Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Drought resistance mechanisms in "Mediterranean" perennial ryegrass (*Lolium perenne* L.) and potential for introgression of "Mediterranean" germplasm into New Zealand commercial cultivars

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

Doctor of Philosophy

in

Plant Science

Massey University
Institute of Agriculture and Environment
College of Sciences
Palmerston North, New Zealand

SAJJAD HUSSAIN

2013

Abstract

The unique topography of New Zealand creates a wide variation in rainfall and temperature between and within the two islands of the country. As a result, successful use of perennial ryegrass (*Lolium perenne* L.), the backbone of New Zealand's agricultural economy, has been restricted to only the higher rainfall and cooler areas of the country. However, there has been only limited analysis of drought resistance in forage grasses at the trait level. This PhD study was conducted on a perennial ryegrass cultivar "Medea" developed in Adelaide in the 1960's from reportedly drought resistant and summer dormant germplasm of North African origin. The main objectives of the study were to compare Medea with a high yielding but drought susceptible current New Zealand cultivar, Grasslands Samson for their drought resistance potential and to evaluate Medea for its suitability for introgression with Grasslands Samson, in a plant improvement programme. Drought resistance strategies of Tolosa, Matrix and Ceres One50 were also evaluated.

In total six glasshouse experiments were conducted. Experiment 1 (April – September 2008) compared winter vegetative growth of potted plants of Grasslands Samson and Medea. Yield of Medea was <50% that of Grasslands Samson, but glasshouse temperature at times exceeded 25°C, so it is possible that this temperature was high enough to partially trigger summer dormancy in Medea.

In Experiment 2 (summer 2008 – 2009) techniques for assessing drought resistance were developed, and in Experiment 2 and Experiment 5 (summer 2009 – 2010) drought resistance strategies exhibited by individual cultivars were evaluated. Experiment 2 included Medea, Grasslands Samson, an unreleased tetraploid breeding line developed from Grasslands Samson and Tolosa. Experiment 5 evaluated Matrix and Ceres One50, in addition to Grasslands Samson and Medea. Drought resistance strategies observed in Medea included deep rootedness and high leaf proline contents, but there was some evidence for lack of transpiration reduction in water deficit stress. Medea had prolific flowering. Grasslands Samson and its tetraploid were more productive than Medea in these experiments. However, Tolosa produced the same shoot DW as Grasslands Samson with greater retention of soil moisture, indicating higher water use efficiency.

Experiment 3 (March 2009 – February 2010) compared five family groups, each comprising a Grasslands Samson and a Medea parent, and three of their F_1 progeny. In this experiment plants were 11 months old when root traits were evaluated and for these older plants, Grasslands Samson had a higher root to shoot ratio and deep rootedness than Medea. Medea plants had similar shoot DW to Grasslands Samson plants during winter, but 46% lower shoot DW in summer. The F_1 progeny showed positive mid-parent heterosis for deep rootedness, but negative mid-parent heterosis for shoot DW, and tended to reflect the prolific flowering of the Medea parent.

Experiment 4 (December 2009 – June 2010) compared six family groups of F_2 progeny for traits related to drought resistance. Although plant numbers were small compared with a commercial breeding programme, it was evident some family groups combined both drought resistance and productivity traits.

Experiment 6 (September 2011 – February 2012) evaluated Grasslands Samson, Medea, and F_1 and F_2 progeny for drought resistance traits. Some useful traits expressed strongly in the F_1 generation reverted to mid-parent values in the F_2 generation. Some genotypes of Grasslands Samson exhibited higher water use efficiency (reduced soil moisture extraction with high shoot DW) and this warrants further research.

It is concluded that some desirable genes for traits contributing to drought resistance, such as deep rootedness and osmotic adjustment might be obtained from Medea. However, the drought resistance strategy of Medea involving reduction in plant size in summer, deep rooting and comparatively high transpiration would have pros and cons for New Zealand farmers as a trait combination. Reduced depletion of soil moisture under water deficit might assist survival of companion plants such as white clover; but high transpiration would decrease water use efficiency. Therefore, improving the water use efficiency of Grasslands Samson or use of material such as Tolosa, which has a comparatively low soil water use per unit of dry matter produced among the cultivars tested, would appear to be a preferred breeding strategy for future breeding programmes in New Zealand.

Acknowledgements

Many thanks are due to my chief supervisor Associate Professor Cory Matthew for his guidance, support and attitude and for going far beyond the call of duty during each phase of my PhD; and to my co-supervisor Dr Sydney Easton for his guidance, support and providing me with plant material to work on.

Thanks to the Massey University Plant Growth Unit staff Steven Ray, Lindsay Silva and Lesley Taylor for providing logistic support during my glasshouse experiments. I am also indebted to Mark Osborne, Simon Osborn, Scott Avery, James Slater, Kay Sinclair and Benoit Pietresson de St. Aubin for their consistent help during soil preparation, data recording, laboratory analysis and the arduous task of retrieval and washing of roots. Most of these colleagues helped me during the very early morning hours when measurements of plant water status were required. Thanks are also due to Chris Rawlinson for providing me with training in the use of laboratory and field equipment, and assistance in conducting laboratory work, in particular proline analysis.

My gratitude also goes to Professor Dr Hossein Behboudian, Dr Muhammad Ashraf, Dr Shahzad Basra, Winthrope Professor Kadambot Siddique, Professor Neil Turner, Professor Emeritus John Boyer, Dr Abraham Blum, Professor Hanif Quazi, Professor Warren Williams and Dr Wajid Hussain for their technical advice on plant physiology and plant breeding aspects of my PhD. Thanks to Dr Zhao He and Ms Denise Stewart for assisting me with thesis formatting and index preparation, and to my friends Drs Mairie Fromont, Edith Khaembah, and Arif Robin; and Abdul Hannan, Muhammad Naeem, Lulu He and Ashiq Saleem for some thesis review work and general technical guidance.

I would also like to thank the Higher Education Commission (HEC) of Pakistan for funding my PhD studies in New Zealand. The T. R. Ellett Agricultural Research Trust is thanked for providing partial funding in my fourth year of study. My travel expenses to Interdrought III in Shanghai in October 2009 were funded by a New Zealand Postgraduate Studies Abroad Award (NZPSAA), the FAO of the United Nations and the Institute of Natural Resources, Massey University. for funding.

Funding received during my PhD programme from the John Hodgson Pastoral Science Scholarship fund is also acknowledged with thanks. Thanks are also due to the Pakistan Agricultural Research Council (PARC) for granting me leave of absence for this PhD study.

Special thanks are also due to my family friends: Mr and Mrs Tariq Mehmood, Dr and Mrs Nasser Shehata, Dr and Mrs Moazzam Zaidi, Mr and Mrs Saqib Sharif, Dr and Mrs Muhammad Shuaib, Mr and Mrs Raza Ullah Khan, Dr Arshad Malik, Dr Muhammad Imran, Jana Muller, Dennis Whiterod, Maria Work and Mohan Ahmad for their support.

Finally, I thank my beloved wife Aqsa Sajjad, my lovely daughter Simra Hussain and my beloved sons Muahmmad Awwab Hussain, and Yusha Awwah Hussain who shared their love during my studies. I am grateful to my parents, especially my mother who passed away in Pakistan during my stay in New Zealand. Her affectionate words by telephone from her death-bed remain with me. My brothers and sisters and the rest of my family members are also thanked. Their consistent encouragement has greatly helped me in the completion of this thesis.

Dedication

To my parents, wife, brothers and sisters

Table of Contents

Abstract i
Acknowledgements iii
Dedication v
Table of Contents vii
List of Tables xiii
List of Figures xvii
List of Appendices xx
Glossary of Abbreviations xxi

Chapter 1 Introduction 1
1.1 General background 1
1.2 Objectives…… 3
1.3 Thesis structure 4

Chapter 2 Literature review 7
2.1 Introduction…. 7
2.2 Definitions of “Drought” and related terms 7
2.3 Fundamentals of plant water relations 9
  2.3.1 Methods of measuring soil and plant water status 10
    2.3.1.1 Amount of water 10
    2.3.1.2 Energy status of water 11
  2.3.2 Plant responses to drought 12
2.4 Previous progress towards drought resistance in forage grasses 16
  2.4.1 Dehydration postponement 16
    2.4.1.1 Improved water uptake 17
    2.4.1.2 Control of transpiration loss 18
  2.4.2 Dehydration tolerance 21
    2.4.2.1 Osmotic adjustment 21
    2.4.2.2 Cell membrane stability 22
  2.4.3 Drought escape 22
  2.4.4 Measurements to quantify drought resistance 23

2.5 New Zealand's climate in relation to the adaptive range of perennial ryegrass 25
2.6 History of perennial ryegrass plant breeding work in New Zealand 31
2.7 Use of hybrids in plant breeding 36
  2.7.1 Concept of heterosis/hybrid vigour 36
  2.7.2 Introgression 38
2.8 Medea as a summer dormant drought resistant cultivar 38
2.9 Conclusions 41

Chapter 3 Comparison of morphogenetic traits in perennial ryegrass (*Lolium perenne* L.) cultivars Grasslands Samson and Medea in winter 43

3.1 Introduction….. 43
  3.1.1 Aims…. 44
3.2 Materials and methods 44
  3.2.1 Morphogenetic data 44
  3.2.2 Leaf gas exchange data 46
  3.2.3 Glasshouse temperature recordings 47
  3.2.4 Statistical analysis 48
3.3 Results………. 48
  3.3.1 Glasshouse temperature data 48
  3.3.2 Leaf morphogenesis 51
  3.3.3 Other morphogenetic traits 52
  3.3.4 Plant dry weight variation for genotypes within cultivars 53
  3.3.5 Trait associations as assessed by correlation analysis and PCA 54
  3.3.6 Leaf gas exchange data 57
3.4 Discussion…… 59
  3.4.1 Plant response to the growth environment 59
  3.4.2 Comparison of morphogenetic traits in Grasslands Samson and Medea... 62
  3.4.3 Comparison of gas exchange traits in Grasslands Samson and Medea... 65
3.5 Conclusions….. 66

Chapter 4 A survey of traits contributing to drought resistance in Medea and some current New Zealand commercial cultivars of perennial ryegrass (*Lolium perenne* L.).. 67

4.1 Introduction….. 67
 4.1.1 Aims for Experiment 2 and Experiment 5 68
4.2 Materials and methods 69
 4.2.1 Experiment 2 (September – December 2008)....................................... 69
  4.2.1.1 Location, design and setting up.. 69
  4.2.1.2 Measurements (Experiment 2) 72
 4.2.2 Experiment 5 (September 2010 – January 2011).................................. 74
  4.2.2.1 Location, design and setting up.. 74
  4.2.2.2 Measurements (Experiment 5) 77
4.3 Results……….. 81
 4.3.1 Experiment 2 81
  4.3.1.1 Glasshouse temperatures... 81
  4.3.1.2 Shoot growth and growth components.. 82
  4.3.1.3 Root development and water uptake 86
  4.3.1.4 Plant water status 90
  4.3.1.5 Stomatal and cellular control 91
 4.3.2 Experiment 5..93
  4.3.2.1 Glasshouse temperatures..93
  4.3.2.2 Shoot growth and growth components...93
  4.3.2.3 Root development and water uptake 98
  4.3.2.4 Plant water status 101
  4.3.2.5 Stomatal and cellular control 104
4.4 Discussion…….. 105
 4.4.1 Methodology development 105
 4.4.2 Statistical interactions involving harvest date...106
 4.4.3 Differences in methodology of watering and their implications on plant growth 107
 4.4.4 Effect of water deficit on plant processes 108
 4.4.5 Cultivar differences in water deficit response 109
4.5 Conclusions…… 114

Chapter 5 Patterns of trait inheritance in Medea × Grasslands Samson F_1 progeny.................................. 115

5.1 Introduction and aims..115
5.2 Materials and methods..115
    5.2.1 Location, design and setting up 115
    5.2.2 Measurements..116
    5.2.3 Statistical analysis..118
5.3 Results………. 119
    5.3.1 Parent / progeny and Family group water deficit responses..............119
    5.3.2 Parent / progeny × family group interaction.......................................125
5.4 Discussion…… 128
    5.4.1 Choice of statistical design 128
    5.4.2 Comparison of Medea and Grasslands Samson 129
    5.4.3 Trait expression in F_1 progeny 130
5.5 Conclusions….. 131

Chapter 6 Evaluation of F_2 Medea × Grasslands Samson hybrids for drought resistance traits....................... 133

6.1 Introduction and aims..133
6.2 Materials and methods..134
    6.2.1 Location, design and setting up 134
    6.2.2 Treatment application and measurements 135
        6.2.2.1 Unwatered plants 138
    6.2.3 Data analysis..138
6.3 Results………. 139
    6.3.1 Family group differences..139
    6.3.2 Analysis of trait expression at the genotype level 142
    6.3.3 Data from unwatered plants..144
6.4 Discussion…… 146
    6.4.1 Findings about proline concentrations and its relationship to OA and plant yield..146
    6.4.2 Trait combinations in F_2..147
    6.4.3 PCA highlights 148
    6.4.4 Findings from unwatered plant..149
6.5 Conclusions….. 149

Chapter 7 Inheritance of drought resistance traits from parents to F_1 and F_2 progeny 151

7.1 Introduction and aims 151

7.2 Materials and methods 151

  7.2.1 Location and experimental set up 151

  7.2.2 Treatment application 152

  7.2.3 Measurements 152

  7.2.4 Data analysis 154

7.3 Results……….. 154

  7.3.1 Glasshouse temperature 154

  7.3.2 Trait characteristics of parents and F_1 and F_2 generations 154

  7.3.3 Traits association analysis as indicated by MANOVA analysis......... 159

  7.3.4 Indices of effectiveness of water use 161

7.4 Discussion…… 162

  7.4.1 Experiment management 162

  7.4.2 Methodology for results presentation 162

  7.4.3 Key findings.. 164

    7.4.3.1 Comparison of Grasslands Samson and Medea..................... 164

    7.4.3.2 Insights from MANOVA 165

    7.4.3.3 Key findings for plant improvement 165

7.5 Conclusions….. 167

Chapter 8 Overview and conclusions.................................... 169

8.1 Rationale for the work 169

8.2 Review of work carried out 171

8.3 Review of experiments and results highlights 172

8.4 Commercialisation potential from the results 175

  8.4.1 Drought resistance traits observed and their implications for New Zealand farm practice 175

    8.4.1.1 Production versus survival... 175

    8.4.1.2 Prolific flowering... 175

8.4.1.3 Physiological traits of Medea (Proline contents, flaccid leaves, stomatal conductance, canopy temperature 175
8.4.1.4 High production per unit of water 176
8.5 Conclusions 177

References 179
Appendices 191

List of Tables

Table 2.1 Terminology for distinguishing different categories of moisture deficit 8

Table 2.2 Turner's (1986) classification of drought resistance mechanisms 14

Table 2.3 Four plant functional domains contributing to differing mechanisms of drought resistance recognized by Turner (1986) and proposed trait measurements to define the drought resistance strategy of test plants 24

Table 2.4 Long-term annual rainfall and January/July temperature data for selected New Zealand sites ranging from high to low rainfall and warm to cool temperature and modelled soil moisture deficit or surplus for months November to March 26

Table 2.5 Extreme soil moisture deficit statistics for 2008 and 2009 in agriculturally important areas of New Zealand 31

Table 3.1 Mean values of leaf length and leaf elongation rate for the first three leaves (leaves 1 – 3) appearing after defoliation on 15 May 2008 51

Table 3.2 Mean values of other morphogenetic traits measured for perennial ryegrass cultivars Grasslands Samson and Medea in Experiment 1 from 15 May to 17 July 2008 53

Table 3.3 Matrix of coefficients of correlation between thirteen selected variables in forty plants of perennial ryegrass cultivars Grasslands Samson and Medea during winter 2008 56

Table 3.4 Principal component coefficients for the first four PCs generated by PCA of morphological data for Grasslands Samson and Medea perennial ryegrass cultivars 57

Table 3.5 Cultivar means for gas exchange parameters measured or calculated by the CIRAS-2 Portable Photosynthesis System for the second youngest leaf of a randomly selected tiller on 10 plants of Grasslands Samson and 10 plants of Medea on 26 August 2008 58

Table 3.6 PC structure for PC1 and PC2 from PCA of gas exchange data for 20 perennial ryegrass plants (10 plants of Grasslands Samson and 10 plants of Medea) 59

Table 3.7 Comparison of temperature regimes for research of Cooper (1964) and for the present experiment 60

Table 4.1 Description of scores of leaf rolling, leaf wilting and degree of blue colour change 80

Table 4.2 ANOVA f-ratios and their P values for measures of shoot growth in Experiment 2 83

Table 4.3 Cultivar and water regime main effect means for measures of shoot growth in Experiment 2 84

Table 4.4 ANOVA f-ratios and their P values for measures of root development and water uptake in Experiment 2 87

Table 4.5 Cultivar and water regime main effect means for measurements of root growth and plant water uptake of Experiment 2 88

Table 4.6 ANOVA f-ratios and their P values for plant water status measurements in Experiment 2 91

Table 4.7 Cultivar and water regime main effect means for plant water status measurements in Experiment 2 91

Table 4.8 ANOVA f-ratios and their P values for measurements of stomatal and cellular control in Experiment 2 92

Table 4.9 Cultivar and water regime main effect means for measurements of stomatal and cellular control in Experiment 2 92

Table 4.10 ANOVA f-ratios and their P values for measures of shoot growth in Experiment 5 94

Table 4.11 Cultivar and water regime main effect means for herbage-yield-related measurements in Experiment 5 95

Table 4.12 ANOVA f-ratios and their P values for measures of root development and water uptake in Experiment 5 99

Table 4.13 Cultivar and water regime main effect measurements of root development and water uptake in Experiment 5 100

Table 4.14 ANOVA f-ratios and their P values for plant water status measurements in Experiment 5 102

Table 4.15 Cultivar and water regime main effect means for measurements of plant water status in Experiment 5 103

Table 4.16 ANOVA f-ratios and their P values for measurements of stomatal and cellular control in Experiment 5 104

Table 4.17 Cultivar and water regime main effect means for measurements of stomatal and cellular control in Experiment 5 105

Table 5.1 List of measurements and their dates for the unstressed phase of Experiment 3 117

Table 5.2 List of measurements and their dates for the mildly stressed phase of watering for Experiment 3 plants 117

Table 5.3 List of measurements and their dates for the severely stressed phase of watering for Experiment 3 plants 118

Table 5.4 Mean, P values and mid-parent heterosis for variables of domain “shoot-growth”... 120

Table 5.5 Mean, P values and mid-parent heterosis for variables of domain “root development and water uptake” 121

Table 5.6 Mean, P values and mid-parent heterosis for variables of domains “plant water status” and “stomatal and cellular control” under stressed, mildly stressed and highly stressed phases of Experiment 3.. 122

Table 5.7 Standard deviations of Medea, Grasslands Samson and progeny means from the population means for traits where statistically significant differences were detected 123

Table 5.8 Standard deviations of family group means from the population means for traits where statistically significant differences were detected.. 124

Table 6.1 Schedule of measurements carried out in the mildly stressed phase of Experiment 4 137

Table 6.2 Schedule of measurements carried out in the severely stressed phase of Experiment 4 138

Table 6.3 Family group means of traits non-destructively measured during the mild water deficit phase of Experiment 4.......................... 139

Table 6.4 Family group means of traits measured during the severely stressed phase of Experiment 4... 140

Table 6.5 Selected statistically significant correlations among the 6 family group means for measurements performed on severely stressed plants 141

Table 6.6 Standard deviations of family group means from the population means for traits where statistically significant differences were detected.. 142

Table 6.7 Coefficients indicating trait contributions to PC scores from PCA of seventeen selected traits across the four plant functional domains.. 144

Table 6.8 Comparison of three unwatered genotypes from Experiment 4 for green and dead dry weight (g plant^{-1}), and soil moisture content (%) at soil depths 1, 2 and 3..145

Table 7.1 Criteria for visually scoring foliage dead leaf percentage 153

Table 7.2 P and f values for the main effects i.e., generations and water regime and their interaction and means values of the four plant populations (Grasslands Samson, Medea, F_1 and F_2) and water regime (Control and Stressed) for traits of domains shoot growth and root development and water uptake..................................156

Table 7.3 P and f values for the main effects i.e., generations and water regime and their interaction and means values of the four plant populations (Grasslands Samson, Medea, F_1 and F_2) and water regime (Control and Stressed) for traits of domain plant water status and stomatal and cellular control...158

Table 7.4 Standardized canonical coefficients for statistically significant canonical factors from MANOVA of traits measuring plant response to water deficit..159

Table 7.5 “Between” canonical structures for statistically significant canonical factors for the four plant populations..................................160

List of Figures

Figure 2.1 Regional variation in (a) mean annual rainfall (mm) and (b) temperature (^oC) of the North and South islands of New Zealand 27

Figure 2.2 Inter-annual variation in monthly rainfall recorded at AgResearch Palmerston North for a four year period from July 2000 - June 2004 30

Figure 3.1 Gas exchange parameters (photosynthesis, evapotranspiration and stomatal conductance) for leaves of cultivars Medea and Grasslands Samson using the CIRAS-2 Portable photosynthesis system 47

Figure 3.2 Hourly temperature data in the glasshouse measured with a Skye Instruments data logger from 19 June to 10 July 2008 49

Figure 3.3 Hourly solar radiation data for the glasshouse measured with a Skye Instruments data logger from 19 June to 10 July 2008 50

Figure 3.4 Comparison of leaf extension duration for the first 4 leaves (L1 to L4) appearing after defoliation on 14 May for the two cultivars, Grasslands Samson and Medea 52

Figure 3.5 Herbage dry weight harvested (DW, g plant^{-1}) for 20 individual plants of Grasslands Samson and 20 plants of Medea cut to ground level on 8 July 2008, plotted against tiller number (TN plant^{-1}) 54

Figure 4.1 Randomized complete block layout used for plants in Experiment 2 69

Figure 4.2 Experiment 2 in October 2008 before the introduction of differential watering 70

Figure 4.3 Plants of Experiment 2 in late November 2008 with 70 cm watering tubes and taps in place 71

Figure 4.4 Randomized Complete Block layout used for plants in Experiment 5 75

Figure 4.5 Arrangement of pots in 200 liter drums fitted with a plastic tap at the bottom in Experiment 5 76

Figure 4.6 Daily maximum and minimum glasshouse temperature during the application of drought treatments in Experiment 2 82

Figure 4.7 Percentage of seed-head weight to shoot DW (H%), in four cultivars, Medea, Grasslands Samson, Samson (4n) and Tolosa, for (a) two water regimes: control and stressed, and (b) Harv1 and Harv2 in Experiment 2 85

Figure 4.8 Percentage of seed-head weight to shoot DW (H%) under control and water stressed conditions of the four cultivars (Medea, Grasslands Samson, Samson (4n) and Tolosa), for (a) two water regimes: control and stressed, and (b) Harv1 and Harv2 in Experiment 2 86

Figure 4.9 Interaction of cultivar × water regime in the four cultivars Medea, Grasslands Samson, Samson (4n) and Tolosa 89

Figure 4.10 Root to shoot ratio and deep root to shoot ratio (expressed as a multiple of 100) of the four cultivars (Medea, Grasslands Samson, Samson tetraploid and Tolosa) for Controlled and Stressed watering 90

Figure 4.11 Daily maximum and minimum glasshouse temperatures for the period 24 November 2010 – 26 January 2011 of Experiment 5 93

Figure 4.12 Comparison between Medea and Grasslands Samson for phenological development of HN, seed-head numbers during spring (November 2010) and summer (December 2010 – January 2011) 96

Figure 4.13 Seed-head number of the four cultivars, Medea, Grasslands Samson, Ceres One50 and Matrix between two harvests of Experiment 5....97

Figure 4.14 Comparison of soil moisture contents at depth 3, under Control and Stress conditions in the four cultivars (Medea, Grasslands Samson, Ceres One50 and Matrix) in Experiment 5 101

Figure 4.15 Comparison of leaf water potential for the four cultivars Medea, Grasslands Samson, Ceres One50 and Matrix at the two harvests (Harv1 and Harv2) in Experiment 5 103

Figure 4.16 A generalized relationship between soil texture and moisture contents at field capacity and permanent wilting point 108

Figure 4.17: A comparative response of shoot growth and root development traits in forage plants for drought and flooding. 109

Figure 5.1 A trend of traits of shoot growth (standardized data) of progeny in comparison with parents 126

Figure 5.2 A trend of traits of root development and water uptake (standardized data) in comparison with parents 127

Figure 5.3 A trend of traits of plant water status and stomatal and cellular control (standardized data) of progeny in comparison with parents....128

Figure 6.1 Layout used for plants in Experiment 4....135

Figure 6.2 Condition of plants from 3 plant genotypes of Family groups 7 and 8 in late March 2010 after remaining unwatered for over 90 days 145

Figure 7.1 A biplot of scores of Generation Canonical 1 with raw data for shoot DW (g) .... .161

Figure 7.2 Variation between individual plants of Grasslands Samson, Medea and their F_1 and F_2 hybrids in soil moisture depletion and herbage production a biplot of SMC d2 (%) and shoot DW (g). 162

List of Appendices

Appendix 3.1 SAS code for analysis of morphogenetic data of Experiment1 191

Appendix 4.1 ELISA scans for endophyte status of Grasslands Samson, Samson 4n and Medea 191

Appendix 5.1 Calculation of “pot field capacity” and for the amount of water to be topped up 192

Appendix 5.2 Partitioning of two ANOVAs in Experiment 3 (Chapter 5). 193

Appendix 6.1 Calculation of contribution of proline to osmotic potential 194

Appendix 8.1 Published paper 195

Glossary of Abbreviations

Abbreviation	**Full name/meaning**	**Units**
2n	Diploid	-
4n	Tetraploid	-
A_{Lf}	Leaf appearance interval	days leaf^{-1}
ANOVA	Analysis of Variance	-
Ci	Internal CO_2 concentration	Ppm
c.w.	Controlled watering	-
d1	Upper soil depth in experimental pots	-
d2	Middle soil depth in experimental pots	-
d3	Lower soil depth in experimental pots	-
DADW	Days after differential watering	
DR:S	Deep root (soil depths 2 and 3) to shoot ratio	-
DW	Herbage dry weight	G
EL	Electrolyte leakage	%
Evp	Evapotranspiration	m mol m^{2}s^{-1}
Fs	Site Filling	
FW	Fresh weight	g or mg
G. Samson	Grasslands Samson	-
Gener	Generation	
H%	Ratio of seed-head weight to shoot dry weight expressed as a percentage	
Harv	Harvest	-
HN	Seed-head number	Count
HN:TN%	Ratio of seed-head number to tillers number expressed as a percentage	
HW	seed-head weight	g
HW:HN	Ratio of seed-head weight to seed-head number	-
IndexDR	Index of deep rooting, i.e. ratio of root weight in depth2 and depth3 to total root weight	-
IndexWU	Index of water use; ratio of shoot dry weight to soil moisture content at soil depth 2	-
IRT	Infrared thermometer	-
Lcs	Leaf colour score	Score
Ldead%	Ratio of dead leaves to shoot dry weightexpressed as a percentage	%
Lds	Visual score for amount of leaf death	Score
LED	Leaf elongation duration	days
LER	Leaf extension rate	mm d^{-1}
LL	Leaf lamina length	mm
Llam%	Ratio of leaf lamina weight to shoot dry weight expressed as a percentage	%
LN	Whole plant leaf number	count
Lrs	Leaf rolling score	Score

LT	Leaf temperature	°C
LW	Leaf width	mm
LWP	Leaf water potential (often denoted Ψ)	MPa
Lws	Leaf wilting score	Score
MANOVA	Multivariate Analysis of Variance	
NLL	Number of live leaves	count
Ns	Non-significant	-
NZ	New Zealand	-
OA	Osmotic adjustment	-
OP	Osmotic potential (often denoted Ψ_p)	MPa
P	Probability	-
PC	Principal component	
PCA	Principal component analysis	-
PEG	Polyethylene glycol	-
Pn	Photosynthetic rate	μ mol m^{2}s^{-1}
PP	Pressure potential	MPa
Proline	Proline contents	mg g^{-1}.DW
Ps:Llam	Pseudostem:leaf lamina ratio	
PsL	Leaf pseudostem length	mm
R:S	Root Shoot Ratio	-
Rc d1	Coarse root weight at depth1	g
Rc d2	Coarse root weight at depth2	g
Rc d3	Coarse root weight at depth3	g
Rep	Experimental replication	-
Rf d1	Fine root weight at depth1	g
Rf d2	Fine root weight at depth2	g
Rf d3	Fine root weight at depth3	g
Rt	Total root weight	g
Rt d1	Total root weight at soil depth 1	g
Rt d2	Total root weight at soil depth 2	g
Rt d3	Total root weight at soil depth 3	g
RTAR	Relative tiller appearance rate	Tiller tiller^{-1} d^{-1}
RWC	Relative water content	%
SAS	Statistical Analysis System	-
SC	Stomatal conductance (often denoted g_c)	m mol m^{2}s^{-1}
SEM	Standard error of mean	-
SMC d1	Soil moisture content at depth1	%
SMC d2	Soil moisture content at depth2	%
SMC d3	Soil moisture content at depth3	%
SMD	Soil moisture deficit	-
SS	Sum of squares (in ANOVA)	
Str	Water deficit treatment	-
Tc-Ta	Canopy-Air temperature difference	°C
TDR	Time domain reflectometer	-
TFW	Turgor fresh weight	mg
T_L	Leaf temperature	°C
TN	Tiller number	count
TW	Tiller weight	g
Var	Cultivar	-
Var × Harv	Cultivar × harvest interaction	-

Var × Wreg	Cultivar × water regime interaction	-
Var × Wreg × Harv	Cultivar × water regime × harvest interaction	-
Wreg	Water regime	-
Wreg × Harv	Water regime × harvest interaction	-
WUE	Water use efficiency	-