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Leaf Rubisco turnover variation in a perennial ryegrass (*Lolium perenne* L.) population: Analysis of quantitative trait loci, implications for productivity, and potential for manipulation

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

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Abstract

The Grasslands II perennial ryegrass (*Lolium perenne* L.) mapping population comprising F_1 progeny with the two parents (one plant each from the cultivars Samson and Impact) (Crush et al., 2007) was used to detect putative quantitative trait loci (QTL) for leaf Rubisco turnover and herbage yield traits. Rubisco turnover was described by three mathematical parameters: *d* (maximum Rubisco content), *g* (time of *d*) and *f* (a measure of curve width). All three parameters exhibited continuous variation among the F_1 progeny. Sixteen QTL were detected, seven for Rubisco turnover and nine for herbage yield traits. Support interval overlap on linkage group (LG) 1 and close location on LG2 for plant dry weight (DW) QTL in this study and in a previous analysis (Sartie, 2007) of the same mapping population suggests DW QTL stability across environments. Some QTL identified by Sartie (2007) were not re-confirmed in this study, but new QTL were identified. This suggests genotype x environment interaction generated by variable expression of genes in different environments. Clusters of QTL with overlapping support intervals were found on LG2 and LG5. The cluster on LG2 included QTL for herbage yield traits leaf lamina length (LL), tiller number (TN), productivity index (PI) and DW. The cluster on LG5 included QTL for DW, PI, TN, and *d*. These two regions offer potential for plant breeding applications. Apart from the QTL for *d* on LG5, there was no co-location of Rubisco turnover and herbage yield QTL. However, principal component analysis indicated plants with lower *d* tended to have higher DW; thus Rubisco turnover effects on plant productivity may relate to energy cost of Rubisco synthesis rather than photosynthetic capacity. DW was generally unrelated to *f* and *g;* therefore, hypothesised nitrogen use inefficiencies arising from premature Rubisco degradation, or retention of Rubisco at leaf senescence, were not confirmed. LG5 and LG7 on which QTL for *d* were located have conserved syntenic regions with rice chromosomes 8 and 9 where QTL for Rubisco content at different stages during heading were mapped by Ishimaru et al (2001a).

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Dedication

To my parents, brothers and sisters

Table of Contents

Abstract i
Acknowledgements ii
Dedication iv
Table of Contents v
List of Tables x
List of Figures xiv
List of Appendices xvi
Glossary of Abbreviations xix
Trait Abbreviations xxii
Definition of Terms xxiii

CHAPTER 1

Introduction 1
- 1.1. General background 1
- 1.2. Summary of objectives 3
- 1.3. Thesis structure 3

CHAPTER 2

Literature review 5
- 2.1. Grassland farming and forage crop improvement 5
- 2.2. Conventional forage breeding methods and challenges 7
- 2.3. DNA technology in forage grass breeding 9
  - 2.3.1. Molecular marker systems in perennial ryegrass 10
  - 2.3.2. Application of DNA marker systems 11
    - 2.3.2.1. Tagging economically important traits 11
    - 2.3.2.2. Marker assisted selection of quantitative traits 12
    - 2.3.2.3. Marker assisted gene introgression 12
    - 2.3.2.4. DNA profiling/DNA finger printing 13
    - 2.3.2.5. Construction of genetic linkage maps 14
- 2.4. Quantitative trait locus analysis 17
  - 2.4.1. Methods for QTL detection 18
  - 2.4.2. Experimental design factors 19

2.4.3. QTL validation 20
2.4.4. Heritability 21
2.4.5. QTL mapping *versus* Association mapping 21
2.5. Improvement of perennial ryegrass for sustainable pastoral systems 22
2.5.1. Molecular approach for the improvement of NUE in perennial ryegrass 22
2.5.2. Agronomic to physiological basis of NUE 23
2.5.3. QTL analysis of physiological traits related to NUE 24
2.6. Nitrogen remobilisation and NUE 26
2.7. Leaf development 27
2.7.1. Senescence and nutrient remobilisation 28
2.7.1.1. Chlorophyll degradation 29
2.7.1.2. Protein degradation pathways 31
2.8. Rubisco 33
2.8.1. Rubisco as a photosynthetic catalyst 33
2.8.2. Rubisco as a potential N storage protein 33
2.8.3. Rubisco turnover 34
2.8.3.1. Rubisco synthesis 34
2.8.3.2. Rubisco degradation 35
2.8.4. Rubisco in leaf ontogeny 38

CHAPTER 3

Rubisco turnover in barley (*Hordeum vulgare* L.) leaves at two levels of nitrogen nutrition 41
3.1. Introduction 41
3.1.1. Hypothesis 42
3.1.2. Experiment objectives 43
3.2. Materials and Methods 44
3.2.1. Plant materials and growth conditions 44
3.2.2. Sampling 45
3.2.3. Determination of total leaf N and Rubisco 45
3.2.3.1. Determination of N 46
3.2.3.2. Determination of Rubisco 46
3.2.4. Statistical analysis 47
3.3. Results 47
3.3.1. Change in fresh weight and total leaf N 47
3.3.2. Change in Rubisco content and model-derived Rubisco turnover characteristics 51

3.3.3. Decline in leaf N with age 56

3.4. Discussion 56

3.5. Conclusions 59

CHAPTER 4

Assessment of genotypic variation of Rubisco turnover in a sample of the Grasslands II perennial ryegrass mapping population........ 62

4.1. Introduction 62

4.2. Objectives 63

4.3. Materials and methods 63

4.3.1. Plant material 63

4.3.2. Growth conditions 64

4.3.3. Sampling 65

4.3.4. Determination of Rubisco 65

4.3.5. Morphological traits 65

4.3.6. Statistical analysis 65

4.4. Results 66

4.4.1. Genotypic variation in Rubisco turnover 66

4.4.2. Trait correlation analysis 67

4.4.3. Principal component analysis of Rubisco turnover, plant morphology and growth 69

4.5. Discussion 70

4.6. Conclusions 74

CHAPTER 5

Analysis of quantitative trait loci (QTL) for Rubisco turnover and herbage production traits in the Grasslands II perennial ryegrass mapping population 75

5.1. Introduction 75

5.2. Objectives 76

5.3. Materials and methods 76

5.3.1. Cultivation of plants 76

5.3.2. Growth conditions 78

5.3.3. Management and chemical input for weed, disease, and pest control 78

5.3.4. Tiller/leaf marking 78

5.3.5. Measurement of morphological traits and dry weight 79

5.3.6. Rubisco measurements 80

5.3.7. Statistical analysis 81

5.3.8. QTL analysis ... 81
5.4. Results ... 82
5.4.1. Phenotypic variation for traits in Experiment 3 ... 82
5.4.2. Correlations among traits measured in Experiment 3 ... 86
5.4.3. Principal component analysis of Experiment 3 traits ... 88
5.4.4. Cross-experiment correlations between morphological traits ... 89
5.4.5. QTL identification ... 93
5.4.5.1. QTL detection for Rubisco turnover parameters ... 93
5.4.5.2. QTL detection for herbage yield traits ... 94
5.5. Discussion ... 101
5.5.1. Repeatability and other experimental design considerations ... 101
5.5.2. Rubisco turnover in the mapping population ... 102
5.5.3. Rubisco turnover and herbage yield parameters ... 103
5.5.4. QTL discovery ... 107
5.5.4.1. Regulation of Rubisco turnover ... 108
5.5.4.2. Co-regulation of Rubisco turnover and herbage yield parameters ... 109
5.5.4.3. QTL for herbage yield traits ... 110
5.5.4.4. Coincidences of QTL for herbage yield traits in the studies ... 112
5.6. Conclusions ... 112

CHAPTER 6

Rubisco turnover characteristics of long-leaved and short-leaved perennial ryegrass populations after two and four cycles of divergent selection ... 115

6.1. Introduction ... 115
6.2. Objectives ... 115
6.3. Materials and methods ... 116
6.3.1. Plant material and growth conditions ... 116
6.3.2. Assessment of herbage yield characteristics ... 117
6.3.3. Sampling for Rubisco analysis ... 118
6.3.4. Determination of Rubisco content ... 118
6.3.5. Photosynthesis measurements ... 118
6.3.6. Statistical analyses ... 119
6.4. Results ... 119
6.4.1. Performance of the breeding populations ... 119
6.4.2. Correlation among Rubisco turnover and herbage yield traits ... 123
6.4.3. Principal component analysis of herbage yield and Rubisco turnover traits ... 123

    6.4.4. Photosynthesis 125
  6.5. Discussion 126
  6.6. Conclusions 130

CHAPTER 7

General discussion and conclusions 131

  7.1. Review of thesis objectives 131
    7.1.1. Rubisco turnover variation 131
    7.1.2. Rubisco turnover characteristics and plant yield 131
    7.1.3. Regulation of Rubisco turnover and NUE 132
      7.1.3.1. Possible syntenic relationships 133
      7.1.3.2. Effect of genotype x environment interactions 134
      7.1.3.3. Heritability estimates of Rubisco turnover 135
  7.2. Rubisco turnover model performance 135
  7.3. Physiological processes and Rubisco 136
  7.4. Conclusions 138
  7.5. Recommendations and further research 138

Bibliography 140

Appendices 173

List of Tables

Table 3.1: Ratio of Rubisco to total leaf N when Rubisco had reached a maximum; N- and N+ denote low N treatment and high N treatment, respectively. **54**

Table 3.2: Irving and Robinson (2006) Rubisco turnover model parameters for leaves of barley plants grown hydroponically under low (N-) and high (N+) N nutrition levels. Values were estimated from log-normal regression in Sigma plot. Values are means (n=3) except for Rubisco in the 4^{th} and 6^{th} leaves which were based on single leaf measurements. **55**

Table 3.3: The amount of total N and Rubisco-N retained in older leaves expressed as a percentage of the maximum measured in younger leaves at approximately 10 days of age. Quantities were calculated at a specified age (*) in each leaf category. **56**

Table 4.1: Genotype effects on Rubisco turnover curve parameters for 16 plant genotypes of the Grasslands II perennial ryegrass mapping population and the two parent plants of the population. Data are the range of genotype means and their SEM: *d* = curve height (mg Rubisco/leaf); *f* = measure of curve width; *g* = time of curve peak (days). **66**

Table 4.2: Correlation of Rubisco turnover and morphological and plant growth parameters for the two parent plants and 16 F_1 progeny of the Grasslands II perennial ryegrass mapping population: Mean values for morphological traits were taken from the autumn data of Sartie (2007). Alg, ligule appearance interval (days/leaf); Alf, leaf appearance interval (days/leaf); LER, leaf elongation rate (cm/day); LED, leaf elongation duration (days); LL, leaf lamina length (cm); TN, tiller number; TW, tiller weight (g); DW, herbage dry weight (g); *d*, curve height (mg Rubisco/leaf); *f*, measure of curve width; *g*, time of curve peak (days). **68**

Table 4.3: Principal component structure for the first five PCs of eleven variables of the two parent plants and 16 F_1 progeny of the Grasslands II mapping population: *d*, curve height (mg Rubisco/leaf); *f*, measure of curve width; *g*, time of curve peak (days); Alg, ligule appearance interval (days/leaf); Alf, leaf appearance interval (days/leaf); LER, leaf elongation rate (cm/day); LED, leaf elongation duration (days); LL, leaf lamina length (cm); TN, tiller number; TW, tiller weight (g); DW, herbage dry weight (g). **69**

Table 5.1: Genotype effects on Rubisco turnover and herbage yield traits for 135 genotypes of the Grasslands II perennial ryegrass mapping population and the Samson (P1) and Impact (P2) parent plants. Data are the range of genotype means and their SEM: TW = tiller weight (mg); LL = leaf lamina length (mm); TN = tiller number; DW = herbage dry weight (g); PI =

productivity index; *d* = maximum Rubisco content (mg/g leaf dry weight); *f* = curve width measure; *g* = time of *d* (days). **84**

Table 5.2: Coefficients of phenotypic correlations of the traits measured in the135 F_1 plants of the Grasslands II perennial ryegrass mapping population and the Impact and Samson parent plants. TW = tiller weight (mg); LL = leaf lamina length (mm); TN = tiller number; DW = herbage dry weight; PI = productivity index; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight). **87**

Table 5.3: Principal component analysis structure of the final data for 135 genotypes of Grasslands II perennial ryegrass mapping population and the Impact and Samson parent plants: TW = tiller weight; LL = leaf lamina length; TN = tiller number; DW = herbage dry weight; PI = productivity index; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight). **88**

Table 5.4: Coefficients of correlations among tiller weight data measured in 135 F_1 plants of the Grasslands II mapping population and the parent plants (Impact and Samson). TW = tiller weight (as in Table 5.2, 5.3), TW_{Large} = average weight of three largest tillers, TW_a and TW_s = tiller weight from Sartie's (Sartie, 2007) autumn and spring data, respectively. **90**

Table 5.5: Cross experiment correlations between Rubisco turnover and herbage yield traits of 135 progeny of the Grasslands II perennial ryegrass mapping population and the two parent plants: Agronomic traits were measured in a previous experiment (Sartie, 2007)^1. DW_a = herbage dry weight; LL_a = leaf lamina length; TN_a = tiller number; TW_a = tiller weight; PI_a = productivity index; Alf_a = leaf appearance interval; Alg_a = ligule appearance interval; LED_a = leaf elongation duration; LER_a = leaf elongation rate; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight).. **91**

Table 5.6: PCA structure involving a combination of traits measured in this study and those measured in the Sartie (2007) autumn study (coefficients in bold italics) for 135 progeny of the Grasslands II perennial ryegrass mapping population and the two parent plants. TW = tiller weight; LL = leaf lamina

length; TN = tiller number; DW = herbage dry weight; PI = productivity index; Alf_a = leaf appearance interval; Alg_a = ligule appearance interval; LED_a = leaf elongation duration; LER_a = leaf elongation rate; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight).. **92**

Table 5.7: QTL identified by simple interval mapping (SIM) and multiple QTL mapping (MQM) for Rubisco turnover and herbage yield traits for 135 progeny of the Grasslands II mapping population and the parent plants (Samson and Impact), assessed in a glasshouse between May and August 2007. TW = tiller weight; LL = leaf lamina length (mm); TN = tiller number; DW = herbage dry weight (g); PI = productivity index; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight); PVE = phenotypic variance explained. **96**

Table 5.8: Genotype class means and allelic effects associated with seventeen significant ($P < 0.05$) QTL for herbage yield and Rubisco turnover traits measured on 135 F_1 plants of the Grasslands II mapping population. Impact parent (I) alleles = a and b; Samson parent (S) alleles = c and d. TW = tiller weight; LL = leaf lamina length (mm); TN = tiller number; DW = herbage dry weight (g); PI = productivity index; *d* = maximum Rubisco content (mg/g of leaf dry weight); *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d*; PK = average of the two Rubisco content determinations closest to *d* (mg/g of leaf dry weight); TL = residual Rubisco content measured of the average of the last two time points (mg/g of leaf dry weight). **100**

Table 5.9: Correlations between principal component (PC) scores without (a) or with (b) plastochron data of Sartie (2007) included in the PCA. **104**

Table 6.1: Mean values, standard error (SEM), and effects of leaf length, cycle number, leaf length*cycle interaction of Rubisco turnover and herbage yield parameters of the four INRA perennial ryegrass breeding populations. Alf: leaf appearance interval (days/leaf), Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum leaf Rubisco concentration (mg/cm^2), *g*: time when *d* occurs (days), *f*: a measure of curve width....... **121**

Table 6.2: Trait correlation structure of the Rubisco turnover and herbage yield parameters of the four INRA perennial ryegrass breeding populations. Alf:

leaf appearance interval (days/leaf), Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum leaf Rubisco concentration (mg/cm^2), *g*: time when *d* occurs (days), *f*: a measure of curve width....... **122**

Table 6.3: Principal component analysis structure of Rubisco turnover and herbage yield parameters of the four INRA perennial ryegrass breeding populations. Alf: leaf appearance interval (days/leaf), Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum leaf Rubisco concentration (mg/cm^2), *g*: time when *d* occurs (days), *f*: a measure of curve width, PVE (%): percent variation explained... **124**

Table 6.4: Means of populations for principal component (PC) scores and statistical significance of population differences based on herbage yield traits and Rubisco turnover data of the four INRA perennial ryegrass breeding populations; LLC2: long-leaved 2nd cycle population, LLC4: long-leaved 4th cycle population, SLC2: short-leaved 2nd cycle population, SLC4: short-leaved 4th cycle population... **125**

List of Figures

Figure 3.1: Log-normal model for the time course of leaf Rubisco concentration (Irving and Robinson, 2006). Under this model, the rate of change in Rubisco concentration at any point in time is the balance between the rate of synthesis and the rate of degradation (assumed to be continous and concentration-dependent). The model is under debate because many researchers believe Rubisco degradation would be enzyme-mediated. In that case the expectation would be for leaf Rubisco concentration to plateau, and then fall suddenly late in the life of the leaf. **42**

Figure 3.2: Experiment 1 barley plants growing under two N treatments in the hydroponic system. Massey University Plant Growth Unit, Palmerston North, New Zealand, June 2006. .... **45**

Figure 3.3: Gel showing protein bands separated by SDS-PAGE and stained by Commassie brilliant blue (CBB). LSU represents Rubisco's large subunit**47**

Figure 3.4: Changes in fresh weight (A) and total N (B) of leaves at different positions in barley plants growing at low (N-) and high N (N+) nutrition levels. Points in each graph are means of 3 replicates (n=3). Bars at the right side of the graphs are the mean SE. **49**

Figure 3.5: The maximum fresh weights (A) and the maximum amount of total N (B) in leaf blades at different positions on the main tiller of barley plants growing at low N (N-) and high N (N+) nutrition levels. Each bar graph represents a mean value ± SE (n=3). Error bars represent 95% confidence interval estimates; bars with different letters represent significantly different means (Student's t-test, $P < 0.05$). **50**

Figure 3.6: Model-derived curves of changes in the content of Rubisco in the 4th (A) and 6th (B) leaf blades of barley plants growing at low N (N-) and high N (N+) nutrition levels. Data points in each graph are based on single leaf measurements. 52

Figure 3.7: Model-derived curves of changes in the content of Rubisco in the 8th (A) and 10th (B) leaf blades of barley plants growing at low N (N-) and high N (N+) nutrition levels. Data points in each graph are means (n=3). **53**

Figure 3.8: The maximum amount of Rubisco N in leaf blades of barley plants growing at low N (N-) and high N (N+) levels of nutrition. Each bar in the 8th and 10th leaves represents a mean value ± SE (n=3). Error bars represent 95% confidence interval of estimates; bars with different letters are significantly different (Student's t-test, $P < 0.05$). **54**

Figure 4.1: Grasslands II mapping population plants growing in the hydroponic system. Massey University Plant Growth Unit, Palmerston North, New Zealand. Experiment 2, November 2006. .... **64**

Figure 5.1: Grasslands II mapping population plants growing in the glasshouse 81 days after transplanting – Massey University Plant Growth Unit, Palmerston North, New Zealand (August 2007). **77**

Figure 5.2: Frequency distribution of the traits measured in the 135 Grasslands II mapping population progeny and the two parent plants. A = tiller weight; B = leaf lamina length; C = tiller number; D = plant dry weight; E = Productivity index; F = Rubisco peak; G = Rubisco curve; H = Time of Rubisco peak. The arrows show the F_1 progeny mean (green) and parental values, Impact (pink) and Samson (blue). **85**

Figure 5.3: Location of QTL for Rubisco turnover and herbage yield traits on the IxS reference genetic map (mainly SSR-based) of perennial ryegrass, based on the results of multiple QTL mapping (MQM). Molecular markers are indicated on the left of each linkage group (LG), and QTL are indicated as filled bars on the right. Bar lengths indicate a LOD drop of 2.0 on either side of the maximum likelihood position. Map distances are indicated by the centimorgan scale at the left. **99**

Figure 6.1: Perennial ryegrass plants of populations SLC2, SLC4, LLC2, and LLC4 in Experiment 4 growing in an environmentally controlled chamber - INRA, Lusignan, France. Light intensity was 500 µmol photons $m^{-2}s^{-1}$ at plant level. SL denotes 'short leaf', LL denotes 'long leaf', C2 indicates second selection cycle, and C4 indicates fourth selection cycle. **117**

Figure 6.2: Changes in net photosynthetic rates in fully expanded 3rd leaves of the four INRA breeding populations. Each point represents a mean value (n=3); Bars above the graphs are the mean SE at each evaluation time. LLC2: long-leaved 2nd cycle population, LLC4: long-leaved 4th cycle population, SLC2: short-leaved 2nd cycle population, SLC4: short-leaved 4th cycle population. **125**

List of Appendices

Appendix 3.1: Changes in fresh weight (g), total N (mg/leaf) and Rubisco content (mg N/leaf) in leaves at different positions of barley plants growing under low N (N-) and high N (N+) concentrations. Values are means (n=3) except for Rubisco in the 4th and 6th leaves which were based on single leaf measurements. N loss (%) represents the proportion of the maximum amount of total N/Rubisco-N lost at the leaf age indicated by the asterisk (*). **173**

Appendix 4.1: Principal component structure of the first five PCs for ten variables measured in 200 genotypes of the Grasslands II mapping population in autumn 2003 (Sartie, 2007). LER, leaf elongation rate (cm/day); LED, leaf elongation duration (days); Alg, ligule appearance interval (days/leaf); LL, leaf lamina length (cm); Alf, leaf appearance interval (days/leaf); TN, tiller number; TW, tiller weight (g); PI, productivity index; CP, crude protein content; DW, herbage dry weight (g)......... **175**

Appendix 4.2: Principal component scores of the first five PCs for ten variables measured in 200 genotypes of the Grasslands II mapping population in autumn 2003 (Sartie, 2007). Variation explained: PC1 (29.8%), PC2 (19.6%), PC3 (14.7%), PC4 (9.4%), and PC5 (8.7%)......................... **176**

Appendix 4.3: ANOVA of Rubisco turnover parameters measured in the 16 genotypes of the Grasslands II mapping population. *d* = curve peak (mg Rubisco-N/leaf); *f* = measure of curve width; *g* = time of curve peak (days)... **181**

Appendix 4.4: Principal component scores of the first five PCs for the 16 genotypes of the Grasslands II mapping population and the two parent plants. Variation explained by each PC; PC1 (35.6%), PC2 (22.2%), PC3 (15.9%), PC4 (10.1%), and PC5 (7.4%). **182**

Appendix 5.1: Mean daily temperature record in the glasshouse during the period of assessment of the Grasslands II perennial ryegrass mapping population for Rubisco turnover and herbage yield traits in 2007. Recording time was May 28 to August 31 2007... **183**

Appendix 5.2: Solar radiation measurements in the glasshouse during the period of assessment of the Grasslands II perennial ryegrass mapping population for Rubisco turnover and herbage yield traits in 2007 .... **183**

Appendix 5.3: The final data of herbage yield and Rubisco turnover measurements of the 135 F_1 and the two parent plants of Grasslands II perennial ryegrass mapping population plants. TW = tiller weight (mg); TW_{Lg} = average weight of the three largest tillers (mg); LL = leaf lamina length (mm); TN = tiller number; DW = herbage dry weight (g); PI = productivity index; *d* = maximum Rubisco content (mg N/g leaf dry weight) ; *f* = curve width measure; *d*/*f* = *d*:*f* ratio; *g* = time of *d* (days); PK = average of the two Rubisco content determinations closest to *d* (mg N/g leaf dry weight); TL = residual Rubisco content measured as the average of the last two time points (mg N/g leaf dry weight)................................... **184**

Appendix 5.4: ANOVA of the herbage yield traits measured in the 135 genotypes of the Grasslands II mapping population in 2007. TW = tiller weight (mg); TW_{Lg} = average weight of the three largest tillers (mg); TN = tiller number; PI = productivity index; DW = herbage dry weight (g); LL = leaf lamina length (mm). **187**

Appendix 5.5: PC scores of the first the first four PCs for herbage yield and Rubisco turnover measurements on the 135 F_1 and the two parent plants of the Grasslands II perennial ryegrass mapping population. Variation explained by each PC: PC1 (35.9%), PC2 (23.3%), PC3 (13.9%), PC4 (10.2%). **188**

Appendix 6.1: ANOVA of the herbage yield traits and Rubisco turnover parameters measured in the four INRA perennial ryegrass mapping populations. Alf: leaf appearance interval (days/leaf), Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum Rubisco content (mg/cm^2), *f*: a measure of curve width, *g*: time when *d* occurs (days). **191**

Appendix 6.2: Correlation analysis of the parameters of four INRA perennial ryegrass breeding populations Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum leaf Rubisco concentration (mg N/g leaf fresh weight), *f*: a measure of curve width, *g*: time of *d* (days). **193**

Appendix 6.3: Correlation analysis of the parameters of four INRA perennial ryegrass breeding populations Alg: ligule appearance interval (days/ligule), LED: leaf elongation duration (days), TN: tiller number, DW: herbage dry weight (g), TW: Tiller weight (g), LL: Leaf lamina length (cm), LER: leaf elongation rate (cm/day), *d*: maximum leaf Rubisco concentration (mg N/g leaf), *f*: a measure of curve width, *g*: time of *d* (days). **194**

Appendix 6.4: PC scores for the first four PCs for herbage yield and Rubisco turnover measurements taken on four INRA perennial ryegrass populations.

Variation explained by each PC: PC1 (32.8 %), PC2 (21.8%), PC3 (17.1%), PC4 (10.5%). **195**

Appendix 6.5: Mean values of light-saturated net photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) of the third leaves of the four INRA perennial ryegrass breeding populations. SLC2: short-leaved 2nd cycle population, SLC4: short-leaved 4th cycle population, LLC2: long-leaved 2nd cycle population, LLC4: long-leaved 4th cycle population. **196**

Glossary of Abbreviations

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
C	Carbon
CAPS	Cleaved amplified polymorphic sequences
CBB	Coomassie Brilliant Blue
CIM	Composite interval mapping
cM	Centimorgan
CO_2	Carbon dioxide
DH	Doubled haploid
DM	Dry matter
DMY	Dry matter yield
DNA	De-oxyribonucleic acid
DW	Dry weight
EST	Expressed sequence tags
FCC	Fluorescent chlorophyll catabolite
Fd	Ferredoxin
GDH	Glutamate dehydrogenase
GISH	Genomic *in situ* hybridization
GOGAT	Glutamate synthetase
GS	Glutamine synthetase
LHCII	Light harvesting complex of photo-system II
LD	Linkage disequilibrium
LDS	Lithium dodecyl-sulphate
LRS	Likelihood ratio statistic
LOD	Logarithm-of-odds
LPSSR	*Lolium perenne* simple sequence repeats
LSU	Large subunit
MAS	Marker assisted selection
MIM	Multiple interval mapping
MQM	Multiple QTL mapping
mRNA	Messenger RNA
N	Nitrogen

NADH	Nicotinamide adenine dinucleotide
NCC	Non-fluorescent chlorophyll catabolite
NH_3	Ammonia
NH_4^+	Ammonium
NIL	Near-isogenic line
NiR	Nitrite reductase
NIRS	Near infra-red spectrometry
NO_3^-	Nitrate
NR	Nitrate reductase
NRE	Nitrogen remobilisation efficiency
NUE	Nitrogen use efficiency
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PS	Photo-system
PVE	Phenotypic variation explained
QTL	Quantitative trait loci/locus
RAPD	Randomly amplified polymorphic DNA
RBC	Rubisco containing bodies
RCBD	Randomised complete block design
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RCC	Red chlorophyll catabolite
RCCR	Red chlorophyll catabolite reductase
ROS	Reactive oxygen species
SAG	Senescence-associated genes
SCAR	Sequence characterised amplified region
SDC	Size/density compensation
SDS-PAGE	Sodium dodecyl-sulphate polyacrylamide gel electrophoresis
SIM	Simple interval mapping
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
SSU	Small subunit
STS	Sequence tagged site

Ub	Ubiquitin
UV-B	Ultraviolet B
WSC	Water soluble carbohydrate

Trait Abbreviations

Alf	Leaf appearance interval (days)
Alg	Ligule appearance interval (days)
CP	Crude protein content
d	Rubisco peak (mg/leaf, mg/g leaf dry weight, mg/cm^2)
DW	Plant dry weight (g)
f	Rubisco curve width (log of days)
g	Time of Rubisco peak (days)
LED	Leaf elongation duration (days)
LER	Leaf elongation rate (cm/day)
LL	Leaf length (cm)
PI	Productivity index
TW	Tiller weight (mg, g)
TN	Tiller number

Definition of Terms

Allele	An alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome.
Epistasis	An interaction between nonallelic genes, especially an interaction in which one gene suppresses the expression of another.
Gene	A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.
Heritability	The proportion of phenotypic variance accounted for by additive genetic effects.
Linkage	The tendency of two or more genes to remain together in the original combination in the same chromosome during the process of inheritance for a number of generations.
Locus	The specific site/place of a gene on a chromosome.
Pleiotropy	The control of more than one phenotypic characteristic by a single gene or set of genes.
Polymorphism	A difference in DNA sequence among individuals, groups, or populations.
Recombination	The process by which new combinations of parental genes or characters arise by independent segregation of unlinked loci or by cross-over between loci that are linked.
Trait	A genetically determined characteristic or condition.