9)		
Tahora-21/900-4	47 ± 7.3^{bc}	3.5 ± 0.38^{a}	34.8 ± 4.15^{a}	3.5 ± 0.30^{a}	1.75 ± 0.123^{ab}	2.5 ± 0.34^{ab}	3.3 ± 0.12^{c}	4.2 ± 0.19^{c}	4.5 ± 0.11^{c}	2.73 ± 0.109^{ab}	10.32 ± 0.881^{c}
				(34.4)	(5.74)	(12.8)	(27.6)	(18.0)	(87.7)		
Trophy R4-1	62 ± 7.3^{b}	2.0 ± 0.38^{b}	36.3 ± 4.15^{a}	2.8 ± 0.30^{b}	1.69 ± 0.123^{b}	2.4 ± 0.34^{ab}	4.1 ± 0.12^{a}	8.4 ± 0.19^{a}	5.0 ± 0.11^{a}	2.81 ± 0.109^{a}	14.60 ± 0.881^{a}
				(15.7)	(5.44)	(11.2)	(63.1)	(70.0)	(143.3)		
Trophy R4-1/900-4	7 ± 3.9^{d}	3.4 ± 0.20^{a}	38.1 ± 2.22^{a}	3.3 ± 0.16^{a}	1.92 ± 0.066^{a}	1.7 ± 0.18^{c}	3.5 ± 0.06^{c}	4.4 ± 0.10^{c}	4.7 ± 0.06^{b}	2.91 ± 0.058^{a}	12.98 ± 0.471^{b}
				(27.1)	(6.83)	(5.5)	(33.4)	(19.5)	(115.1)		
p	*<0.001*	*<0.001*	*0.007*	*<0.001*	*<0.001*	*0.003*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.3: POW-2 BC$_1$ hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	sqrt MS	log FDW	NRD	sqrt MA	log PL	FLO	log LA	SDi	IL
POW-2	19±10.5^{b}	1.5±0.40^{b}	3.0±0.32^{b} (8.7)	0.7±0.32^{b} (2.1)	3.90±0.543^{c}	4.1±0.36^{a} (16.4)	1.8±0.12^{c} (6.2)	5.9±2.57^{c}	3.9±0.10^{b} (49.9)	2.21±0.092^{c}	1.42±0.995^{c}
Trophy R4-1	62±8.2^{a}	2.0±0.30^{b}	6.0±0.25^{a} (36.1)	2.8±0.25^{a} (15.7)	5.55±0.422^{b}	3.4±0.28^{a} (11.6)	4.1±0.09^{a} (63.1)	70.4±2.00^{a}	5.0±0.08^{a} (143.3)	2.81±0.072^{b}	14.60±0.774^{a}
Trophy R4-1/POW-2	53±5.8^{a}	3.2±0.20^{a}	5.5±0.18^{a} (30.6)	3.1±0.18^{a} (21.8)	7.35±0.301^{a}	3.2±0.20^{a} (10.0)	3.4±0.07^{b} (28.6)	31.2±1.43^{b}	5.0±0.06^{a} (149.5)	3.01±0.051^{a}	9.83±0.552^{b}
p	*0.009*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.113*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.4: POW-4 BC$_1$ hybrid and parent morphological trait means (±SEM). Trait means that have been either log, square, or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	sq PS	SD	MS	log FDW	log NRD	log MA	sqrt PL	sqrt FLO	log LA	SDi	IL
POW-4	5534±1076.2^{ab}	2.8±0.34^{ab}	19.0±5.13^{c}	1.9±0.35^{bc}	2.05±0.099^{a}	2.5±0.38^{a}	4.0±0.34^{d}	2.7±0.26^{d}	4.8±0.11^{a}	2.78±0.116^{a}	5.18±1.012^{d}
	(74)			(6.6)	(7.74)	(12.1)	(16.0)	(7.3)	(126)		
Tahora-21	7895±825.8^{a}	2.0±0.34^{bd}	38.7±5.13^{ab}	1.8±0.35^{c}	1.38±0.099^{d}	2.9±0.38^{a}	6.6±0.34^{b}	6.7±0.26^{b}	3.9±0.11^{c}	1.84±0.116^{c}	13.54±1.012^{ab}
	(88)			(6.0)	(3.97)	(18.4)	(43.0)	(45.0)	(50)		
Tahora-21/POW-4	5475±825.8^{b}	2.8±0.26^{a}	45.1±3.94^{a}	2.5±0.27^{ab}	1.62±0.076^{c}	2.2±0.29^{a}	7.0±0.26^{b}	6.5±0.20^{b}	4.3±0.08^{b}	2.15±0.089^{b}	12.17±0.777^{bc}
	(74)			(12.6)	(5.03)	(9.4)	(48.9)	(42.4)	(75)		
Trophy R4-1	4103±825.8^{b}	2.0±0.34^{bd}	36.3±5.13^{ab}	2.8±0.35^{a}	1.69±0.099^{bc}	2.4±0.38^{a}	8.0±0.34^{a}	8.4±0.26^{a}	5.0±0.11^{a}	2.81±0.116^{a}	14.60±1.012^{a}
	(64)			(15.7)	(5.44)	(11.1)	(64.2)	(70.0)	(143)		
Trophy R4-1/POW-4	4671±575.3^{b}	2.6±0.18^{abc}	34.8±2.74^{b}	2.9±0.19^{a}	1.86±0.053^{ab}	2.5±0.20^{a}	5.9±0.18^{c}	5.1±0.14^{c}	4.8±0.06^{a}	2.93±0.062^{a}	11.10±0.541^{c}
	(68)			(19.1)	(6.44)	(12.0)	(34.9)	(26.5)	(121)		
p	*0.016*	*0.041*	*0.003*	*0.006*	*<0.001*	*0.521*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm^2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.5: POW-8 BC$_1$ hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	log SD	MS	log FDW	log NRD	log MA	sqrt PL	FLO	sqrt LA	SDi	log IL
POW-8	42±7.3^{c}	0.8±0.16^{ab}	18.8±3.99^{b}	1.9±0.27^{b}	1.54±0.111^{bc}	2.0±0.36^{a}	3.5±0.33^{d}	10.8±2.75^{d}	9.5±0.57^{c}	3.04±0.097^{a}	1.43±0.092^{c}
		(2.2)		(6.5)	(4.66)	(7.4)	(12.6)		(90.5)		(4.17)
Tahora-21	89±5.6^{a}	0.6±0.12^{b}	38.7±3.02^{a}	1.8±0.21^{b}	1.38±0.084^{c}	2.9±0.28^{a}	6.6±0.25^{b}	45.6±2.09^{b}	7.2±0.43^{d}	1.84±0.735^{c}	2.59±0.070^{a}
		(1.8)		(6.0)	(3.97)	(18.4)	(43.0)		(51.3)		(13.29)
Tahora-21/POW-8	47±3.8^{c}	1.1±0.08^{a}	33.4±2.06^{a}	3.0±0.14^{a}	1.80±0.057^{a}	2.1±0.19^{a}	5.8±0.17^{c}	29.0±1.42^{c}	10.0±0.29^{c}	2.77±0.050^{b}	2.37±0.048^{b}
		(2.9)		(19.4)	(6.06)	(8.0)	(33.7)		(100.4)		(10.72)
Trophy R4-1	62±5.6^{b}	0.7±0.12^{b}	36.3±3.02^{a}	2.8±0.21^{a}	1.69±0.084^{ab}	2.4±0.28^{a}	8.0±0.25^{a}	70.4±2.09^{a}	12.1±0.43^{a}	2.81±0.735^{ab}	2.65±0.070^{a}
		(1.9)		(15.7)	(5.44)	(11.1)	(64.2)		(146.7)		(14.20)
Trophy R4-1/POW-8	60±3.8^{b}	1.1±0.08^{a}	32.3±2.06^{a}	3.1±0.14^{a}	1.82±0.057^{a}	2.3±0.19^{a}	5.4±0.17^{c}	29.5±1.42^{c}	11.1±0.29^{b}	2.94±0.050^{a}	2.37±0.048^{b}
		(3.0)		(21.5)	(6.20)	(10.0)	(29.1)		(122.3)		(10.65)
p	*<0.001*	*0.001*	*0.004*	*<0.001*	*<0.001*	*0.153*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.6: POW-10 BC$_1$ hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	sqrt MS	log FDW	log NRD	log MA	log PL	sqrt FLO	log LA	sqrt SDi	sqrt IL
POW-10	52±6.8^{bc}	2.0±0.43^{b}	3.8±0.38^{b}	1.7±0.31^{c}	1.41±0.107^{b}	2.7±0.32^{a}	2.5±0.11^{d}	2.6±0.19^{e}	4.2±0.10^{c}	1.59±0.034^{c}	2.23±0.138^{d}
			(14.7)	(5.5)	(4.11)	(14.2)	(11.6)	(7.0)	(66.9)	(2.52)	(4.96)
Tahora-21	89±6.8^{a}	2.0±0.43^{b}	6.2±0.38^{a}	1.8±0.31^{c}	1.38±0.107^{b}	2.9±0.32^{a}	3.7±0.11^{b}	6.7±0.19^{b}	3.9±0.10^{d}	1.35±0.034^{d}	3.66±0.138^{ab}
			(38.0)	(6.0)	(3.97)	(18.3)	(41.7)	(45.0)	(49.9)	(1.82)	(13.42)
Tahora-21/POW-10	53±3.5^{c}	3.5±0.22^{a}	6.5±0.19^{a}	3.1±0.16^{ab}	1.83±0.055^{a}	2.3±0.16^{a}	3.5±0.06^{c}	5.2±0.10^{c}	4.5±0.05^{b}	1.57±0.017^{c}	3.49±0.071^{bc}
			(41.8)	(22.0)	(6.22)	(10.0)	(33.6)	(27.2)	(89.7)	(2.48)	(12.20)
Trophy R4-1	62±6.8^{bc}	2.0±0.43^{b}	6.0±0.38^{a}	2.8±0.31^{b}	1.69±0.107^{a}	2.4±0.32^{a}	4.1±0.11^{a}	8.4±0.19^{a}	5.0±0.10^{a}	1.67±0.034^{b}	3.80±0.138^{a}
			(36.1)	(15.7)	(5.44)	(11.2)	(63.1)	(70.0)	(143.3)	(2.80)	(14.41)
Trophy R4-1/POW-10	64±3.5^{b}	3.7±0.22^{a}	5.9±0.19^{a}	3.4±0.16^{a}	1.88±0.055^{a}	2.2±0.16^{a}	3.4±0.06^{c}	4.9±0.1^{d}	4.8±0.05^{a}	1.75±0.017^{a}	3.36±0.071^{c}
			(35.0)	(31.1)	(6.57)	(9.1)	(30.9)	(23.8)	(124.6)	(3.06)	(11.31)
p	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.175*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.7: POW-139 BC$_1$ hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	sqrt MS	log FDW	log NRD	log MA	sqrt PL	log FLO	log LA	SDi	IL
Tahora-21	88±5.3^{a}	2.8±0.44^{ab}	4.6±0.32^{b} (20.9)	1.9±0.30^{c} (6.6)	1.74±0.115^{a} (5.68)	3.0±0.32^{a} (20.2)	4.2±0.39^{d} (17.4)	2.6±0.07^{e} (13.8)	4.4±0.09^{c} (81.6)	2.61±0.108^{bc}	6.21±0.827^{c}
POW-139	64±6.9^{b}	2.0±0.33^{b}	6.2±0.32^{a} (38.0)	1.8±0.30^{c} (6.0)	1.38±0.115^{b} (3.97)	2.9±0.32^{a} (18.3)	6.6±0.30^{bc} (43.0)	3.8±0.05^{b} (44.4)	3.9±0.07^{d} (49.9)	1.84±0.083^{d}	13.54±0.635^{ab}
Tahora-21/POW-139	47±3.68^{c}	3.4±0.23^{a}	6.2±0.17^{a} (38.5)	3.0±0.16^{ab} (20.3)	1.84±0.062^{a} (6.32)	2.5±0.17^{a} (12.0)	5.9±0.21^{c} (34.8)	3.2±0.04^{d} (24.9)	4.4±0.05^{c} (77.6)	2.43±0.058^{c}	12.51±0.442^{b}
Trophy R4-1	62±5.3b	2.0±0.33^{b}	6.0±0.32^{a} (36.1)	2.86±0.30^{b} (15.7)	1.69±0.115^{a} (5.44)	2.4±0.32^{a} (11.2)	8.0±0.30^{a} (64.2)	4.2±0.05^{a} (69.5)	5.0±0.07^{a} (143.3)	2.81±0.083^{ab}	14.60±0.635^{a}
Trophy R4-1/POW-139	63±3.7b	3.4±0.23^{a}	6.1±0.17^{a} (37.3)	3.4±0.16^{a} (30.8)	1.86±0.062^{a} (6.40)	2.3±0.17^{a} (9.5)	6.7±0.21^{b} (44.2)	3.4±0.04^{c} (31.2)	4.8±0.05^{b} (118.6)	2.92±0.058^{a}	13.32±0.442^{ab}
p	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*	*0.123*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm2); SDi, stolon diameter (mm); IL, internode length (mm).

Table A4.8: POW-140 BC_1 hybrid and parent morphological trait means (±SEM). Trait means that have been either log or square-root transformed to meet the assumptions of ANOVA are noted in the headings. Back-transformed means are presented in parentheses. Entries with the same letter within columns show no significant differences in means at the 5% level.

Family	PS	SD	MS	log FDW	log NRD	log MA	sqrt PL	log FLO	log LA	SDi	sqrt IL
POW-140	86 ± 8.1^{ab}	2.0 ± 0.35^{b}	15.3 ± 3.92^{b}	1.4 ± 0.31^{b} (4.1)	1.39 ± 0.120^{b} (4.02)	2.2 ± 0.36^{a} (8.7)	4.0 ± 0.34^{c} (16.2)	2.5 ± 0.08^{e} (11.7)	4.3 ± 0.11^{c} (73.8)	2.62 ± 0.098^{ab}	2.09 ± 0.147^{d} (4.35)
Tahora-21	89 ± 8.1^{a}	2.0 ± 0.35^{b}	38.7 ± 3.92^{a}	1.8 ± 0.31^{b} (6.0)	1.38 ± 0.120^{b} (3.97)	2.9 ± 0.36^{a} (18.4)	6.6 ± 0.26^{b} (43.0)	3.8 ± 0.06^{b} (44.4)	3.9 ± 0.08^{d} (49.9)	1.84 ± 0.075^{c}	3.66 ± 0.113^{ab} (13.42)
Tahora-21/POW-140	50 ± 4.4^{d}	3.0 ± 0.19^{a}	38.7 ± 2.10^{a}	2.9 ± 0.17^{a} (18.9)	1.81 ± 0.064^{a} (6.10)	2.2 ± 0.19^{a} (9.0)	6.0 ± 0.18^{b} (36.1)	3.2 ± 0.04^{d} (24.3)	4.4 ± 0.06^{c} (82.0)	2.42 ± 0.052^{b}	3.37 ± 0.078^{c} (11.38)
Trophy R4-1	62 ± 8.1^{cd}	2.0 ± 0.35^{b}	36.3 ± 3.92^{a}	2.8 ± 0.31^{a} (15.7)	1.69 ± 0.120^{a} (5.44)	2.4 ± 0.36^{a} (11.1)	8.0 ± 0.26^{a} (64.2)	4.2 ± 0.06^{a} (69.5)	5.0 ± 0.08^{a} (143.3)	2.81 ± 0.075^{a}	3.80 ± 0.113^{a} (14.41)
Trophy R4-1/POW-140	67 ± 4.4^{bc}	3.0 ± 0.19^{a}	34.5 ± 2.10^{a}	3.1 ± 0.17^{a} (22.1)	1.90 ± 0.064^{a} (6.70)	2.2 ± 0.19^{a} (8.8)	6.3 ± 0.18^{b} (39.2)	3.4 ± 0.04^{c} (30.8)	4.7 ± 0.06^{b} (113.3)	2.83 ± 0.052^{a}	3.42 ± 0.078^{bc} (11.71)
P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.211*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

PS, pollen stainability (%); SD, stolon density; MS, maximum plant spread (cm); FDW, foliar and stolon dry weight (g); NRD, maximum nodal root diameter (mm); MA, minimum anchoring (%); PL, peduncle length (mm); FLO, florets per inflorescence; LA, leaf area (mm2); SDi, stolon diameter (mm); IL, internode length (mm).

Appendix 5 BC$_1$ experiment mean data matrix for PCA and cluster analysis

Table A5.1: BC$_1$ mean data matrix for PCA and cluster analysis of all trial entries generated from means calculated using ANOVA analysis. PCA number and PCA group were generated from the analyses.

Family	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL	PCA No.	PCA Group
900-3	70.00	2.00	13.00	2.84	3.79	11.81	9.94	6.35	64.14	2.35	2.49	G1	1
900-4	36.50	1.75	19.75	3.87	4.69	6.25	8.71	7.70	45.02	2.46	5.63	G2	1
POW-10	51.75	2.00	14.75	5.50	4.13	14.49	11.61	6.98	66.89	2.52	4.85	G3	1
POW-139	63.50	2.75	21.00	6.79	5.74	20.65	16.46	13.94	81.61	2.60	5.91	G4	1
POW-140	86.00	2.00	15.25	4.25	4.04	9.09	14.86	11.83	73.85	2.61	4.23	G5	1
POW-2	18.67	1.50	9.00	2.47	3.83	16.57	6.20	5.86	49.90	2.21	1.35	G6	1
POW-4	74.00	2.75	19.00	7.36	7.77	13.85	15.89	7.35	126.34	2.76	4.87	G7	1
POW-8	42.33	2.25	18.75	6.59	4.68	7.77	12.32	10.60	88.50	3.03	4.17	G8	1
Tahora-21	88.57	3.55	38.71	6.03	3.97	18.34	41.68	44.98	49.90	1.84	13.54	G9	2
Tahora-21/900-3	42.82	2.00	40.18	19.36	5.15	10.88	37.52	28.10	82.19	2.56	12.00	G10	2
Tahora-21/900-4	46.83	3.50	34.83	20.27	3.97	12.77	27.58	18.04	87.71	2.73	10.32	G11	2
Tahora-21/POW-10	53.20	3.53	42.33	18.86	5.74	10.03	33.65	27.23	89.66	2.49	12.44	G12	2
Tahora-21/POW-139	47.31	3.39	39.00	12.60	6.06	11.99	33.58	25.38	77.56	2.43	12.51	G13	2
Tahora-21/POW-140	49.77	3.00	38.69	18.86	5.15	9.03	34.67	24.75	82.02	2.42	11.71	G14	2
Tahora-21/POW-4	70.40	2.80	45.10	12.60	5.03	9.39	46.67	42.42	74.51	2.15	12.17	G15	2
Tahora-21/POW-8	46.71	3.07	33.43	6.03	5.03	8.03	32.30	28.40	95.20	2.77	11.06	G16	2
Trophy R4-1	62.37	2.00	36.25	17.59	7.29	12.00	64.22	69.96	143.31	2.81	14.60	G17	3
Trophy R4-1/900-3	14.15	3.07	33.21	22.18	6.83	11.16	37.48	23.49	135.78	3.03	11.03	G18	3
Trophy R4-1/900-4	6.84	3.36	38.14	28.33	6.70	10.28	35.50	19.54	115.12	2.91	12.98	G19	3
Trophy R4-1/POW-10	64.00	3.69	35.69	33.78	6.57	9.96	32.06	23.79	124.59	3.07	11.62	G20	3
Trophy R4-1/POW-139	63.00	3.36	37.57	32.11	6.50	9.54	44.25	31.69	118.63	2.92	13.32	G21	3
Trophy R4-1/POW-140	67.43	3.00	34.50	24.22	6.44	9.39	39.19	31.56	113.30	2.83	12.02	G22	3
Trophy R4-1/POW-2	53.25	3.23	31.08	22.77	6.40	9.12	30.46	30.67	149.46	3.01	9.83	G23	3
Trophy R4-1/POW-4	66.77	2.64	34.79	20.88	6.20	8.73	34.93	26.51	121.39	2.93	11.10	G24	3
Trophy R4-1/POW-8	60.16	3.13	32.27	22.37	5.44	5.48	29.13	28.72	118.16	2.94	11.44	G25	3

Table A5.2: Mean data matrix for PCA and cluster analysis of Tahora BC_1 entries generated from means calculated using ANOVA analysis. PCA number and PCA group were generated from the analyses

Family	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL	PCA No	PCA Group
900-3	70.00	2.00	13.00	2.84	3.79	11.81	9.94	6.35	64.14	2.35	2.49	G1	3
900-4	36.50	1.75	19.75	3.87	4.69	6.25	8.71	7.70	45.02	2.46	5.63	G2	3
POW-10	51.75	2.00	14.75	5.50	4.13	14.49	11.61	6.98	66.89	2.52	4.85	G3	3
POW-139	63.50	2.75	21.00	6.79	5.74	20.65	16.46	13.94	81.61	2.60	5.91	G4	2
POW-140	86.00	2.00	15.25	4.25	4.04	9.09	14.86	11.83	73.85	2.61	4.23	G5	3
POW-2	18.67	1.50	9.00	2.47	3.83	16.57	6.20	5.86	49.90	2.21	1.35	G6	3
POW-4	74.00	2.75	19.00	7.36	7.77	13.85	15.89	7.35	126.34	2.76	4.87	G7	2
POW-8	42.33	2.25	18.75	6.59	4.68	7.77	12.32	10.60	88.50	3.03	4.17	G8	3
Tahora-21	88.57	3.55	38.71	6.03	3.97	18.34	41.68	44.98	49.90	1.84	13.54	G9	1
Tahora-21/900-3	42.82	2.00	40.18	19.36	5.15	10.88	37.52	28.10	82.19	2.56	12.00	G10	1
Tahora-21/900-4	46.83	3.50	34.83	20.27	3.97	12.77	27.58	18.04	87.71	2.73	10.32	G11	1
Tahora-21/POW-10	53.20	3.53	42.33	18.86	5.74	10.03	33.65	27.23	89.66	2.49	12.44	G12	1
Tahora-21/POW-139	47.31	3.39	39.00	12.60	6.06	11.99	33.58	25.38	77.56	2.43	12.51	G13	1
Tahora-21/POW-140	49.77	3.00	38.69	18.86	5.15	9.03	34.67	24.75	82.02	2.42	11.71	G14	1
Tahora-21/POW-4	70.40	2.80	45.10	12.60	5.03	9.39	46.67	42.42	74.51	2.15	12.17	G15	1
Tahora-21/POW-8	46.71	3.07	33.43	6.03	5.03	8.03	32.30	28.40	95.20	2.77	11.06	G16	1

Table A5.3: Mean data matrix for PCA and cluster analysis of all Trophy BC$_1$ entries generated from means calculated using ANOVA analysis. PCA number and PCA group were generated from the analyses

Family	PS	SD	MS	FDW	NRD	MA	PL	FLO	LA	SDi	IL	PCA No.	PCA Group
900-3	70.00	2.00	13.00	2.84	3.79	11.81	9.94	6.35	64.14	2.35	2.49	G1	3
900-4	36.50	1.75	19.75	3.87	4.69	6.25	8.71	7.70	45.02	2.46	5.63	G2	3
POW-10	51.75	2.00	14.75	5.50	4.13	14.49	11.61	6.98	66.89	2.52	4.85	G3	3
POW-139	63.50	2.75	21.00	6.79	5.74	20.65	16.46	13.94	81.61	2.60	5.91	G4	2
POW-140	86.00	2.00	15.25	4.25	4.04	9.09	14.86	11.83	73.85	2.61	4.23	G5	3
POW-2	18.67	1.50	9.00	2.47	3.83	16.57	6.20	5.86	49.90	2.21	1.35	G6	3
POW-4	74.00	2.75	19.00	7.36	7.77	13.85	15.89	7.35	126.34	2.76	4.87	G7	2
POW-8	42.33	2.25	18.75	6.59	4.68	7.77	12.32	10.60	88.50	3.03	4.17	G8	3
Trophy R4-1	62.37	2.00	36.25	17.59	7.29	12.00	64.22	69.96	143.31	2.81	14.60	G9	1
Trophy R4-1/900-3	14.15	3.07	33.21	22.18	6.83	11.16	37.48	23.49	135.78	3.03	11.03	G10	1
Trophy R4-1/900-4	6.84	3.36	38.14	28.33	6.70	10.28	35.50	19.54	115.12	2.91	12.98	G11	1
Trophy R4-1/POW-10	64.00	3.69	35.69	33.78	6.57	9.96	32.06	23.79	124.59	3.07	11.62	G12	1
Trophy R4-1/POW-139	63.00	3.36	37.57	32.11	6.50	9.54	44.25	31.69	118.63	2.92	13.32	G13	1
Trophy R4-1/POW-140	67.43	3.00	34.50	24.22	6.44	9.39	39.19	31.56	113.30	2.83	12.02	G14	1
Trophy R4-1/POW-2	53.25	3.23	31.08	22.77	6.40	9.12	30.46	30.67	149.46	3.01	9.83	G15	1
Trophy R4-1/POW-4	66.77	2.64	34.79	20.88	6.20	8.73	34.93	26.51	121.39	2.93	11.10	G16	1
Trophy R4-1/POW-8	60.16	3.13	32.27	22.37	5.44	5.48	29.13	28.72	118.16	2.94	11.44	G17	1

Appendix 6 Correlations

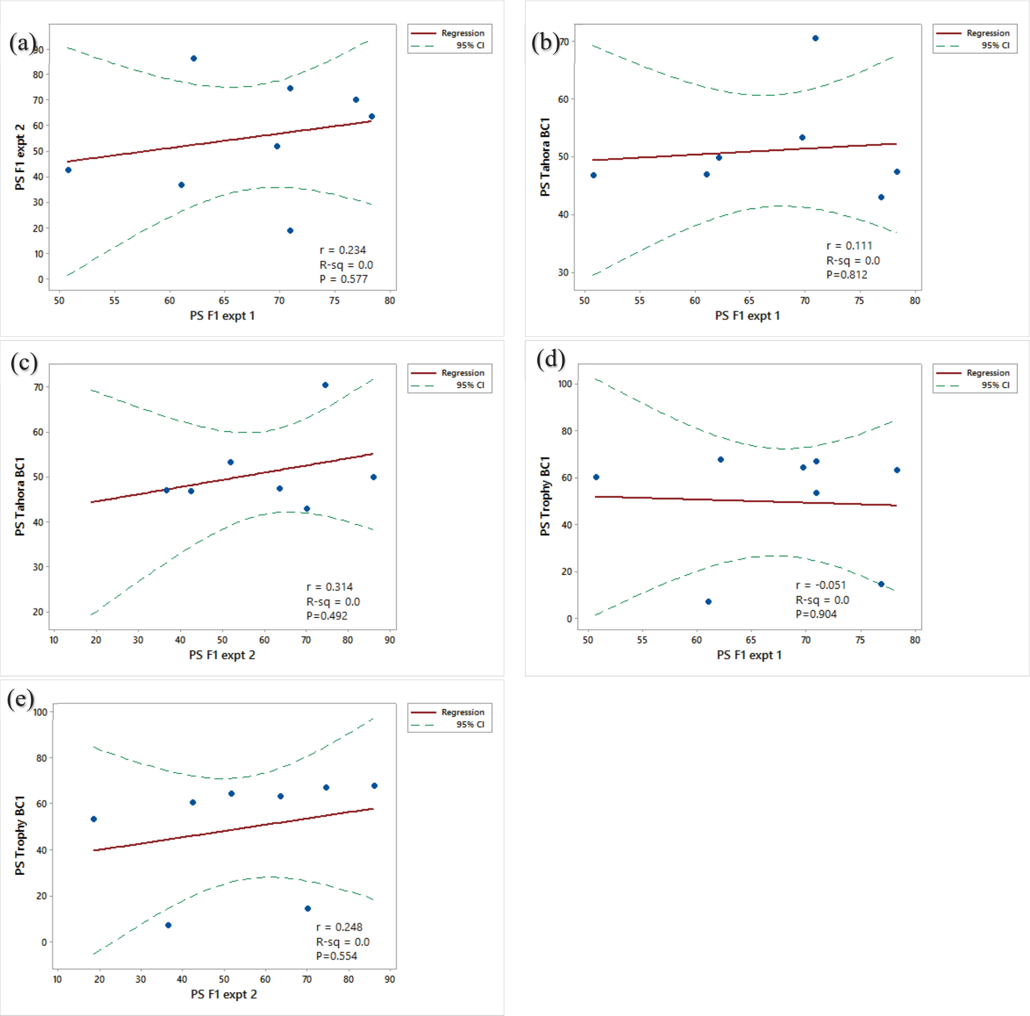

Figure A6.1: Correlations of mean Pollen Stain (%) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

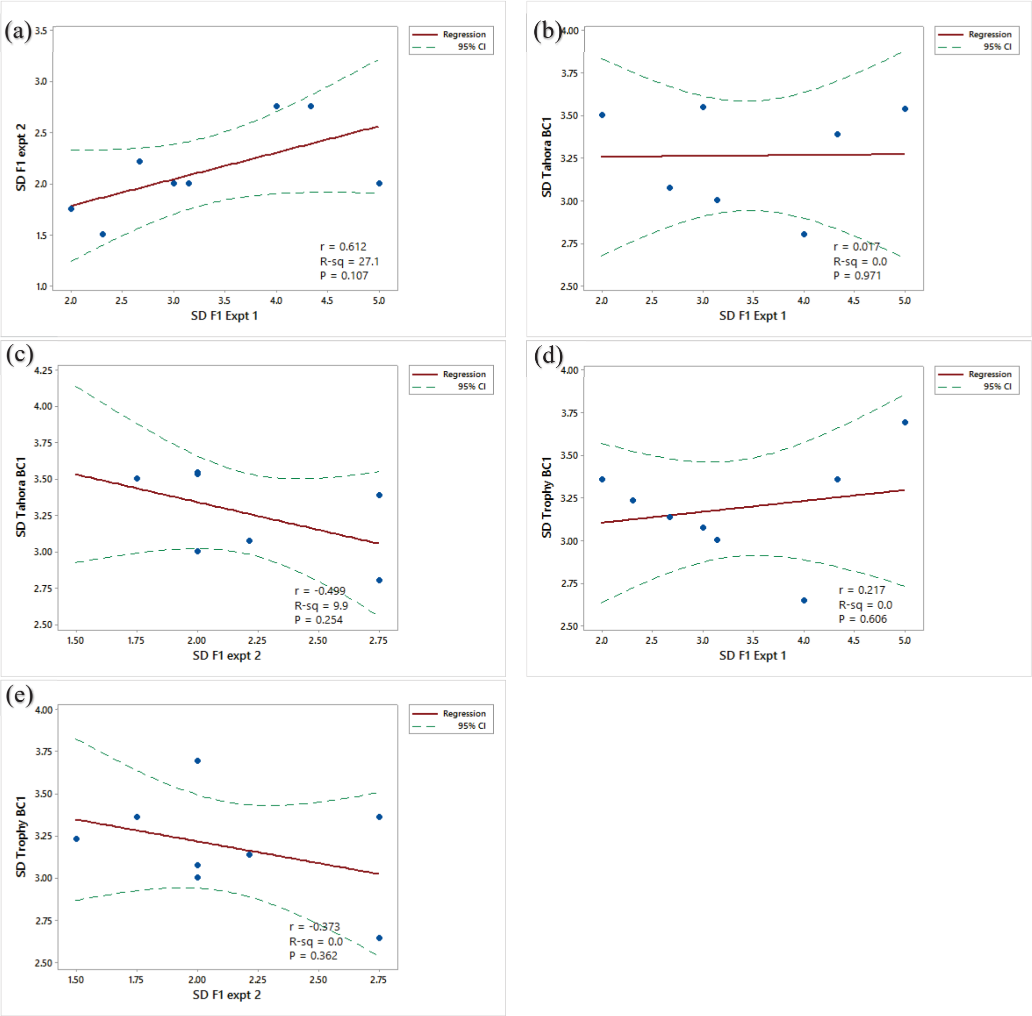

Figure A6.2: Correlations of mean Stolon Density between (a) F$_1$ hybrids in experiment 2 and F$_1$ hybrids in experiment 1, (b) Tahora BC$_1$ families and F$_1$ hybrid parents in experiment 1, (c) Tahora BC$_1$ families and F$_1$ hybrid parents in experiment 2, (d) Trophy BC$_1$ families and F$_1$ hybrid parents in experiment 1, (e) Trophy BC$_1$ families and F$_1$ hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

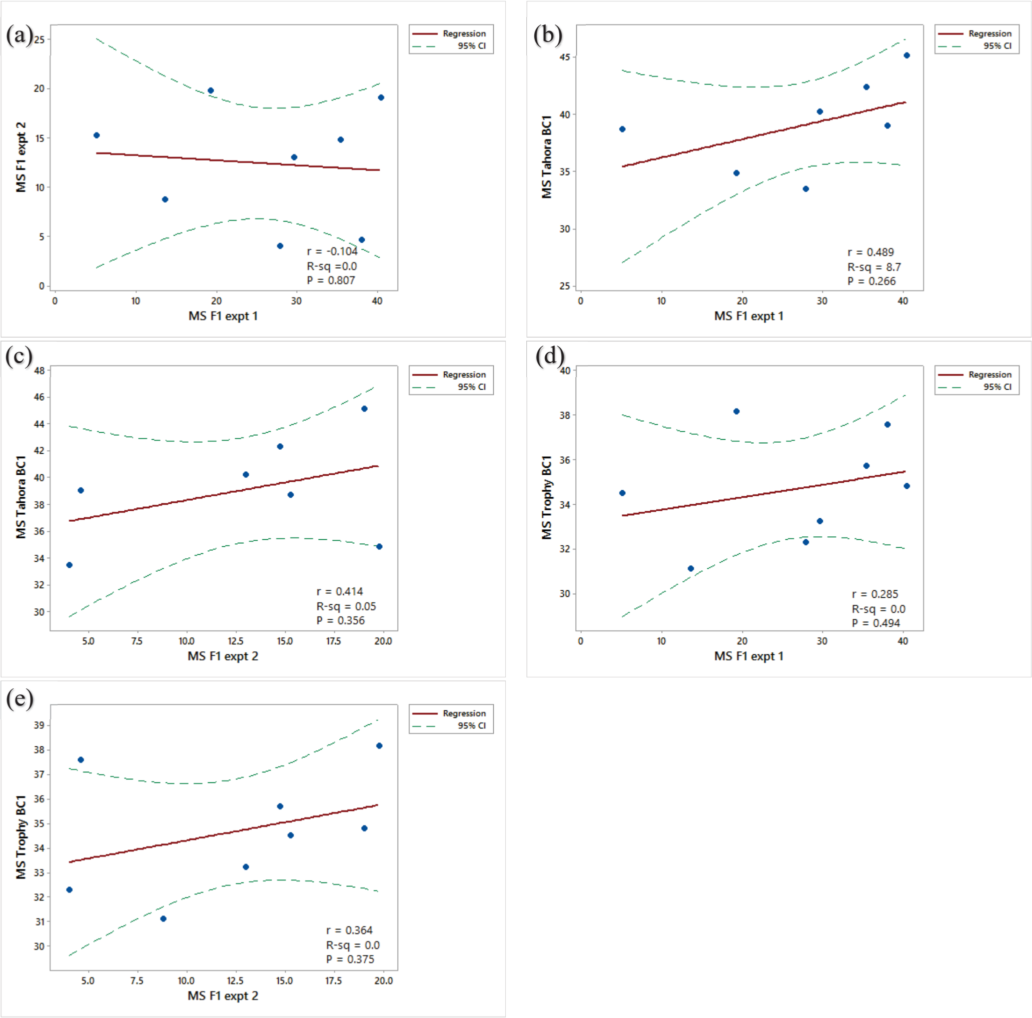

Figure A6.3: Correlations of mean Maximum Plant Spread (cm) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

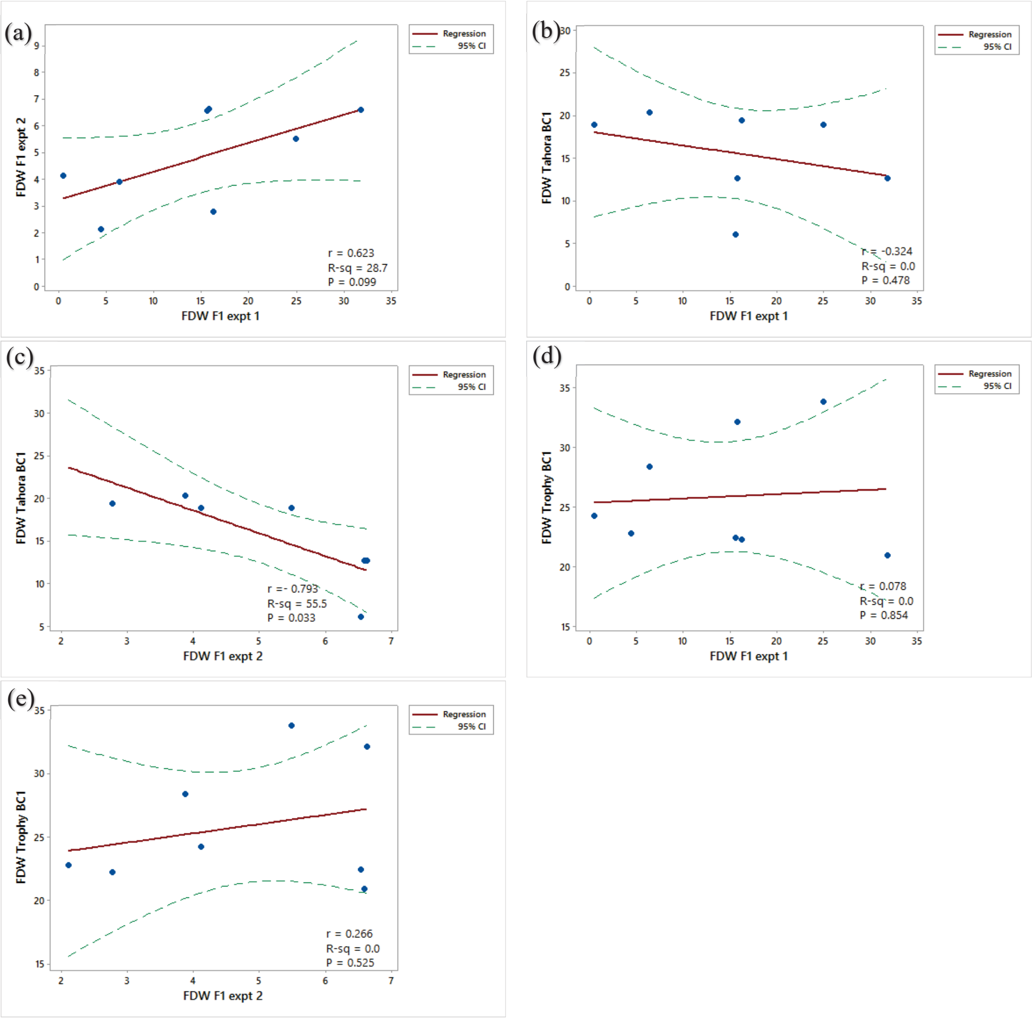

Figure A6.4: Correlations of mean Foliar Dry Weight (g) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

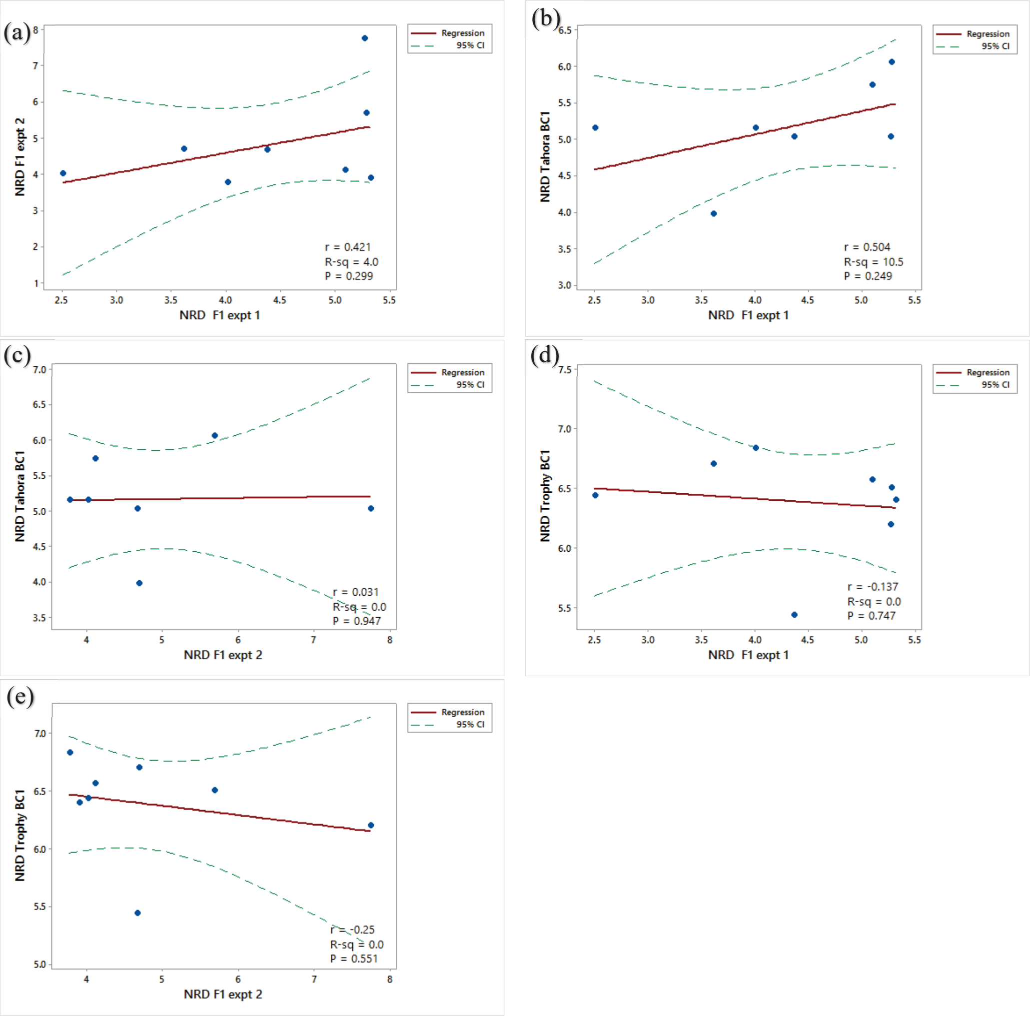

Figure A6.5: Correlations of mean Maximum Nodal Root Diameter (mm) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F1 hybrid parents in experiment 1, (e) Trophy BC1 families and F1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

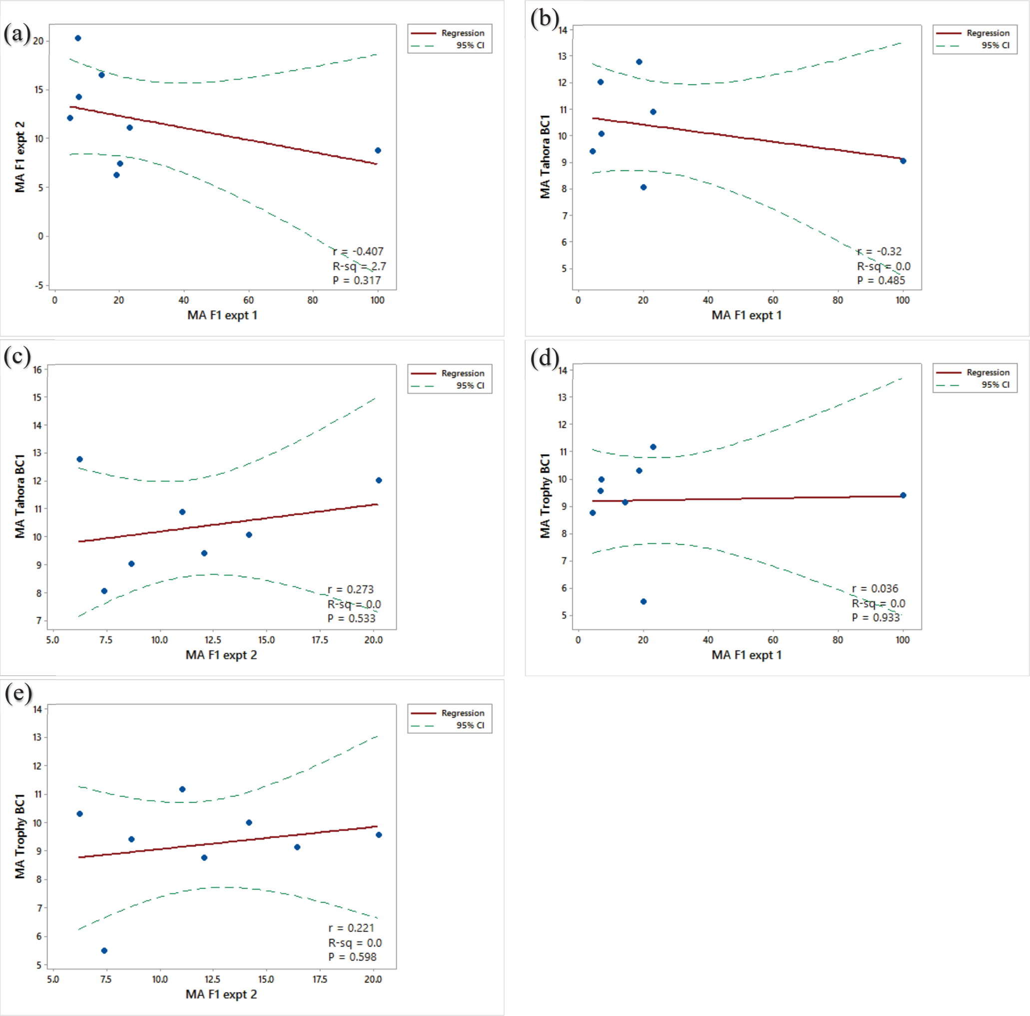

Figure A6.6: Correlations of mean Minimum Anchoring (%) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

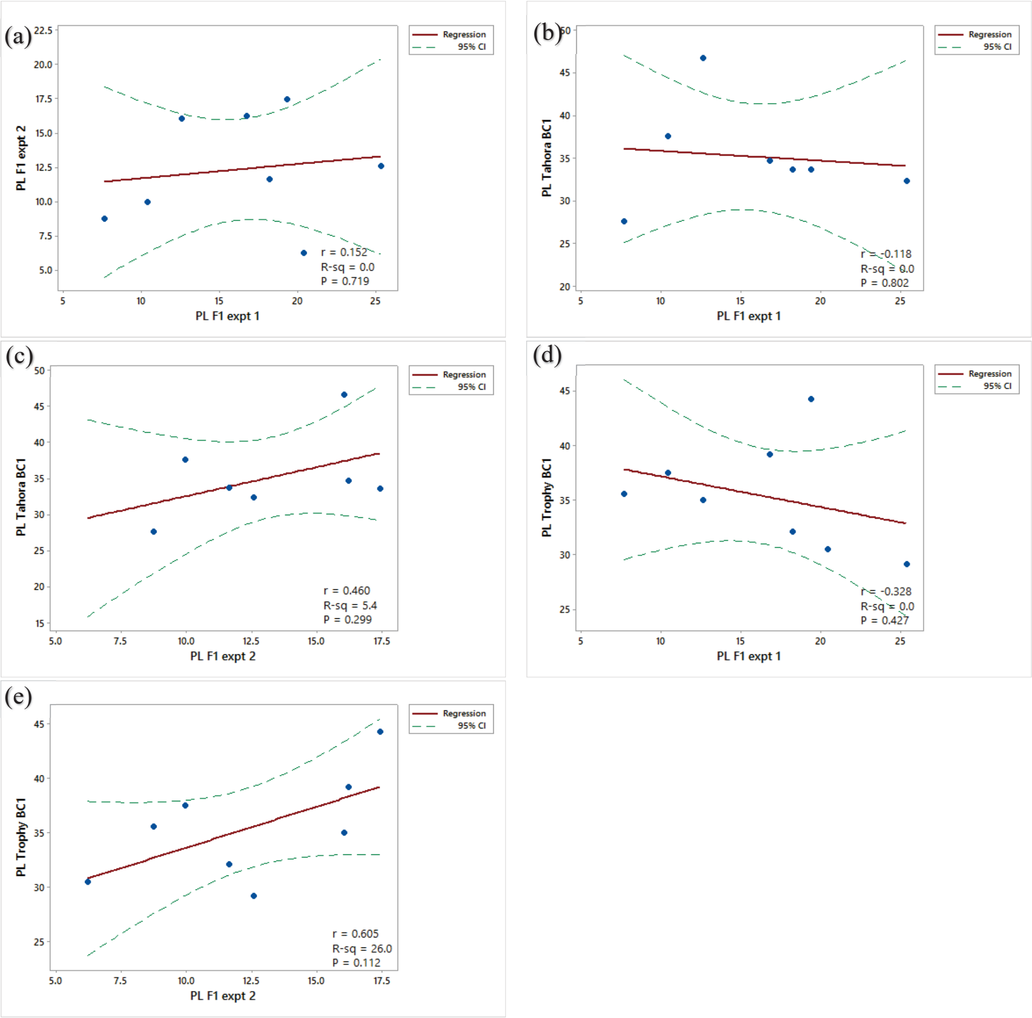

Figure A6.7: Correlations of mean Peduncle Length.(mm) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph

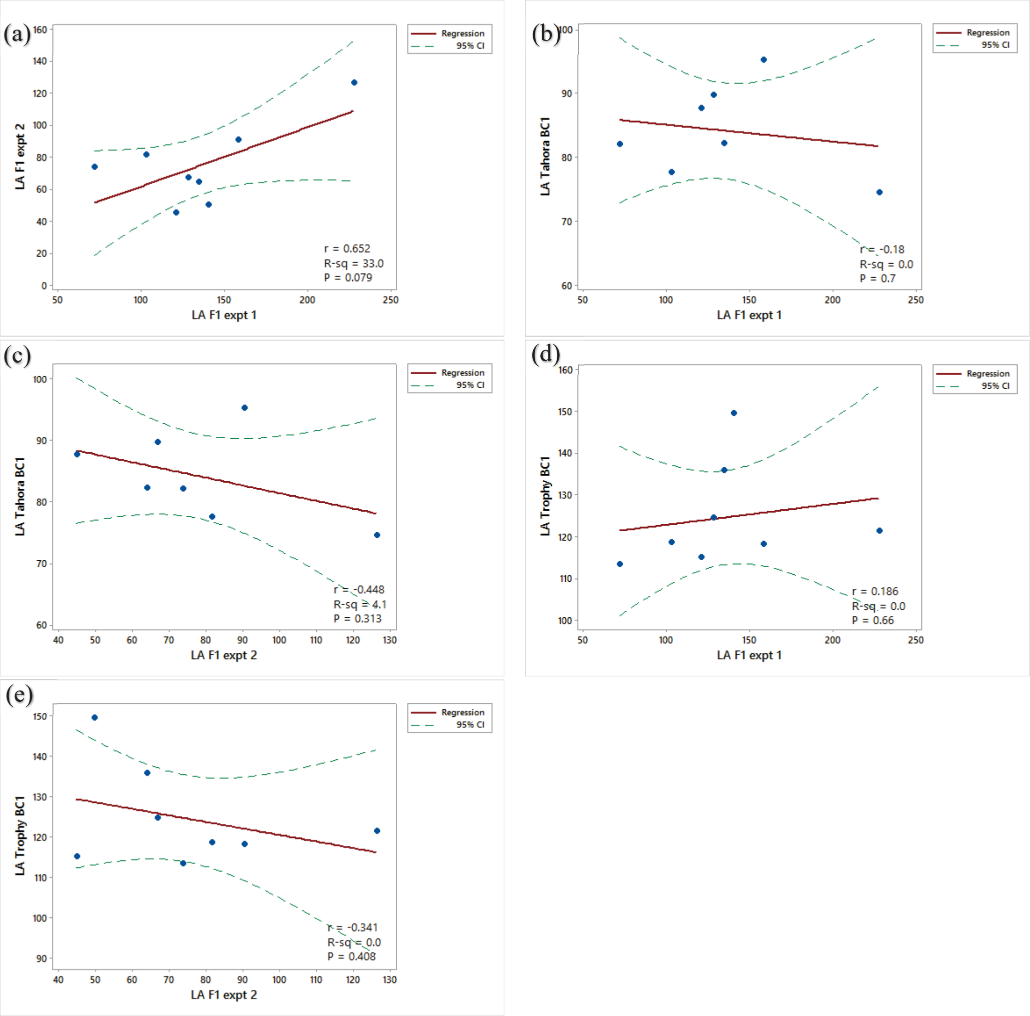

Figure A6.8: Correlations of mean Leaf Area (mm^2) between (a) F$_1$ hybrids in experiment 2 and F$_1$ hybrids in experiment 1, (b) Tahora BC$_1$ families and F$_1$ hybrid parents in experiment 1, (c) Tahora BC$_1$ families and F$_1$ hybrid parents in experiment 2, (d) Trophy BC$_1$ families and F$_1$ hybrid parents in experiment 1, (e) Trophy BC$_1$ families and F$_1$ hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

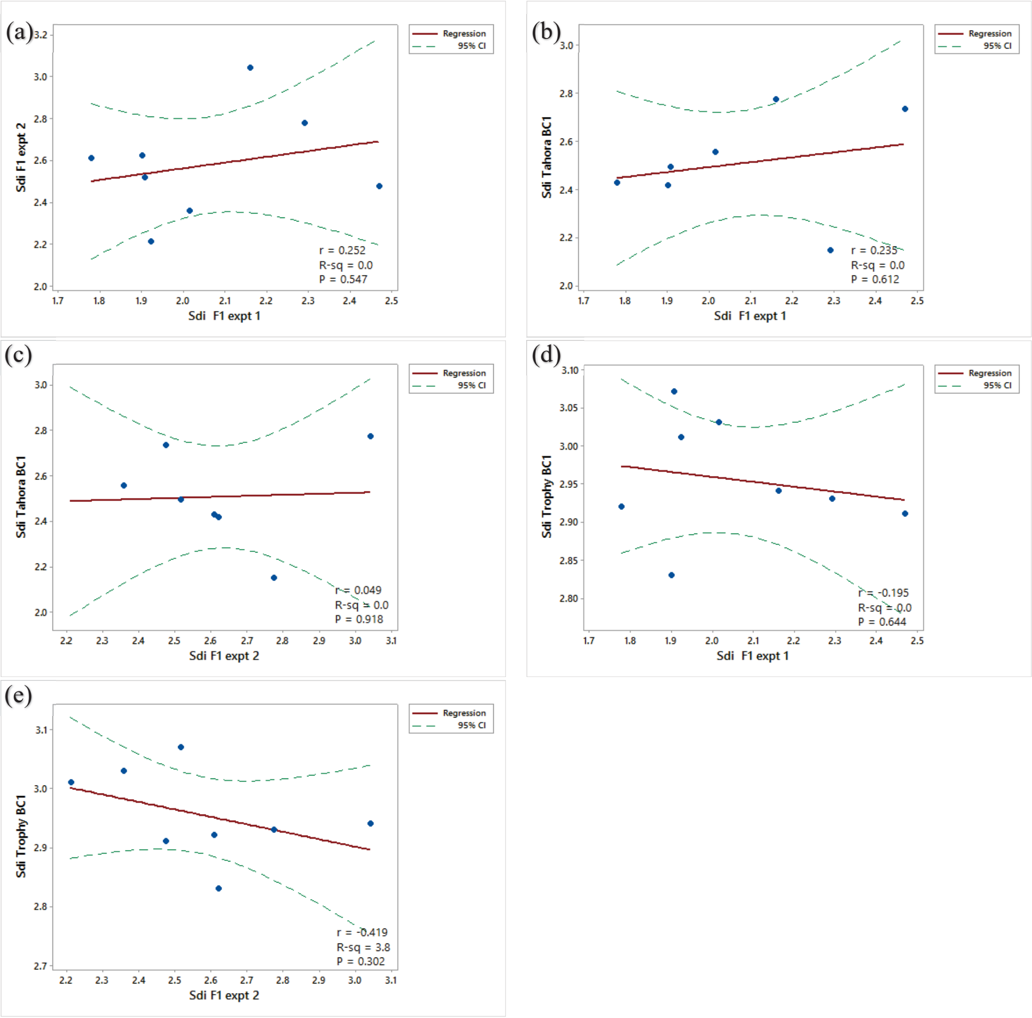

Figure A6.9: Correlations of mean Stolon Diameter (mm) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.

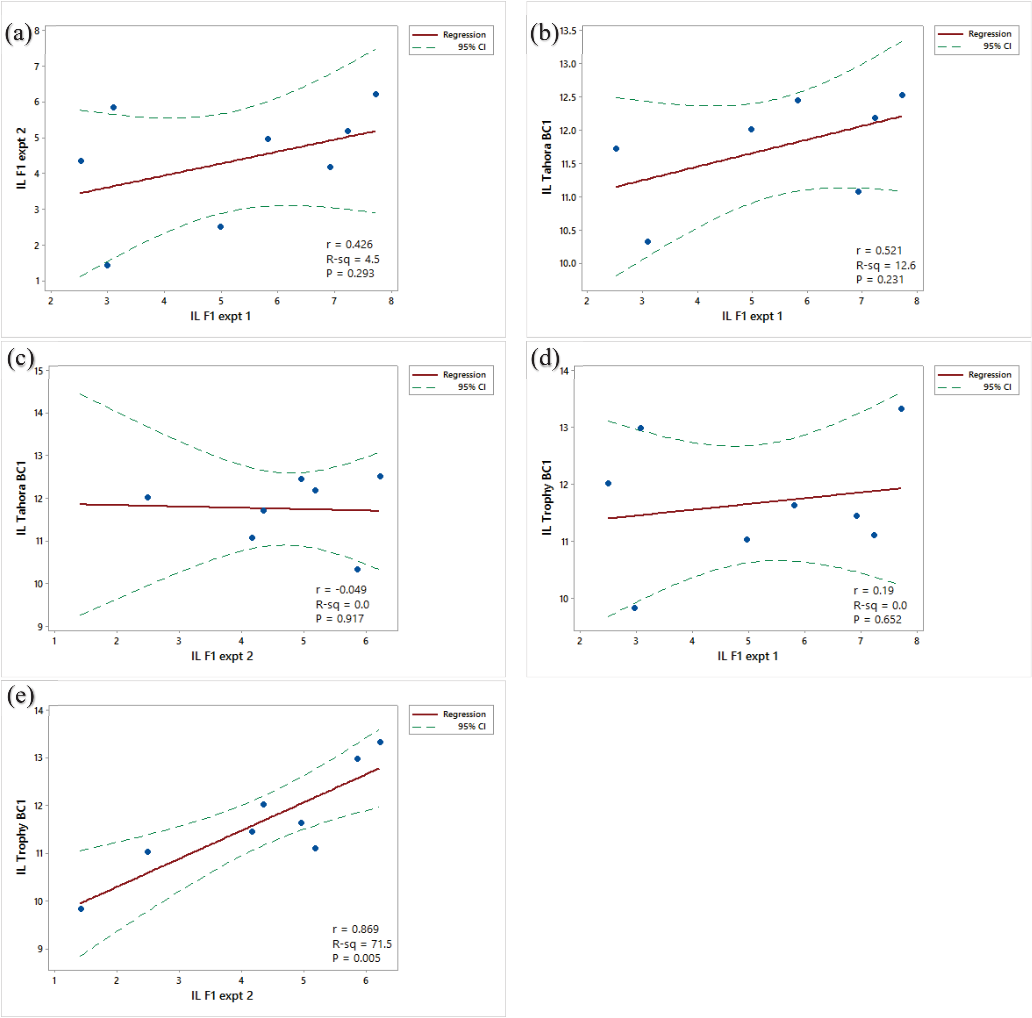

Figure A6.10: Correlations of mean Internode Length (mm) between (a) F_1 hybrids in experiment 2 and F_1 hybrids in experiment 1, (b) Tahora BC_1 families and F_1 hybrid parents in experiment 1, (c) Tahora BC_1 families and F_1 hybrid parents in experiment 2, (d) Trophy BC_1 families and F_1 hybrid parents in experiment 1, (e) Trophy BC_1 families and F_1 hybrid parents in experiment 2. The Pearson correlation coefficient (r), R^2 value (%), and significance (P) of the correlations are noted on each graph.