Phenotypic examination of variation occurring both among families and among genotypes within a *T. repens* x (*T. ambiguum* x *T. occidentale*) BC$_1$F$_2$ hybrid population.

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

In

Plant Breeding

At Massey University, Palmerston North,

New Zealand.

Stephen Charles Slack

2017
Abstract
Development of white clover cultivars with increased vegetative persistence, particularly in dryland farming systems has been a major goal within breeding programmes, however little useful genetic variation for survival and growth in these environments has been found. Consequently, it has become necessary to look towards white clovers wild relatives as sources of genetic variation. *T. repens* x (*T. ambiguum* x *T. occidentale*) tri-species hybrids have been developed, however, their morphologies have not been evaluated, and little is known about optimal breeding strategies in these populations.

An experiment was designed to characterise the magnitude of phenotypic variation for a range of root, shoot, and floral traits, and to ascertain optimal breeding strategies within a *T. repens* x (*T. ambiguum* x *T. occidentale*) BC₁F₂ hybrid plant population. The experiment was designed such that it could be analysed in two ways;

a) Investigated levels of phenotypic variation occurring among hybrid families, compared to representatives of their F₁ parents

b) Investigated levels of phenotypic variation among individual hybrid genotypes, again compared to representatives of their F₁ parents.

Analysis (a) found a relative lack of among hybrid family variation. With significant (*P*<0.05) family variance components for 11 of the 18 traits measured, and generally only occurring between the upper and lower extremes. Repeatability estimates on a family mean basis were low (less than 0.51 for all traits).

Analysis (b) found significant (*P*<0.05) genotypic variance components for all of the traits measured. Repeatability estimates ranged from 0.47-0.88, indicating a relatively high level of genetic determination for the majority of traits.

Pattern analysis allowed the identification of hybrid genotypes showing the combined expression of key shoot, and root traits. These genotypes may provide a route to hybrid clover cultivars showing increased vegetative persistence via increased nodal and tap-root size, combined with good dry matter production.
The wide range of phenotypic variation and high repeatability estimates among hybrid genotypes, combined with the relative lack of variation and low repeatability estimates among hybrid families allowed us to conclude that phenotypic recurrent selection based on individual genotypes should be practised in these early generation hybrids populations.
Acknowledgements

Firstly, I would like to thank my supervisor Dr Warren Williams, and his wife Isabelle for their ongoing enthusiasm, support, patience, and mentorship, not just with this thesis, but also professionally, I cannot thank you both enough.

Thank you to Professor Cory Matthew for his input and guidance, particularly with respect to statistical analysis and his helpful comments on the manuscript.

Thanks to Dr Zulfi Jahufer for his support and advice on experimental design and analysis.

Thanks to Dr Jim Crush, and AgResearch as a whole for all of their support over the course of this project.

Finally, thank you to my family and friends, particularly Amanda, who has tolerated my preoccupation with this project over the last couple of years.
# Table of contents

Abstract .......................................................................................................................................... ii

Acknowledgements ....................................................................................................................... iv

List of tables ................................................................................................................................ viii

List of figures ................................................................................................................................. ix

List of appendices ......................................................................................................................... x

List of plates ................................................................................................................................ xi

List of abbreviations ..................................................................................................................... xii

Chapter 1 Introduction ................................................................................................................. 1

1.1 Background ............................................................................................................................ 1

1.2 Gaps in knowledge ............................................................................................................... 2

1.3 Current context ..................................................................................................................... 3

1.4 Hypothesis and objectives .................................................................................................. 3

Chapter 2 Literature review ........................................................................................................ 5

2.1 White clover ........................................................................................................................ 5

2.1.2 White clover morphology ............................................................................................ 5

2.1.3 Traits affecting drought tolerance of white clover ...................................................... 6

2.2 Genus *Trifolium*—wild relatives of white clover ............................................................ 7

2.3 White clover ancestry ......................................................................................................... 9

2.3.2 Genetic control of chromosome pairing in *T. repens* ................................................ 11

2.4 Interspecific hybridisation and its potential in *Trifolium* ................................................ 12

2.4.2 *T. repens* x *T. occidentale* .................................................................................... 12

2.4.3 *T. repens* x *T. ambiguum* .................................................................................... 13

2.4.4 Breeding with tetraploid *T. ambiguum* .................................................................... 13

2.4.5 Breeding with diploid *T. ambiguum* ....................................................................... 15

2.4.6 Breeding with hexaploid *T. ambiguum* ................................................................... 15

2.5 The use of *T. occidentale* as a genetic bridge for the introgression of *T. ambiguum* alleles ........................................................................................................................................ 16

2.6 Hybrid morphology ........................................................................................................... 17

2.7 Future options and concluding remarks ........................................................................... 17

Chapter 3 Materials and methods .............................................................................................. 19

3.1 Plant population development ......................................................................................... 19

3.2 Trial Site ............................................................................................................................ 20

3.3 Plant material .................................................................................................................... 20

3.4 Experimental design ......................................................................................................... 21

3.5 Plant establishment .......................................................................................................... 22
5.4.1 Associations among shoot traits ................................................................. 60
5.4.2 Associations among root traits ................................................................. 61
5.4.3 Associations among selected shoot and root traits................................. 62
5.5 Implications for plant breeding................................................................. 63
5.6 Conclusions ......................................................................................... 64
Chapter 6. References ........................................................................ 66
Appendices ......................................................................................... 76
List of tables

Table 1: Species of section Trifoliastrium; life form, chromosome number, distribution, habitat, and characteristics. Adapted from (Williams, 2014) ................................................................. 9

Table 2: Experimental entries, their parentage, and expected genomic constitution. .... 21

Table 3: Means, ranges, and variance components (\(\sigma^2\)) with associated standard errors (±SE) for various traits measured from 20 T. repens x (T. ambiguum x T. occidentale) BC\(_1\)F\(_2\), one T. ambiguum x T. occidentale, and one T. repens cv. Crusader families grown in sand.⁠†……………….. 32

Table 4: Means, ranges, and variance components (\(\sigma^2\)) with associated standard errors (±SE) for various traits measured from 120 T. repens x (T. ambiguum x T. occidentale) BC\(_1\)F\(_2\), six T. ambiguum x T. occidentale, and six T. repens cv. Crusader progeny grown in sand.⁠†……………….. 37

Table 5: Phenotypic correlation coefficients among the morphological traits measured from 120 T. repens x (T. ambiguum x T. occidentale) BC\(_1\)F\(_2\), six T. ambiguum x T. occidentale, and six T. repens cv. Crusader progeny grown in sand.⁠†…………………………………….. 39

Table 6: Within-group genotype means for each shoot trait based on the four clusters generated from cluster analysis of 120 BC\(_1\)F\(_2\), 6 AAOO, and 6 T. repens genotypes grown in sand.⁠† ................................................................. 40

Table 7: Within-group genotype means for each root trait based on the four clusters generated from cluster analysis of 120 BC\(_1\)F\(_2\), 6 AAOO, and 6 T. repens genotypes grown in sand.⁠† ................................................................. 43

Table 8: Within-group genotype means for each shoot and root trait based on the four clusters generated from cluster analysis of 120 BC\(_1\)F\(_2\), 6 AAOO, and 6 T. repens genotypes grown in sand.⁠† ................................................................. 47
List of figures

Figure 1. Distribution of T. repens x (T. ambiguum x T. occidentale) BC₁F₂ hybrid genotype BLUPs for selected traits in the BC₁F₂ population. Means for parental (AAOO and T. repens) and BC₁F₂ populations are shown by arrows. ................................................................. 38

Figure 2. Biplot generated using standardized Best Linear Unbiased Predictor values of genotype shoot trait means from 120 BC₁F₂, 6 AAOO (pink), and 6 T. repens (red) genotypes grown in sand. Components I and II account for 59 and 16% of total variation, respectively. The different symbols indicate genotype Groups 1 to 4 generated from cluster analysis (a), whilst the different numbers represent individual genotypes (b). The vectors represent the shoot traits: LL, leaflet length (mm); LW, leaflet width (mm); PTL, petiole length (mm); LDW, leaf dry-weight (g); IL, internode length (mm); SL, stolon length (mm); SD, stolon diameter (mm); SDW, stolon dry-weight (g). The arrow (⇢) indicates the labels of directional vectors that are not legible. .................................................................................................................... 42

Figure 3. Biplot generated using standardized Best Linear Unbiased Predictor values of genotype root trait means from 120 BC₁F₂, 6 AAOO (pink), and 6 T. repens (red) genotypes grown in sand. Components I and II account for 41 and 21% of total variation, respectively. The different symbols indicate genotype Groups 1 to 5 generated from cluster analysis (a), whilst the different numbers represent individual genotypes (b). The vectors represent the root traits: SA, stolon anchoring (mm); NRD, nodal root diameter (mm); NRL, nodal root length (mm); TRD, tap root diameter (mm); TRL, tap root length (mm); RDW, root dry weight (g). The arrow (⇢) indicates the labels of directional vectors that are not legible. .............................................. 45
List of appendices

Appendix 1: Experimental design 1† ............................................................. 76
Appendix 2: Sandpit experimental design 2† .......................................................... 77
List of plates

Plate 1: Experimental area at AgResearch Grasslands, Palmerston North ........................... 23
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Parameter</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>First filial generation</td>
<td></td>
</tr>
<tr>
<td>BC&lt;sub&gt;1&lt;/sub&gt;F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>First generation backcross one hybrid</td>
<td></td>
</tr>
<tr>
<td>BC&lt;sub&gt;1&lt;/sub&gt;F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Second generation backcross one hybrid</td>
<td></td>
</tr>
<tr>
<td>AAOO</td>
<td>(T. ambiguum x T. occidentale) hybrid</td>
<td></td>
</tr>
<tr>
<td>cv.</td>
<td>Cultivar</td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>Open pollinated</td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>Pollen stainability</td>
<td>%</td>
</tr>
<tr>
<td>PDL</td>
<td>Peduncle length</td>
<td>mm</td>
</tr>
<tr>
<td>FPI</td>
<td>Florets per inflorescence</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>Leaflet length</td>
<td>mm</td>
</tr>
<tr>
<td>LW</td>
<td>Leaflet width</td>
<td>mm</td>
</tr>
<tr>
<td>LL:LW</td>
<td>Leaflet length to leaflet width ratio</td>
<td></td>
</tr>
<tr>
<td>PTL</td>
<td>Petiole length</td>
<td>mm</td>
</tr>
<tr>
<td>SD</td>
<td>Stolon diameter</td>
<td>mm</td>
</tr>
<tr>
<td>IL</td>
<td>Internode length</td>
<td>mm</td>
</tr>
<tr>
<td>SL</td>
<td>Stolon length</td>
<td>mm</td>
</tr>
<tr>
<td>SA</td>
<td>Stolon anchoring</td>
<td>mm</td>
</tr>
<tr>
<td>NRD</td>
<td>Nodal root diameter</td>
<td>mm</td>
</tr>
<tr>
<td>NRL</td>
<td>Nodal root length</td>
<td>mm</td>
</tr>
<tr>
<td>TRL</td>
<td>Tap root length</td>
<td>mm</td>
</tr>
<tr>
<td>TRD</td>
<td>Tap root diameter</td>
<td>mm</td>
</tr>
<tr>
<td>LDW</td>
<td>Leaf dry weight</td>
<td>g</td>
</tr>
<tr>
<td>SDW</td>
<td>Stolon dry weight</td>
<td>g</td>
</tr>
<tr>
<td>RDW</td>
<td>Root dry weight</td>
<td>g</td>
</tr>
</tbody>
</table>