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**Response to Water Deficit Stress
in the Native New Zealand Tussock,
*Festuca novae-zealandiae***

Submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

*Department of Plant Biology and Biotechnology
Massey University
Palmerston North
New Zealand*

Grant Andrew Abernethy

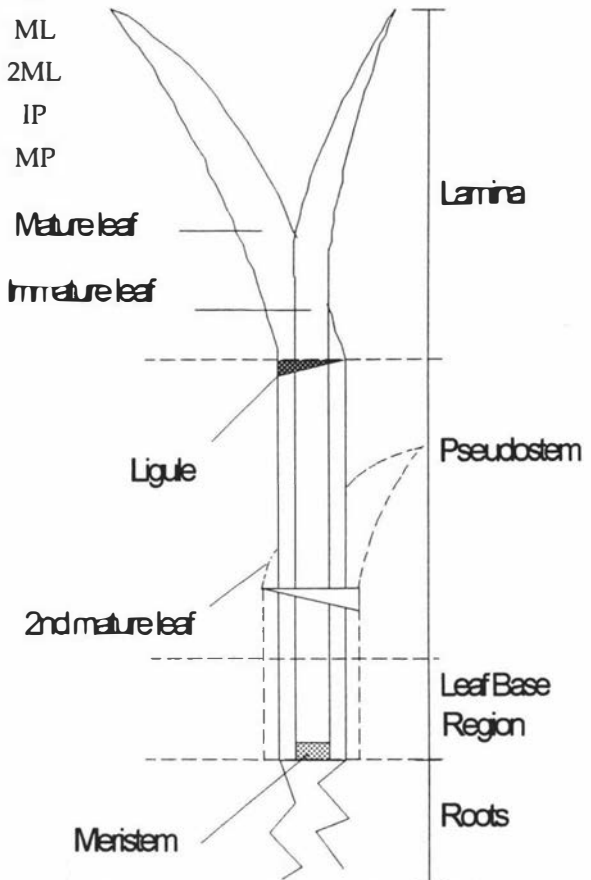
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Tiller region	Abbreviation
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Leaf Base Region	LB
Immature Lamina	IL
Mature Lamina	ML
Second Mature Lamina	2ML
Immature Pseudostem	IP
Mature Pseudostem	MP



Common Abbreviations

D_s	Mean degree of leaf tip scorching
H_L	Leaf hydration
LER	Leaf elongation rate
LWP	Leaf xylem water potential
M_T	Mean tiller mass
N_T	Mean number of green leaves per tiller
NSD	Not significantly different
RWC	Relative water content
SWC	Soil water content
T_{Num}	Mean number of green tillers per plant

Dedication

To my parents,
Maxwell and Stephanie Abernethy,
whose encouragement, support and advice
has made this dissertation possible.

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Abstract

Responses to water-deficit stress by a xerophytic tussock native to sub-alpine grasslands of New Zealand have been investigated. *Festuca novae-zealandiae* possesses some structural adaptations such as curled laminae, limited stomatal distribution, and dense trichomes characteristic of dry-land adapted species. These features were compared with those of a mesophyte, *F. arundinacea*. In addition to structural adaptations, evidence that metabolic changes contribute to the drought tolerance of *F. novae-zealandiae* was sought.

Plants of *F. arundinacea* and *F. novae-zealandiae* were grown in glasshouse pots and were subjected to water-deficit. Concentrations of the phytohormone ABA and the osmoprotectants proline and glycine-betaine increased in mature laminae of *F. arundinacea* as SWC declined to 8%. In *F. novae-zealandiae*, ABA concentration increased from 25-50 ng/gDW in two steps. At the first step (at 9-10% SWC), the increase was greatest in leaf bases (to 150 ng/gDW), and the second increase (at 6% SWC) was greatest in laminae (to 320 ng/gDW) and non-existent in leaf bases. In water-sufficient tillers, proline concentration was elevated in leaf bases (5-8 mg/g DW) compared to laminae (2 mg/g DW) and increased to 28 mg/g DW in leaf bases, and to 14 mg/g DW in laminae as SWC declined to 8-9%. Glycine-betaine concentration (40-100 μ mol/gDW) was unchanged by water-deficit stress.

Leaf and tiller counts, and estimates of leaf tip scorching indicated that leaf death progressed with increasing water-deficit. Although leaf elongation declined, it did not cease until the last tillers had died. SDS-PAGE and Western analysis of soluble proteins using antisera to ubiquitin indicated that protein turnover increased in lamina, but not in leaf bases in response to water-deficit. It was concluded that in response to water-deficit stress, leaf bases of *F. novae-zealandiae* retained turgor and remained viable at the expense of existing leaf laminae, which died back.

Southern analysis suggested that the *F. novae-zealandiae* genome contains sequence(s) with homology to group 2 LEA proteins. Western analysis using antisera raised against the dehydrin consensus sequence KIKEKLPG revealed several related proteins in seeds of *F. novae-zealandiae*, but these were not induced by water-deficit stress in leaves.

Table of Abbreviations

Abbreviation	Meaning
2ML	Second mature lamina
A _x	Absorbance at x nm
ABA	Abscisic acid ((±) 5-[1-Hydroxy-2,6,6,-trimethyl-4-oxocyclohex-2-en-1-yl]-3-methyl-[2Z,4E]-pentadienoic acid)
ANOVA	Analysis of variance
APS	Ammonium persulphate
AU	Absorbance unit
BCIP	5-bromo 4-chloro 3-indolyl phosphate
Bisacrylamide	N N'-Methylene bis-acrylamide
BSA	Bovine serum albumin
C _x	Watered control plant number x
c.f.	Compare with
D _s	Mean degree of leaf tip scorching
D _x	Drought treatment plant number x
DMSO	Dimethylsulphoxide
DW	Dry weight
EDC	1-Ethyl-3(3-dimethylaminopropyl)-carbodiimide
ELISA	Enzyme-linked immuno-sorbent assay
FDW	Freeze-dried weight
FW	Fresh weight
G-B	Glycine-betaine
GC	Gas chromatography
H _L	Leaf hydration
HPLC	High performance liquid chromatography
IAA	Indole acetic acid
IL	Immature lamina
IP	Immature pseudostem
kD	Kilodaltons
LER	Leaf elongation rate
LWP	Leaf water potential
MAb	Monoclonal antibody
ML	Mature lamina
MP	Mature pseudostem
N _T	Mean number of green leaves per tiller
n	Number of replicates
NBT	Nitro-blue tetrazolium, <i>p</i> -toluidine salt
NMR	Nuclear magnetic resonance
NPK	Nitrogen, phosphate, potassium ratio
NSD	Not significantly different
P	Probability

Abbreviation	Meaning
PBSalt	50mM sodium phosphate buffer, pH 7.4, containing 250mM NaCl
Pers. comm.	Personal communication
PMSF	Phenylmethanesulphonyl fluoride
Pro	Proline
PVC	Polyvinyl chloride
PVDF	Polyvinylidene difluoride
QAC	Quaternary ammonium compound
R _f	Relative distance, compared to solvent front
RIA	Radioimmunoassay
RSD	Relative standard deviation
RuBisCO	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RWC	Relative water content
s.e.	standard error of the mean
SDS	Sodium dodecyl sulphate
SDS- PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscope
Std. Dev.	Standard deviation
SWC	Soil water content
TDR	Time-domain reflectometer
TEMED	N N N' N'-tetramethylethylenediamine
TLC	Thin layer chromatography
Tris	Tris(hydroxymethyl)methylamine
T _{Num}	Mean number of green tillers per plant
Tween-20	Polyoxyethylenesorbitan monolaurate
UV	Ultra-violet radiation

S.I. Unit Abbreviations

The System Internationale (S.I.) symbol for the unit litre is by convention a lower case "L". However, in typed manuscript it is possible to misread this lower case letter as either the numeral "1", or as an upper or lower case "l". Therefore, to avoid such difficulties, the uppercase character has been used in this dissertation. Note that this convention applies to S.I. units sharing the base unit litre, such as millilitre (mL) or microlitre (μ L). All other quantities used in this volume are expressed in conventional S.I. units.

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