

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.

**QUANTITATIVE ANALYSIS OF GENETIC
VARIABILITY IN FLORAL AND GERMINATIVE
CHARACTERISTICS OF MEADOWFOAM
(*LIMNANTHES ALBA*)**

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Plant Science
at Massey University

Canhong Cheng

1997

Abstract

Genetic and physiological aspects of flowering, seeding, seed shattering and dormancy of meadowfoam have been studied to provide information on the new crop meadowfoam, and to assist in its domestication and early breeding.

Two hundred and fifty-nine plants were chosen randomly to form an unbiased sample representing the germplasm of eight divergent meadowfoam accessions, which had been intermated to form a composite. Thirteen characters associated with flowering and seeding were involved. Both principal components and clustering proved to be efficient analyses which maximized the discrimination of differences amongst plants in this segregating population. These procedures recognized 190 phenotypes within this germplasm. A high level of significant genotypic variance in all characters was found. Moreover, environmental variances were generally lower in size, so that most characters had high heritability ($h^2 > 0.800$). Early seed set was an exception, with heritability of only 0.446. The results showed that meadowfoam should respond well to selection.

A special feature of this germplasm analysis was the use of cluster analysis, to define “natural” groups. This involved truncating the cluster dendrogram on the basis of minimum F-probability of amongst-cluster to within cluster mean-squares. Further refinement was based on the maximum genotypic variance (σ_G^2), phenotypic variance (σ_P^2) and heritability (h^2), at or near the original truncation.

Another innovation was the use of principal components analysis to identify patterns of fresh flowers, total flowers and seed set. Variability in floral initiation, general flowering time or seed set was randomly distribution, which indicated that the base population was panmictic: one large segregating gamodeme. This pattern analysis was superior to regressions (over time), because there were few time-nodes, and it avoided the need to find optimum functions.

Next, germination and dormancy of this gene-pool were studied, requiring extensive use of the Richards functions and MANOVA. These analyses provided better discrimination amongst treatments than did the univariate character “final germination level”.

Dormancy-breaking for meadowfoam was found to be possible by using KNO_3 and/or GA_{4+7} in darkness at $10/5^\circ\text{C}$, without prechilling. This special test assesses whole-seed germinative maturity; the difference between this and standard germination provides an estimate of dormancy. The results indicated that biosynthesis of GAs and/or sensitivity to GAs were probably involved in meadowfoam germination.

A representative sample gene-pool for study of seed development was established using principal components, giving eight half-sib lines. Harvest ripeness (decline in seed moisture to 12.5%) was compared with seed growth (dry mass), and line differences were found. Standard and special germination tests showed that primary dormancy developed in seeds before they were harvest ripe. Dormancy varied in the eight lines.

ABA content was assayed by ELISA, and principal components and cluster analyses followed using ABA peak measurements (peak height, width and time reaching peak). There were four ABA groups for the eight lines. However, a clear link between ABA pattern and dormancy breaking was not established. Difference in sensitivity to ABA may explain why this was the case.

The results indicated the presence of considerable genotypic variation, allowing estimates of heritability for the development traits. Although the contrast of variance from $\hat{Y}$ (regression) with pooled $\sigma^2_{\hat{Y}}$ was novel, it conformed with biological expectation, and compared well with results from wheat. Heritability was high for harvest-ripe seed mass (0.9238), showing a strong genetic control. In contrast, seed moisture heritability was low (0.3292), indicating considerable environmental influence. Heritability of germinative maturity (dormancy-breaking test on 5 weeks storage seed) was very high (0.9437), indicating that this is a credible measure of true physiological maturity. The heritability of dormancy-breaking was also high (0.9306) after 5 weeks storage. Conversely, after 15 weeks storage, both germinative maturity and dormancy-breaking heritability were low. This indicated that prolonged storage effects were more environmental than genetic.

The general levels of genetic influences (variances and heritabilities) indicate that there is much potential for changing (domesticating) meadowfoam in these characters.

Acknowledgments

I gratefully acknowledge the untiring advice, understanding, constructive criticism and encouragement from Dr. I. L. Gordon, my chief supervisor.

My gratitude and appreciation is also extended to the following people for their contributions toward the completion of this thesis.

My supervisor, Dr. D. J. Woolley for his advice, understanding, criticism and readiness to help.

Dr. P. Coolbear for helpful advice and discussion, especially in the planning of the treatments of seed germination.

Mrs. K.A. Hill, Messers C. R. McGill, M. Currie, C. Rawlingson for occasional technical assistance. Mr. G. Oliver, Ms. L. Barton, B. Rodgers, and G. Gielen, for proof-reading of this thesis.

Past and present members and postgraduate students of the Plant Science, and fellow Chinese students for their friendship and encouragement.

The National Climate Laboratory, HortResearch-Batchelar, for providing one of the controlled environment rooms for me to conduct the experiment of seed development. The Doctoral Scholarship and Graduate Research Awards of Massey University, AGMART and the Department of Plant Science for financial and facility support.

My husband, Hailong, my son, Samuel, and my parents for their love, understanding and encouragement during my PhD study.

Table of contents

Abstract.....	ii
Acknowledgments	iv
Table of contents	v
List of tables.....	x
List of figures.....	xiii
Chapter 1. Introduction.....	1
Chapter 2. Review of Literature	4
2.1 Background of Meadowfoam.....	4
2.2 Seed development	6
2.3 Seed Dormancy	8
2.3.1 Definition and types of dormancy.....	8
2.3.2 Development of primary dormancy	9
2.3.2.1 Genetic and correlative effects on dormancy	9
2.3.2.2 The environmental control of dormancy inception	11
2.4 Effects of hormones on seed dormancy	12
2.4.1 Absciscic acid (ABA).....	13
2.4.2 Gibberellic acid (GA).....	17
2.4.3 Cytokinins	20
2.5 Environmental effects on dormancy release	23
2.5.1 Low temperature (chilling)	23
2.5.2 Alternating temperature	24
2.5.3 Light.....	25

2.5.4 Afterripening	26
2.6 Breaking of dormancy by potassium nitrate	29
2.7 Quantitative genetic analysis.....	32
2.7.1 Estimation of genetic variances	32
2.7.2 Heritability and its standard error.....	34
2.7.3 Heritability of dormancy and germinability	36
2.8 Multivariate analysis	36
2.8.1 MANOVA	37
2.8.2 Discriminant analysis	38
2.8.3 Principle component analysis	39
2.8.4 Cluster analysis	40
2.9 Quantification of biology	41
 Chapter 3. Meadowfoam germplasm	44
3.1 Introduction.....	44
3.2 Material and methods.....	45
3.2.1 Plant material.....	45
3.2.2 Characters studied.....	45
3.2.2.1 Flowering and seed set	46
3.2.2.2 Yield and yield components after harvest.....	46
3.2.2.3 Seed shattering	46
3.3 Data analysis	47
3.3.1 Multiple regression.....	47
3.3.2 Pattern analysis.....	47
3.3.3 Clustering phenotypes	48
3.3.4 Estimation of variances.....	51
3.3.5 Distribution of data.....	52
3.4 Results	52
3.4.1 Pattern analysis.....	52
3.4.2 Distribution of data.....	56

3.4.3 Clustering phenotypes	59
3.4.3.1 Second principal components analysis	59
3.4.3.2 Clustering and truncation of dendrogram	61
3.4.4 Quantitative genetic analysis	63
3.4.4.1 Variance components	63
3.4.4.2 Heritability	64
3.5 Discussion.....	78
3.5.1 Distribution of data.....	78
3.5.2 Pattern analysis and clustering.....	79
3.5.2.1 Principal components analysis.....	79
3.5.2.2 Cluster analysis	80
3.5.2.3 Truncation of dendrogram via “natural groups”	80
3.5.3 Genetic analysis.....	82
3.5.3.1 Genetic variances	82
3.5.3.2 Heritability	82
Chapter 4. Seed germination.....	85
4.1 Introduction.....	85
4.2 Materials and Methods.....	86
4.2.1 Experiment design	86
4.2.2 Seed germination and treatments.....	87
4.2.3 Data analysis.....	88
4.2.3.1 Curve fitting for seed germination	88
4.2.3.2 Analysis of variance	92
4.2.3.3 Multivariate function analysis -- extra issues.....	93
4.2.3.3.1 Tests of Wilks' Lambda for special cases	93
4.2.3.3.2 Discriminant analysis.....	94
4.3 Results.....	97
4.3.1 Curve fitting	97
4.3.2 Analysis of variance	97

4.3.2.1 Viability	104
4.3.2.2 Final germination	105
4.3.3 Germination profiles	108
4.3.3.1 Main effects	108
4.3.3.2 First-order interaction.....	114
4.3.3.3 Second-order interaction	118
4.3.3.4 Third-order interaction	124
4.4 Discussion	128
4.4.1 Optimum dormancy-breaking germination	128
4.4.2 The effect of seed-colour on germination.....	130
4.4.3 Biological applications of the quantitative analysis of seed germination.....	130
4.4.4 Mechanisms on seed dormancy of meadowfoam.....	133
4.4.5 Germination testing and dormancy	135
 Chapter 5. Seed development and afterripening.....	136
5.1 Introduction.....	136
5.2 Material and methods	138
5.2.1 Choosing eight lines	138
5.2.2 Seed development.....	139
5.2.3 Afterripening	141
5.2.4 Data analysis.....	141
5.2.4.1 Curve fitting	141
5.2.4.2 Criteria of “best fit”	142
5.2.4.3 Regression equations.....	142
5.2.4.4 Tests for outlying observations.....	143
5.2.4.5 Profile analysis	145
5.2.4.6 Estimate of heritability	146
5.3 Seed ABA analysis.....	148
5.3.1 ABA extraction and purification.....	148
5.3.2 ELISA assay procedure	149

5.3.3	Validation of ELISA assay for ABA of meadowfoam seed	151
5.3.4	Analysis of ABA Curves	153
5.4	Results.....	155
5.4.1	Seed dehydration	155
5.4.2	Seed dry mass.....	165
5.4.3	Seed dormancy	167
5.4.4	Seed germination (afterripening)	168
5.4.4.1	Functions of seed germination comparison	173
5.4.4.2	Dormancy release by afterripening.....	174
5.4.5	Measurement and comparison of seed ABA.....	178
5.5	Discussion	185
5.5.1	Seed development.....	185
5.5.2	Seed ABA content	186
5.5.3	The relationship between endogenous ABA and seed dormancy	187
5.5.4	Heritability measurements	191
Chapter 6	General discussion	193
6.1	Potential for meadowfoam domestication.....	193
6.2	The role of statistical analysis in plant breeding	196
6.3	Scope for future research.....	198
References		199
Appendices		225

List of tables

Table 3.1	The description of parental lines.	45
Table 3.2	The standardized β of multiple regression for seed yield against yield components.	48
Table 3.3	Flowering and seeding behaviours, as observed through PC analysis on fresh flowers, total flowers and seed set.	49
Table 3.4	General ordination of plant via PC analysis over thirteen characters.	50
Table 3.5	Sources of variation, degrees of freedom, expectations of mean squares and variance component of CRD analysis.	51
Table 3.6	Factor structure matrix and standard coefficient for fresh, total flowers and seed set.	53
Table 3.7	Frequency of plants (%) around mean value for 13 characters.	56
Table 3.8	Factor structure matrix for thirteen characters.	59
Table 3.9	Standard coefficient for thirteen characters.	60
Table 3.10	Number of clusters and phenotype groups at various cluster stages.	61
Table 3.11	Significances of differences between genotypic variances and phenotypic variances, and the heritability at stages 58 and 124.	63
Table 3.12	Genotypic variances, phenotypic variances, the heritability and their standard errors for 13 characters at stage 58 (the optimum cut-off point of clustering).	64
Table 4.1	The interpretation system of the importance of parameters to discriminant scores (I.L. Gordon, pers. comm.).....	96
Table 4.2	The observations of final germination and the parameters of Richards function and their standard errors for 192 experimental units.	98
Table 4.3	Significance in analysis of variance for seed germination.	102
Table 4.4	The effect of interaction between light, chilling and seed colour on seed viability.	104
Table 4.5	Main effect of imbibants on final germination of seeds.	106
Table 4.6	Main effects of temperature and chilling on final germination.	106

Table 4.7	Main effects of light and seed-colour as well as interaction amongst light, chilling and seed-colour treatments on final germination.	106
Table 4.8	Final germination of seed at various temperature, light, chilling, imbibant and seed-colour.....	107
Table 4.9	Parameter combinations of Richards' function for seed germination.	110
Table 4.10	The effects of temperature, light, chilling, imbibant and seed colour on the time required to reach 5% and 95% of upper asymptote of germination.	111
Table 4.11	The importance of parameters of Richards' function contributed to discriminant scores of MANOVA.....	117
Table 4.12	Two discriminant scores for combination of imbibant and seed-colour.....	118
Table 5.1	Comparison of regressions for the (+)ABA standards with or without added seed extract.....	153
Table 5.2	Estimated statistics of final curves (linear form) describing changes in seed moisture during seed development in 8 lines.....	155
Table 5.3	The differences between functions of 8 lines for seed moisture.	164
Table 5.4	The number of days from anthesis to harvest ripeness and seed moisture (%) at 86 days after anthesis for 8 lines.	164
Table 5.5	Variance components and heritability estimates for seed moisture at 86 days after anthesis.....	164
Table 5.6	Estimated statistics of final curves (linear form) describing changes in seed dry mass during seed development in 8 lines.....	166
Table 5.7	The differences between functions of 8 lines for seed dry mass.	166
Table 5.8	Seed dry mass (mg) at harvest ripeness (HRDW) and at 86 days after anthesis for 8 lines.....	166
Table 5.9	Variance components and heritability estimates for seed dry mass at 86 days after anthesis.....	167
Table 5.10	Estimated statistics of final curves describing changes in seed germination during seed development various treatments and lines.....	168
Table 5.11	The differences between germination functions of 8 lines for three treatments.	173
Table 5.12	The differences between functions of the three treatments for 8 lines.....	174

Table 5.14 The differences of germination after 5 and 15 weeks storage amongst various treatments for 8 lines. 176

Table 5.15 Variance components and heritability estimates of seed germination for three treatments at 5 and 15 weeks storage. 176

Table 5.16 PC analysis for ABA peaks of 8 lines. 178

Table 5.17 Factor structure matrix and factor scores for ABA peaks of 8 lines. 178

List of figures

Figure 3.1	Scatterplots of plants for fresh flowers against PC1 and PC2.	54
Figure 3.2	Scatterplots of plants for total flowers against PC1 and PC2.....	55
Figure 3.3	Scatterplots of plants for seed set against PC1 and PC2.....	55
Figure 3.4	Histogram and frequency polygon for PC1 and PC2 of fresh flowers, total flowers and seed set.	57
Figure 3.5	Histogram and frequency polygon for 7 characters.	58
Figure 3.6	Variance components and heritability for FFPC1 (median flowers).	65
Figure 3.7	Variance components and heritability for FFPC2 (flowering extremities). ..	66
Figure 3.8	Variance components and heritability for TFPC1 (flower production).....	67
Figure 3.9	Variance components and heritability for TFPC2 (early or/and late flowers).68	
Figure 3.10	Variance components and heritability for SETPC1 (median and late seed set).....	69
Figure 3.11	Variance components and heritability for SETPC2 (early seed set).....	70
Figure 3.12	Variance components and heritability for maximum seed set (SETMAX).71	
Figure 3.13	Variance components and heritability for harvest seed shattering (HSH). 72	
Figure 3.14	Variance components and heritability for seed set after harvest (LSET). . 73	
Figure 3.15	Variance components and heritability for flowers plant-1 after harvest (FLOWS).	74
Figure 3.16	Variance components and heritability for seeds plant-1 after harvest (SEEDS).....	75
Figure 3.17	Variance components and heritability for 100 seed weight (SEEDWT)... 76	
Figure 3.18	Variance components and heritability for seed yield plant-1 after harvest (YLD).....	77
Figure 4.1	The fitted curves of seed germination fitting in Richards function.	103
Figure 4.2	The mean Richards functions of seed germination for four imbibants.....	112
Figure 4.3	The mean Richards functions of seed germination for two temperature treatments.	112

Figure 4.4	The mean Richards functions of seed germination for darkness and light conditions.	113
Figure 4.5	The mean Richards functions of seed germination for two chilling treatments.	113
Figure 4.6	The mean Richards functions of seed germination for green and brown seed.	114
Figure 4.7	The mean Richards functions of seed germination for interaction between temperature and light.	115
Figure 4.8	The mean Richards functions of seed germination for interaction between temperature and seed colour.	115
Figure 4.9	The mean Richards functions of seed germination for interaction between temperature and imbibant.	119
Figure 4.10	The mean Richards functions of seed germination for interaction between darkness and imbibant.	120
Figure 4.11	The mean Richards functions of seed germination for interaction between imbibant and seed colour.	121
Figure 4.12	The results of Hotelling T ² for imbibant x seed-colour in discriminant plane (No significant differences within a circle).	122
Figure 4.13	The mean Richards functions of seed germination for interaction between temperature, chilling and seed colour.	123
Figure 4.14	The mean Richards functions of seed germination for interaction between darkness, chilling and seed colour.	125
Figure 4.15a	The mean Richards functions of seed germination for interaction between temperature, light, imbibant and seed colour.	126
Figure 4.15b	The mean Richards functions of seed germination for interaction between temperature, light, imbibant and seed colour.	127
Figure 5.1	A typical curve of (+)-ABA standard by ELISA.	152
Figure 5.2	Validation of the method of ABA analysis for meadowfoam seeds.	152
Figure 5.3	The functions of seed dry mass and seed moisture for Line 1.	156
Figure 5.4	The functions of seed dry mass and seed moisture for Line 2.	157
Figure 5.5	The functions of seed dry mass and seed moisture for Line 3.	158
Figure 5.6	The functions of seed dry mass and seed moisture for Line 4.	159

Figure 5.7	The functions of seed dry mass and seed moisture for Line 5.....	160
Figure 5.8	The functions of seed dry mass and seed moisture for Line 6.....	161
Figure 5.9	The functions of seed dry mass and seed moisture for Line 7.....	162
Figure 5.10	The functions of seed dry mass and seed moisture for Line 8.....	163
Figure 5.11	The functions of seed germination various treatments for Line 1.	169
Figure 5.12	The functions of seed germination various treatments for Line 2.	169
Figure 5.13	The functions of seed germination various treatments for Line 3.	170
Figure 5.14	The functions of seed germination various treatments for Line 4.	170
Figure 5.15	The functions of seed germination various treatments for Line 5.	171
Figure 5.16	The functions of seed germination various treatments for Line 6.	171
Figure 5.17	The functions of seed germination various treatments for Line 7.	172
Figure 5.18	The functions of seed germination various treatments for Line 8.	172
Figure 5.19	Time-dependent changes in ABA content of developing seeds for Line 1.	180
Figure 5.20	Time-dependent changes in ABA content of developing seeds for Line 2.	180
Figure 5.21	Time-dependent changes in ABA content of developing seeds for Line 3.	181
Figure 5.22	Time-dependent changes in ABA content of developing seeds for Line 4.	181
Figure 5.23	Time-dependent changes in ABA content of developing seeds for Line 5.	182
Figure 5.24	Time-dependent changes in ABA content of developing seeds for Line 6.	182
Figure 5.25	Time-dependent changes in ABA content of developing seeds for Line 7.	183
Figure 5.26	Time-dependent changes in ABA content of developing seeds for Line 8.	183
Figure 5.27	Distribution of the lines according to their response patterns on the basis of the first two PC scores.	184
Figure 5.28	Dendrogram of cluster analysis by Ward's method for ABA content patterns during seed development.....	184