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

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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°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.

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Chapter 1. Introduction

Meadowfoam (*Limnanthes alba* Benth) is a herbaceous winter annual, native to the Pacific Northwest United States of America. Substantial interest in meadowfoam as a source of industrial oil was aroused when the oil was used to derive liquid wax esters which had properties very similar to those of jojoba (*Simmondsia chinensis*) (Miwa and Wolff, 1962). Efforts to reduce the harvest of whales brought new attention to meadowfoam as a potential substitute for sperm whale oil (Jolliff, 1984).

The seed oil of meadowfoam is a source of highly saturated long chain (C_{20} and C_{22}) fatty acids (Chang and Rothfus, 1977). These particular fatty acids constitute more than 90% of the lipids as compared with 40% for herring oil; the amount of eicosenoic acid (20:1) is especially high (Jolliff *et al.,* 1981). Because seed oil of meadowfoam exhibits stability at high temperature and pressure, this oil has potential industrial applications, including high-quality waxes, lubricants in high-performance engines, detergents and plasticisers (Higgins *et al.*, 1971). Currently, meadowfoam oil crops are primarily consumed by the cosmetics industry (Jolliff per. comm., 1996).

Meadowfoam has a growing season and harvest period similar to winter grains. The plants appear to be efficient in synthesis of dry matter and to produce a high ratio of seed to vegetative dry matter (Jolliff *et al.*, 1981). In addition, there is no crop residue problem after meadowfoam harvest, since the plant is not fibrous, and threshing pulverises the dry vegetation, leaving only a trace of fine particles. Furthermore, de-oiled seed residue can be converted to a feedstuff for broiler chicks, weanling rabbits and sheep (Throckmorton *et al.*, 1980; 1981). Because of this potential, meadowfoam should be considered as a great prospect for diversification of New Zealand's agriculture. Limited experience in New Zealand suggests that meadowfoam grows well, revealing genuine potential as a new crop.

Meadowfoam is still a wild plant. Its domestication will not be easy, because it has some traits which provide a challenge, such as seed dormancy, seed shattering and low yield. Domestication of any wild plants for development as a new crop is of particular interest to

plant breeders. The source of germplasm, with favourable genetic variability, will play a key role in determining the efficiency and potential accomplishments during domestication. Other very important issues are the breeding method and system of evaluation. Meadowfoam has great morphological and physiological variation, possibly as a result of the adaptation to its native conditions (Gentry and Miller, 1965). Variation has occurred in response to seasonal rain, elevation and soil composition. The wild populations of meadowfoam are also variable in plant characteristics (Brown *et al.*, 1979; Brown and Jain, 1979; Chozin, 1990). This variation has provided a basis for progress in selecting for characteristics favourable for field production, resulting in the early release of improved varieties. Jolliff (1989) reported that no major biological barriers to high oil yield per acre have been encountered with meadowfoam. Therefore, the short life cycle and the environmental and genetic flexibility suggest possibilities of rapid progress in meadowfoam improvement. Moreover, modern plant breeding can also afford opportunities for more realistic exploitation than has been possible in the past. Successful domestication by plant breeding is a major contribution to modern agriculture and therefore, society. For example, corn and sorghum have both become hybridised crops since 1930, with yields increasing some 471% and 455%, respectively, over a 60-year period; and wheat yield, though essentially all varietal, has increased some 191% during this same period (Maunder, 1992).

Developing an understanding of the genetic and physiological bases of these traits may assist in its domestication and in the development of cultivars of meadowfoam. Quantitative genetic studies have revealed that genetic variability for most traits is adequate to expect response to selection. Krebs and Jain (1985) have suggested that studies of genetic variation of meadowfoam in agronomic traits provide insight on which attributes have greatest effect on overall crop performance. Environment is another important factor to contribute high yields in crop production. Thus, understanding the functional relationships between environmental conditions and meadowfoam responses may help in modeling growth and development. It may also be useful when recommending cultural and management practices, and determining the adaptability of this crop to new environments.

The present study examined a diverse gene-pool and investigated its variability of genetics and biology in flowering and seeding, as part of the knowledge needed to assess potential opportunities for domestication of meadowfoam. The objectives of this study were:

1) to investigate the gene-pool for flowering, seeding and seed shattering;

2) to estimate heritability for these traits and group phenotypes based on genetic variance components, and make recommendation to plant breeders;

3) to identify optimum germinating test procedures for dormancy breaking and investigate effects of putative maturity (seed-colour) on seed germination;

4) to study seed development, maturity and germinability of meadowfoam, in order to estimate the duration of dormancy; and

5) to establish the relationship between seed dormancy and the levels of endogenous ABA in meadowfoam seeds.

Chapter 2. Review of Literature

2.1 Background of Meadowfoam

The Genus *Limnanthes* (family Limnanthaceae), described in 1833 by Robert Brown, is native to northern California, southern Oregon, Vancouver Island and British Columbia (Mason, 1952). The family is divided into Inflexae and Reflexae, on the basis of a morphological character (petals folding inward versus outward during seed maturation); no attempt to make intersectional crosses were successful (Mason, 1952). The genus is a low-growing, herbaceous winter annual, diploid (n = 5) plant that is well adapted to the Mediterranean climate of the Pacific Coast (Jolliff *et al.,* 1981). They usually grow in dense colonies in moist habitats such as vernal pools, or along temporary streams and wet meadows. The common name "meadowfoam" was given because the canopy of light coloured flowers at full bloom is nearly solid.

Gentry and Miller (1965) explored the feasibility of developing meadowfoam as a crop plant. The plants appeared to be efficient in synthesis of dry matter and produced a high ratio of seed to vegetative dry matter (Jolliff *et al.*, 1981). *Limnanthes alba* (the Inflexae section) was suggested as having the most adaptive potential for good performance under cultivation, because it represents a rapid evolution (Gentry and Miller, 1965), more erect habit and better seed retention, compared to other accessions (Higgins *et al.* 1971). The great diversity of genetic resources in this genus has been explored by population genetic studies (Jain *et al.*, 1977). Considerable genetic variation for morphological traits (leaf hairness, clump density, leaf size, budding time and flowering time) in *Limnanthes alba* has also been reported (Chozin, 1990). Meadowfoam as a new oilseed crop is being developed at Oregon State University (Jolliff, 1981).

The major agronomic problems associated with the initial use of wild meadowfoam populations were poor seed retention, low yield, prostrate growth habit and short plants. The yield components of meadowfoam are number of flowers per area, number of seeds per flower, seed weight and oil content per seed. Flower number in Mermaid and Floral

meadowfoam appears to be the component of seed yield which is most responsive to management plus most consistently associated with oil yield (Jolliff, 1992). Higgins *et al.*, (1971) assumed that harvestable yields of 1700 kg ha^{-1} or more in meadowfoam appeared attainable on a commercial basis through plant improvement and the use of sound cultural practices. Agronomic research, however, revealed the potential to improve meadowfoam oil yield through genetic and management manipulation of yield components (Jolliff, 1992).

Seed yields of all species of meadowfoam grown at all locations have been extremely variable between years. In Corvallis, seed yield trials from 1975 through 1990 with Mermaid, a registered meadowfoam cultivar, have ranged from 788 - 1852 kg ha^{-1} (Franz *et al.*, 1992). Meadowfoam responds readily to environmental changes (Jolliff, 1981). The relationship between environmental factors and meadowfoam growth and development need to be delineated so that agronomic management can be applied to optimize seed yields. Consistent with historical yield improvement in field crops, management seems to play an equally important role as genetics in increasing meadowfoam oil yield (Jolliff, 1981).

Excessive seed shatter occurs before the crop is mature enough for mechanical harvest (Jolliff *et al.*, 1981; Higgins *et al.*, 1971). However, use of improved cultivars and timely harvest have given greater than 95% seed recovery from either direct combine harvesting on research plots or windowing and then combining on a commercial field scale (Jolliff, 1981). Maturity of meadowfoam is reached quickly especially under warm condition (Higgins *et al.*, 1971). Thus, timing of harvest to minimize seed losses is critical.

Sensitivity of the seed to high soil temperature was observed. Optimum temperatures for seed germination of 10 species of meadowfoam ranged from 5°C - 15°C (Toy and Willingham, 1966). The seed of some species was induced into secondary dormancy when imbibed at 27°C (Toy and Willingham, 1967). Seed of *L. gracilis* var *L. striata* germinated best at about 4.4°C, which was induced into secondary dormancy as the soil temperature rise above 15.6°C (Higgins *et. al.* 1971). The resulting dormancy prevents the seed from germinating even after the soil temperature drops. The seed dormancy problem

was encountered with some *L. alba* plant introduction materials at the Corvallis, Oregon, research station but the dormancy characteristic was reduced to less than 10% during the mass selection phase of the development of the cultivar Foamore (the first named meadowfoam cultivar) (Jolliff *et. al.* 1981). The response to soil temperature is an important factor in relation to proper planting time.

The flowers of *L. alba* are protandrous (Mason, 1952), with anthesis occurring one to four days before the stigma matures. Viable pollen is too heavy to be carried by the wind, so insects are required for pollination. In Oregon, honey-bee foraging is sometimes adversely affected by weather conditions. For this reason, the development of a self-pollination, variety of the *L. alba* plant-type is of special importance in the development of higher yielding meadowfoam varieties under Oregon conditions (Jolliff *et al.,* 1981). The limited experience in New Zealand suggests that bee pollination will not be a problem (Gordon, pers.comm.).

Meadowfoam has a growing season and harvest period similar to winter grains and has shown adaptability to cultivation in a wide range of soils and climates (Gentry and Miller, 1965). Furthermore, the wild populations of meadowfoam are quite variable in plant characteristics (Brown *et. al.*, 1979). Thus, the short life cycle and the environmental and genetic flexibility may suggest possibilities of rapid progress in crop improvement. Genetic contributions to meadowfoam seed yield increase during 1983-1990 has averaged approximately 87 $kg.ha^{-1}.yr^{-1}$ (Jolliff, 1992), compared to 61.6 $kg.ha^{-1}.yr^{-1}$ for U.S. maize during 1930-1980 when hybrids were developed (Russell, 1985).

2.2 Seed development

There are three major phases of seed development, characteristic of most seed plants, *e.g.*, histodifferentiation, expansion, and maturation drying (Bewley and Black, 1994). They are marked by distinctive changes in fresh weight, dry weight, and water content. During histodifferentiation and early cell expansion, there is a rapid increase in whole seed fresh weight and water content. Generally, a period of rapid dry-weight gain

follows (when whole seed fresh weight is relatively stable); this takes place during the latter part of the seed expansion (or maturation) phase of development. Most seeds lose water during this phase as reserves are deposited primarily within storage tissues, displacing water from the cells. This decline in water content slows as the seed approaches its maximum dry weight. Then, as the seed undergoes maturation drying and approaches either quiescence or dormancy, there is a period of fresh weight loss accompanied by a rapid decline in whole seed water content.

Plant growth regulators play important roles in the regulation of certain aspects of seed growth and development. Hormones in developing seed may be involved in several processes, as follows: (1) Growth and development of the seed, including the arrest of growth prior to seed maturation; (2) The accumulation of the storage reserves; (3) Growth and development of the extraseminal tissues; (4) Storage for later use during germination and early seedling growth; and (5) Various physiological effects on tissues and organs close to the developing fruit (Bewley and Black, 1994). The roles of seed hormones in development are given in more detail in Section 2.4.

Seeds do not usually germinate during development on the parent plant, but undergo a process of maturation, generally including desiccation, before being shed. During development, there is commonly an increase in the ABA content of a seed, both within the embryo itself and in the surrounding structures. It is generally accepted that ABA plays an important role in preventing germination during seed development, that it declines during late maturation (drying), and that at the same time the sensitivity to its presence is decreased (details in Section 2.4.1).

Maturation drying is the normal terminal event in the development of many seeds, which results in a gradual reduction in metabolism as water is lost from the seed tissues and the embryo passes into a metabolically inactive state. Upon hydration under suitable conditions, the seed commences germination -- if it is not dormant. There is now substantial evidence that the "switch" from a developmental to a germinative mode is elicited by the intervening maturation drying event (Bewley and Black, 1994).

Maturation is generally terminated by some degree of drying. Harvest ripeness has been defined at 12.5% grain moisture in wheat (Gordon *et al.*, 1979). These authors used a Gompertz function to describe the grain growth, and the result showed that at a grain moisture content between 20% to 12.5%, the grain dry weight was still increasing. At 12.5%, the grain weights reached about 92 - 99% of the upper asymptotes in the four wheat cultivars (Gordon *et al.*, 1979). Sereeprasert (1990) also reported that harvest ripeness occurred after dry weight maturity (time of 90% maximum dry weight) in wheat. The maximum potential longevity in wheat and barley seeds is achieved not at physiological maturity (maximum dry weight) but at a later stage in development (Pieta Filho and Ellis, 1991; Ellis and Pieta Filho, 1992).

Maturity with respect to embryo maturity and dry weight growth has also been reported in wheat (Gordon *et al.*, 1979; Sereeprasert, 1990). Maturity in relation to the development of the embryo (embryo maturity) was indirectly expressed by the time when the grains acquired 90% potential germination with dormancy broken (Sereeprasert, 1990). Dry weight maturity was defined as the time when grains reached 90% of their maximum dry weight (Sereeprasert, 1990). Gordon *et al.*, (1979) pointed out that grain maturation was a multi-faceted process, with flexible synchronizations amongst maturation traits at any point in time, such as at harvest ripeness.

2.3 Seed Dormancy

2.3.1 Definition and types of dormancy

Seed dormancy has been defined as: "the absence of germination of an intact, viable seed under a favourable germination conditions within a specified time lapse" (Hilhorst, 1995). Seed quiescence is a state of arrested development maintained solely by unfavourable environmental conditions such as inadequate water supply (Villiers, 1972).

A simple, generally accepted classification of dormancy factually distinguishes two types of dormancy: primary dormancy and secondary dormancy (or induced dormancy) (Karssen, 1982a). Primary dormancy occurs during seed development and maturation,

whereas secondary dormancy can only occur after seed dispersal, is often subject to annual dormancy cycles in the seed bank and is reversible. The term relative dormancy is used to describe the situation where germinability is retained at lower temperatures and dormancy is manifested at higher temperatures (Vegis, 1964; Bewley and Black, 1982).

Another form of dormancy with a clear mechanistic principle is displayed by seeds with hard seed coats (Kelly *et al.*, 1992). In general, seeds with this form of dormancy possess hard or/and thick seed coats, pericarps or other structures that impose a high mechanical resistance on an often non-dormant embryo, or block water uptake or/and oxygen diffusion. Only removal of this restraint may enable germination to proceed.

2.3.2 Development of primary dormancy

The stage in the seed's development and maturation at which primary dormancy sets in varies from species to species. In the case of *Acer*, embryo dormancy appears to develop before the end of seed maturation since embryos isolated from maturing seeds fail to germinate (Juntilla, 1973). In *Avena fatua*, when isolated from the caryopsis 10 days after fertilization and placed in a liquid medium , the embryo is already dormant, but it can be stimulated to germinate by application of gibberellic acid (Andrews and Simpson, 1969).

2.3.2.1 Genetic and correlative effects on dormancy

It has long been known that the entry into dormancy is under genetic control. Pure lines of certain species have been isolated that show contrasting degrees of dormancy. There is a wide range of dormancy encountered in natural populations of wild oats (*Avena fatua)*, from types which have a short-lived dormancy to those whose dormancy is very prolonged (Sawhney and Naylor, 1979). From these a line with a deep embryo dormancy has been inbred. Pure lines have also been isolated for two groups whose grains respond to temperature in distinct ways, one showing little or no dormancy at temperatures in the range 4-23 °C and the second exhibiting dormancy at intermediate temperatures. In lettuce, different lines of the cultivar Grand Rapids exist, some showing the classical

dormancy symptoms and others with no dormancy at all (Bewley and Black, 1985). In wheat, red-grain had deeper and longer embryo dormancy than white-grain (Gordon, 1979). Grain redness has been suggested to be a relevant concomitant selection criterion against sprouting damage.

It is quite clear, nevertheless, that genotypic expression in the embryo is plastic in response not only to environmental regulation but also to physiological factors which operate in the mother plant (*e.g.* correlative effects) (Bewley and Black, 1982). In some species there are clearly influences of the seeds on each other. Spikelets of *Avena ludoviciana* produce a small distal caryopsis with a fairly deep dormancy and a large, proximal one which is less dormant (Morgan and Berrie, 1970). But if the latter is removed during grain development, the remaining distal caryopsis is much less dormancy at maturity. This suggests that caryopsis may compete for factors whose supply can determine the degree of dormancy. These factors might include hormones, but no information is available as to how these are distributed to different seeds in an inflorescence or to different inflorescences.

A number of previous reports have demonstrated the heritability of dormancy characteristics in seeds, even in genetically uniform seed lots. For example, the chilling requirements for the promotion of germination of individual *Rumex* seeds varied from about 1 day to 3 weeks at 1.5 °C (Totterdell and Roberts, 1979). After-ripening periods varied from zero to about 130 days in rice seeds (cv. Masalaci) when stored at 13.5% moisture content and 27 °C (Roberts, 1963). Witcombe and Whittington (1971) clearly demonstrated the genetic basis for differences in germination responses between individuals in a seed population of *Sinapis arvensis*, using sensitivity to gibberellin as a selection tool. Probert *et al.* (1989) has devised simple selection criteria using ecologically important parameters to create genetic seed stocks.

2.3.2.2 The environmental control of dormancy inception

There is no doubt that environmental factors play an important role in forcing seeds into dormancy. Depth of primary dormancy depended highly on temperature during growth and development of the parent plants in *Avena fatua* (Adkins and Simpson, 1988; Sawhney and Naylor, 1979). Elevated temperatures corresponded to seeds which were less dormant. In *Avena fatua*, a lower temperature (20°C) after grain set induces deeper dormancy, *i.e.* grains which retain their dormancy for at least 19 weeks, whereas dormancy of grains developing at the higher temperature (28°C) lasts for only 4 weeks after harvest (Sawhney and Naylor, 1979). Embryos taken from barley plants growing under relatively low temperatures (*i.e.* about 21°C) were found to be less germinable than those from plants held at higher temperatures (Norstog and Klein, 1972). Similarly, higher temperatures late in the grain's maturation promote low dormancy in wheat (Belderok, 1961; Reddy *et al.*, 1985). A low germination temperature is effective for breaking dormancy and thus less dormancy is expressed. Gordon (1978), however, found that lack of germination in the cool environment was due to embryo immaturity rather than dormancy, and this happened for all four genotypes of wheat studied.

Thermoperiod (*i.e* the day and night temperature) also influences dormancy. For example, seeds of *Anagallis arvenis* have a very low dormancy when produced on a regime of 30/25°C (day/night), fairly high dormancy at 25/20 °C, and extreme dormancy at 20/15 °C (Lipp *et al.*, 1963). The thermoperiodic regime determines dormancy in *Aegilops kotschyi*, in which more deeply dormant seeds are produced by mother plants exposed to cycles of 17/12°C than by those at 27/22°C (Sidhu and Cavers, 1977).

Photoperiodic effects on the inception of seed dormancy are well known in several species. *C. album* has deeply dormant seeds when the fecund plants are held under long days, but nondormant seeds under short days (Karssen, 1970). Not only is the dormancy pattern affected by daylength but also the structure of the seeds, for those maturing in long days are smaller and thicker coated than those in short days.

Light, quite apart from the photoperiod, has an important role in dormancy induction in several species. Seeds of *Arabidopsis thaliana* maturing in white fluorescent light have little dormancy when harvested, whereas those which have experienced incandescent light remain deeply dormant for at least several months (Hayes and Klein, 1974). The explanation for this effect is that white fluorescent light is relatively rich in the red wavelengths whereas incandescent light has a relatively high component of far-red light. Under the former illumination conditions there is a higher concentration of the active Pfr form (active phytochrome) of phytochrome set up in seeds than there is under the latter type of light. Seeds with a high Pfr content can germinate in darkness while those with a low Pfr concentration remain dormant (details in Section2.5.3).

2.4 Effects of hormones on seed dormancy

Early work on hormones in seeds gave rise to a general scheme to explain dormancy on the basis of changing levels of growth promoting and inhibiting hormones. Convincing direct evidence that such effects reflect the action of naturally occurring hormones within the seeds is hard to produce. Attempts to correlate changes in dormancy with changes in endogenous hormone levels mostly fail to present reliable evidence for a causal relationship. The development of hormone-deficient mutants and new methods of hormone determinations, in particular the use of immunological techniques, made it possible to provide direct evidence for the involvement of hormones in germination and dormancy related processes. It has been demonstrated that abscisic acid (ABA) was responsible for the onset of primary dormancy on the parent plant (Karssen *et al.*, 1983), whereas gibberellins (GAs) were not involved in this stage (Hilhorst and Karssen, 1992). On the contrary, GAs were required for germination (Karssen *et al.*, 1989). A role for both these hormones in the control of α-amylase gene transcription has been identified (Zwar and Hooley, 1986). The exact role, if any, for cytokinins in seed dormancy is not clear (see Section 2.4.3).

The hormonal status of a seed, as established during its development and maturation, can certainly influence the degree of dormancy. However, in many cases no relationship has

been found between levels of hormones in the seed and the level of dormancy (Black, 1980/81; Cohn, 1987). Discussions initiated by Trewavas (1981) resulted in a greater awareness of the importance of sensitivity. Changes in sensitivity have been shown to contribute to the control of several processes (Trewavas, 1991).

2.4.1 Abscisic acid (ABA)

ABA has long been associated with dormancy, mainly because the hormone could be detected in both developing and mature seeds while it was known that it was inhibitory to germination when applied exogenously. Freshly harvested seeds of many species which show dormancy, such as ash, sycamore (*Acer pseudoplatanus*) and hazel do contain relatively high levels of ABA (Sondheimer *et al.*, 1968; Webb and Wareing, 1972; Williams *et al.*, 1973). That ABA does impose primary dormancy on seeds has been elegantly demonstrated by Karssen (1982b) using single-gene mutants of *Arabidopsis thaliana*. In the wild-type seeds the ABA concentrations reached 200-500 ng g^{-1} fresh weight, whereas in the mutants the concentration never exceeded 10 ng g^{-1}. The wild-type seeds developed dormancy during a period of after-ripening but the mutants did not. Reciprocal crosses between ABA-deficient mutants (*aba*) and wild type in *Arabidopsis thaliana* show that dormancy is initiated only when the embryo itself can produce ABA (Hilhorst and Karssen, 1992). Mendelian backcrossing experiments confirmed that the induction of dormancy was correlated with ABA derived from the embryo and not with maternal production or applied ABA, thus implying lack of transport of any active ABA between coats and embryo (Karssen, 1982b). Evidence also comes from studies of ABA-deficient mutants of plants, such as *Arabidopsis thaliana* (Karssen *et al.*, 1987) and *Lycopersicon esculentum* (Groot and Karssen, 1992). However, from studies of the *sitiens* mutant it appears that in tomato, as in *Arabidopsis*, mainly embryonic ABA is required for dormancy to set in, but maternal ABA can also contribute an effect (Groot *et al.*, 1991).

ABA contents have been measured in developing seeds of a large number of species. Characteristically, ABA contents rise during late maturation, when seed water content decreases (Bewley and Black, 1994). Sometimes two ABA peaks occur, *e.g.* in *Phaseolus*

vulgaris (Hsu, 1979) and *Pisum sativum* (Wang *et al.*, 1987). In developing seeds, ABA, as an inhibitor of germination, prevents the embryo from passing directly from embryogenesis to germination (Bewley and Black, 1985; Sussex, 1975). Immature seeds of soybean can be induced to germinate by treatments which deplete the endogenous pool of ABA (Ackerson, 1984). It has been suggested for seeds of *A. thaliana* (Karssen *et al.*, 1987) that ABA may act to induce dormancy, but may not be required to maintain it since it has substantially declined when maturity is reached. For tomato seeds, however, it is hypothesized that the high levels of ABA that occur during seed development in wild-type seeds induce an inhibition of cell elongation of the radicle that can still be observed after long periods of dry storage (Groot and Karssen, 1992). It was also concluded that *in situ* ABA synthesis was required to impose and maintain embryo dormancy (Le Page-Degivry and Garello, 1992).

ABA has been detected in embryo, endosperm and testa and in fruit tissues such as pericarp and locular tissue. The tissue location of ABA depends on the species. In the endospermic seeds (*e.g.* the cereals), most of ABA is in the bulk tissue, the starchy endosperm, but at times comparable concentrations are found in the embryo and the aleurone tissue (Garcia-Maya *et al.*, 1990; Napier *et al.*, 1989). In wheat, the bulk of ABA in an ear was in the grain (95%) and the husk contained only 5% of the total ABA; within the grain, the embryo contributed 19%, the testa and endosperm contributed 76% (King, 1976).

The germination of many mature, nondormant embryos can be inhibited by exogenous application of ABA (Milborrow, 1974). Exogenous ABA also appears to specifically inhibit the rise in activities of several enzymes, *e.g.* α-amylase, malate synthase, isocitrate lyase, Rubisco, endo-ß-mannanase, involved in postgerminative breakdown of reserves (Quatrano, 1986; Williamson *et al.*, 1985; Dulson *et al.*, 1988; Jacobsen and Beach, 1985; Nolan and Ho, 1988). In *Helianthus annuus*, application of fluridone (an inhibitor of carotenoid biosynthesis) to developing seeds prevents both ABA synthesis and development of embryo dormancy (Le Page-Degivry *et al.*, 1990). Fluridone does not prevent dormancy induction when it is applied after the rise of the ABA level.

Garciarrubio *et al.*, (1997) suggested that ABA appears to inhibit seed germination by restricting the availability of energy and metabolites.

At present virtually nothing is known about the mechanism of dormancy induction in seeds (Black, 1991). Obviously, the action of ABA has been searched in regulation of gene expression. Indeed, the induction of several kinds of polypeptides is stimulated by ABA (Skriver and Mundy, 1990; Thomas *et al.*, 1991), whereas genes coding for certain reserve-mobilizing enzymes are repressed by adding ABA to isolated embryos (Black, 1991). There is no evidence that ABA plays a similar role in the induction of dormancy, but it is suggested that ABA participates in dormancy by up-regulating the expression of certain genes (Hilhorst and Karssen, 1992). Several cDNA clones have been prepared from mRNAs which are abundant only in high-dormant wheat embryos under the influence of ABA (Morris *et al.*, 1991). These findings suggest that ABA-responsive genes are expressed more readily in embryos of dormant grains, raising the possibility that a set of "dormancy proteins" exists. A likely option is that inhibition of precocious germination by ABA provides the signal for dormancy induction (Black, 1991).

Studies on precocious germination of surgically excised cotton (*Gossypium hirsutum*) embryos (Ihle and Dure, 1972), the inhibition of GA_3-induced synthesis of α-amylase in barley aleurone cells (Ho and Varner, 1976; Higgins *et al.*, 1982; Chandler *et al.*, 1984) and the application of fluridone to cultured soybean cotyledons (Bray and Beachy, 1985) lead to the conclusion that the inhibition is at the level of mRNA translation. In high-dormancy wheat embryos several mRNAs are abundant under the influence of ABA (Morris *et al.*, 1991). Mundy (1984) reported that ABA induced the α-amylase inhibitor in barley aleurone layers and suggested that the inhibitor functioned as an active mediator of amylase activity during the development and germination of barley seeds. Jacobsen and Beach (1985), using isolated nuclei from the aleurone layer of mature barley, demonstrated that tABA not only prevents gibberellic acid promoted accumulation of α-amylase and other gene transcripts, but also prevents the GA-suppression of total and ribosomal transcripts. Similar results have been obtained by Zwar and Hooley (1986) using wild oat aleurone protoplasts. It is not known if ABA plays any part in the control of

α-amylase formation and secretion in the normal postgerminative mobilization processes of a mature seed.

However, clear correlation between ABA contents and germinability are rare. Verry (1978) reported that the concentration of ABA in developing wheat grains was not related to either dormancy or maturation and dehydration of the grain. Pea (*Pisum sativum*) accumulates ABA in the seed during development, but it does not exhibit a true physiological dormancy (Eeuwens and Schwabe, 1975). Radley (1979) also found no obvious relationship between changes in ABA and germinability of wheat grains. However, that dormancy induction depends not only on ABA synthesis but also partially or wholly on seed sensitivity to ABA is clearly demonstrated by Walker-Simmons (1987) in a comparison of a sprouting-resistant and a sprouting-susceptible wheat cultivar. Although dormancy varies, the ABA concentrations are closely similar. Isolated embryos of both cultivars differ, however, in their reaction to ABA. Embryo germination of the high-dormancy (non-sprouting) cultivar is inhibited by ABA concentrations which do not affect embryos from the low-dormancy grains (Walker-Simmons, 1987; Morris *et al.*, 1989).

A decrease in endogenous levels of ABA is not always correlated with germination (Kermode *et al.*, 1989). Finkelstein *et al.*, (1985) reported that the effectiveness of ABA varies, however, particularly in respect of the sensitivity of embryos of different ages. Rape embryos, at ages of up to 40 days are prevented from germinating by 1 mM ABA but older embryos require higher concentrations. In alfalfa (*Medicago sativa*), sensitivity to ABA decreased linearly during the course of seed development (Xu and Bewley, 1990). Similarly, ABA sensitivity decreased in *Cucumis melo* seeds, but most prominently during the second half of development (Welbaum *et al.*, 1990). These examples make it clear that correlations between ABA action and developmental events will have to include both ABA content and sensitivity to ABA.

2.4.2 *Gibberellic acid (GA)*

Immature seeds generally contain much higher GA levels than any other tissues, but GA levels often drop to zero at seed maturity (Graebe, 1987; Hutchison *et al.*, 1988). The total endogenous levels of GA-like substances reached a maximum at 19 days after anthesis and pollination in *Pharbitis*, which is just prior to the maximal fresh weight of seeds, reached about 23 days after anthesis (Barendse *et al.*, 1983). GA quantitation at various stages of seed development indicates a constant turn-over of GAs throughout the process of maturation (Sponsel and MacMillan, 1980). Studies with seeds of *P. sativum* show good correlation between the biologically active GAs (*e.g.* GA_9 and GA_{29}), and the active growth phase of the seed. As seeds mature, inactive GAs (*e.g.* GA_8, GA_{20} and GA-catabolites) are produced.

The apparent relation between seed development and GA biosynthesis in *Pharbitis* and wheat would suggest a regulatory role for GAs with respect to seed development. However, Zeevaart (1966) has shown that a reduction of the GA content of *Pharbitis nil* seeds by the growth retardant (CCC) did not affect seed development but only caused a delay in seed germination and subsequent development, suggesting a role for GAs in seed germination only. Studies with the GA-deficient mutants of *Arabidopsis* and tomato support Zeevaart's conclusions (Barendse *et al.*, 1986; Groot *et al.*, 1987). Development of wild type seeds does not differ from heterozygous GA containing seeds in a GA-deficient maternal environment. The endogenous GAs may stimulate seed and fruit development, but their presence is not essential (Barendse *et al.*, 1991). In *Arabidopsis* it was also shown that in an *Aba/Aba* background, it is irrelevant for the induction of dormancy whether or not the seeds are able to synthesize GAs (Karssen and Laçka, 1986). It is concluded that GA is not involved in the induction of dormancy in developing seeds (Karssen and Laçka, 1986).

In seeds of the gibberellin deficient mutant of *Arabidopsis thaliana* and tomato, GAs are absolutely required for germination (Karssen and Laçka, 1986; Koornneef *et al.*, 1981). Moreover, the action of ABA on dormancy induction causes a much higher GA

requirement for germination in mature seeds than in ABA-deficient seeds (Hilhorst and Karssen, 1992). ABA action during seed development increases the GA-requirement during germination. Therefore, the classic theory of dormancy control which supposes a balance between simultaneously acting promotive and inhibiting hormones has to be substituted by a kind of "remote control" in which the GA requirement of germination of ripe seeds is controlled by the ABA levels during seed development *via* ABA-induced dormancy (Karssen and Laçka, 1986).

Karssen *et al.*, (1989) suggested that endogenous GAs have at least two separate promotive actions on seed germination: 1) on reserve food mobilization, and 2) on embryo growth. In general, mobilization of reserve food in seeds is a post-germination event. Mobile food components are transported from storage cells in endosperm, perisperm or cotyledons to the growing seedlings. However, in some species part of the process occurs prior to visible protrusion of the radicle. Such is most evident in species where mature seeds contain an underdeveloped embryo. Prior to the start of germination the embryos of, *e.g.* celery (Jacobson *et al.*, 1976), wheat (Gordon, 1978) and *Trollius ledebouri* (Hepher and Roberts, 1985) have to grow at the expense of the endosperm. In barley, tomato and celery, it has been proven that breakdown of endosperm is controlled by the embryo and that the embryo could be replaced by GA for at least a considerable part (Jacobson *et al.*, 1976). Breaking wild oat embryo dormancy with GA may mainly be a substitute for sugar requirement (Foley, 1992). GA determinations in barley (Gaskin *et al.*, 1984) and the mutant studies in tomato (Groot *et al.*, 1987) presented final proof that embryo control is executed by endogenous GA. In tomato seeds, GAs play a role in growth of the embryo (Hilhorst and Karssen, 1992).

Evidence that the increase in activity of several hydrolases induced by GA actually occurs as a result of *de novo* synthesis, rather than by activation of preformed enzyme, has been obtained in various ways. Gibberellin regulates the synthesis of α-amylase in germinating wheat grain (Rowsell and Goad, 1964) and in barley (Varner, 1964; Varner and Chandra, 1964; Filner and Varner, 1967; Jacobsen and Varner, 1967). Groat and Briggs (1969) pointed that gibberellin originated from embryo was important in regulating the synthesis

of α-amylase in the aleurone layer in malting grain. Khan *et al.* (1973) demonstrated that in the absence of GA_3 the embryoless grain is unable to synthesise α-amylase.

In tomato it was shown that the mechanical resistance of isolated micropylar endosperm halves could be decreased by applying GA_{4+7}. Isolated endosperms from wild type seeds also required exogenous GAs to reduce their mechanical resistance (Hilhorst and Karssen, 1992). These observations led to the conclusion that GAs were synthesised in the embryo and transported to the endosperm where a process was induced that altered the mechanical properties of the endosperm layers. Groot *et al.*, (1988) showed that GA induced the enzymatic hydrolysis of the galacto-manman rich endosperm cell walls. Especially the enzyme endo-ß-mannanase was under complete GA-control. Hydrolysis of thick mannan or galactomannan rich endosperm cell walls is mostly associated with reserve food mobilization, which is essentially a post-germination event.

Much effort has been devoted to link the effect of gibberellins to membrane metabolism. Evins and Varner (1971) reported that gibberellin acid increase the rate of synthesis of the endoplasmic reticulum in barley aleurone cells. They suggested that the increase in endoplasmic reticulum synthesis precedes and is probably required for the GA-induced synthesis and release of hydrolytic enzymes. Baulcombe and Buffard (1983), using hybridization and *in vitro* translation techniques, demonstrated that GA_3 influenced the actual level of α-amylase mRNA, thus one control point for the hormone is at the transcriptional level. This has been confirmed in more detail in the barley system by Deikman and Jones (1986).

The aleurone tissue of immature wheat grains is usually non-responsive to gibberellin, with respect to α-amylase production. In the immature grains the responsiveness can be induced by drying (Nicholls, 1979; King and Gale, 1980; Armstrong *et al.*, 1982). A possible mechanism for the change in the sensitivity of aleurone cells might be through structural alterations in cell membranes (Armstrong *et al.*, 1982).

The study on activities of several gibberellins in stimulating germination of wild-type and GA-deficient *gal* seeds of *Arabidopsis thaliana* demonstrated that GA_4 and GA_7-

isolactone had the highest activity and GA_7 and GA_9 the lowest; activities of GA_1 and GA_3 were intermediate (Derkx *et al.*, 1994). Chilling released dormancy of hazel seeds, but hardly increased GA levels. However, after transfer to germination conditions, levels of GA_1 rose 40-fold and levels of GA_9 at least 300-fold (Williams *et al.*, 1974). It was hypothesized that the actual breaking of dormancy involves the activation of GA production. Differences in activity to various GAs illustrate the ability of binding sites to distinguish between subtle variations in molecular structure. Although virtually nothing is known about low-abundance proteins that possess genuine receptor characteristics, it may be proposed that the affinity of GA receptors for various GAs varies or that different GA receptors exist. Differences in affinity of GA receptors for various GAs are illustrated by parallel positions of GA dose response curves along the X-axis (Firn, 1986; Weyers *et al.*, 1987). Combined application of pure GAs presented no indications that more than one GA receptor is involved (Derkx and Karssen, 1994).

In *Arabidopsis* the stimulation of germination by light depends on the ability of the seeds to synthesize GA, but light also enhances the sensitivity of the seeds to GA. Dry storage and pre-incubation at 2°C also increase the responsiveness of *Arabidopsis* seeds to GA (Karssen *et al.*, 1989). Tetcyclacis, an inhibitor of the GA biosynthetic pathway, antagonizes the stimulative effect of light on germination in *A. thaliana* seeds (Rademacher *et al.*, 1984). Therefore, *de novo* synthesis of GAs might be involved.

2.4.3 Cytokinins

Studies on endogenous levels and exogenous application of cytokinins (CKs) strongly suggest that they are involved in the control of seed dormancy. Kahn (1971, 1975) has assigned to the cytokinins a "permissive" role in seed germination. GAs are the primary stimuli in germination of lettuce seed but that a CK/inhibitor interaction system provides an overriding control mechanism. The results from celery cultivars with deeper dormancy are consistent with this hypothesis (Thomas, 1992a). Applied cytokinins usually display low activity in dormancy and germination control compared to either ABA or GA. Their activities are prominent when combined with other promotive agents such as GA, light

and ethylene (Taylorson and Hendricks, 1977). The role of cytokinins could be to control the movement of gibberellins and other plant chemicals within and from the embryo thus triggering other biochemical processes leading to germination (Thomas, 1977). The involvement of cytokinins in dormancy breaking has been investigated repeatedly. As in the case of ABA and GA the correlations between the breaking of dormancy and the role of level of cytokinins in seeds are not always convincing.

There is much evidence that cytokinins are involved in germination and early post-germinative events. It is reported that the cytokinins may be involved in nutrient mobilisation to potentiate the embryo for germination, and it is indicated by the fact that they accelerated triglyceride catabolism in caryopses of *Triticum vulgare* (Tavener and Laidman, 1972). Eastwood *et al.* (1969) reported that the endosperm of wheat contain a compound which has cytokinin activity and is responsible for the induction of essential metabolic processes in the aleurone cells of the germinating wheat grain. A factor from the endosperm, which can be replaced by cytokinins, initiates the mobilization of triglyceride reserves and promotes the retention of mineral ions in aleurone tissue (Laidman *et al.*, 1974). It has been demonstrated that the embryo is a site of biosynthesis and there is a polarized movement of cytokinin from the embryo to the cotyledons in the early stage of germination (Nandi and Palni, 1989; Villalobos and Martin, 1992). Brits *et al.*, (1995) suggest that CKs control at least three major processes of germination in *Leucospermum*: 1) early mobilization of protein and lipid reserves in the axis and later in cotyledons, 2) cotyledon expansion which causes the endotesta to split permitting radicle protrusion and 3) later, radicle growth.

Although cytokinins have been detected in most plant organs and in a great number of living organisms (Letham, 1978; Van Staden and Davey, 1979), it is noticeable that reproductive structures are not only rich in endogenous cytokinins but also contain a great diversity of these compounds. Two types of cytokinins are found in seeds and fruits: free cytokinins, which are readily extractable with organic solvents, and bound cytokinins which have been detected in tRNA hydrolysates (Van Staden *et al.*, 1982). Villalobos and Martin (1992) suggested that the greater efficacy attributed to the dihydro compounds may

be due to the presence of free bases, which individually or in mixture could exert in greater effect on the hydrolytic activities involved in the mobilization processes.

Many interesting plant developmental processes are accompanied by changes in cytokinin concentrations. Davey and Van Staden (1979) demonstrated that the highest cytokinin activity within the seeds coincided with maximum endosperm volume in *Lupinus albus*. In wheat grain, cytokinins were also localized largely in the endosperm, and cytokinin was higher in normal grains compared with the naturally embryoless seeds (Thomas *et al.*, 1978). A transient peak of biologically active cytokinins has been detected in developing wheat grains during the first six days after fertilization (Wheeler, 1972; Durley and Morris, 1983; Morris *et al.*, 1993). Compared with developing seeds the mature dry seed contains low levels of endogenous cytokinins (Davey and Van Staden, 1979; Summons *et al.* 1980). It has been suggested that CK activity at early stage of seed development is responsible for enhanced seed size by increasing cell number, resulting in larger storage capacity (Rock and Quatrano, 1995). Endosperm cell division is associated with high endogenous cytokinins in maize (Morris *et al.*, 1993; Dietrich *et al.*, 1995). It has been reported that the application of exogenous cytokinins increased crop yield (Trckova *et al.*, 1992; Morris *et al.*, 1993).

Red light affected the behaviour of cytokinins. Kinetin only promotes the germination of lettuce seeds if they are exposed to a low level of red light, indicating that a minimal level of Pfr must be present (Black *et al.*, 1974). In Scots pine seed, CK content increased following red irradiation, with a transient increase in the isopentenyladenosine content, leading the authors to suggest that this CK could be a signal transducer of active phytochrome and that the increase in its content is the first hormonal change in the dormancy breaking process (Qamaruddin and Tillberg, 1989). Rapid increases in *n*-butanol-soluble CKs following irradiation were associated with concomitant decreases in water-soluble CKs, suggesting a red light-induced CK conversion (Thomas, 1992b). Several investigations have indicated that the transient CK increases in seeds following dormancy-breaking treatments are due to their release from bound forms rather than to *de novo* synthesis, but light treatment does not induce CK synthesis in seeds (Thomas, 1992b). An increase was observed in the appearance of free bases in the cotyledons that

was parallelled by an increase in the transport of those substances from the axis to the cotyledons (Villalobos and Martin, 1992). Far red light applied after red light treatment reversed the effects of the latter, which indicated the involvement of phytochrome in the processes responsible for the accumulation and transport of cytokinins (Villalobos and Martin, 1992).

2.5 Environmental effects on dormancy release

2.5.1 Low temperature (chilling)

Many species show dormancy release when they are exposed to low temperatures. In general, the chilling temperatures required to break dormancy are around 5 °C. In apple, the chilling for 60 days suffices to remove the embryo dormancy (Visser, 1956). In contrast, dormancy in some herbaceous species is broken by just a few days of low temperature (7 and 14 days, respectively, in *Poa annua* and *Delphinium ambiguum*) and only 12 hours in wheat (Bewley and Black, 1985).

Many seeds appear to have a well regulated mechanism for chill timing, but this is not always the case. If the prechill temperature is too high, or the chill period is interrupted in some species (*e.g. Rumex* or *Euonymus europaea*) there may be induction of secondary dormancy (Bewley and Black, 1985). In *Rumex* onset of secondary dormancy is inhibited by light (Totterdell and Roberts, 1979).

Singh and Paleg (1984a) found that low temperature (5°C) significantly increases gibberellic acid sensitivity (measured as α-amylase production). They postulated that the low temperature effect probably involved an increase in active hormone (GA_3) receptor sites. Bewley and Black (1982) have discussed a possible mechanism for chilling effects based on membrane phospholipid phase changes at critical temperatures. Singh and Paleg (1984b) further observed that low temperature caused the changes in the content of the lipids, especially phospholipids. These results suggest that GA_3 receptor sites are membrane based as had been proposed by Wood and Paleg (1972, 1974). The contention that sensitivity to GA_3 involves membranes was supported by Armstrong *et al.* (1982).

2.5.2 Alternating temperature

It has long been known that higher germination at alternating temperatures compared with constant temperatures. Probert *et al.* (1986; 1987) have demonstrated that the response to diurnal temperature cycles in seeds of *Dactylis glomerata* and *Ranunculus sceleratus* is dependent on the presence of Pfr. Probert *et al.* (1986) have suggested that the capacity for dark germination at alternating temperatures may be explained by the sensitivity of some individuals to low levels of pre-existing Pfr.

There is an interesting and important relationship between the effects of amplitude (the difference between the maximum and minimum temperature), thermoperiod (time in hours each day above the mean temperature) and number of temperature cycles required. In some species the loss of dormancy is greater when the time spent at the lower temperature in each cycle is more than the time spent at the higher temperature, *e.g.* in *Capsella Bursa-pastoris* (Popay and Roberts, 1970a), *C. polyspermum* (Vincent and Roberts, 1977); in other species the alternating temperature regime results in more germination if the greater proportion of the cycle is spent at the higher temperature, *e.g.* in *Senecio vulgaris* L. (Popay and Roberts, 1970b) and *C. album* (Vincent and Roberts, 1977); and in yet others, *e.g. Lycopus europaeus* L., *Silene dioica* (L.) Clariv., it does not seem to matter whether the low temperature is given for a longer or shorter period (Thompson, 1974). Based on their studies on *Rumex*, Roberts and Totterdell (1981) stressed that for maximum germination at increasingly large amplitudes it was necessary to reduce the period spent at the warm phase of diurnal cycles. Provided the adjustment was made, the number of cycles required to trigger germination was lowered with increasing amplitude. These observations help to explain the connection between the effects of a series of diurnal cycles and the effects of single brief high temperature shifts shown to be effective in stimulating germination in a number of species sensitive to alternating temperatures and light (Takaki *et al.*, 1981; Taylorson and DiNola, 1989).

2.5.3 Light

Light is an extremely important factor for releasing seeds from dormancy. Seeds of many species are affected by exposure to white light (*i.e.* sunlight) for just a few minutes or seconds (*e.g.* lettuce) (Borthwick *et al.*, 1954), whereas others require intermittent illumination (*e.g. Kalanchoë blossfeldiana)* (Bewley and Black, 1985). Dormant *Oryzopsis miliaceae* germinates in response to a few minutes of light but does not do so under continuous exposure (Koller and Negbi, 1959). Photoperiodic effects also exist, so that species require exposure to long days (*e.g. Begonia evansiana*) and others to short days (*e.g. Tsuga canadensis*) (Bewley and Black, 1994). Conversely, some seeds are normally dark germinators (*e.g. Nemophila insignis* and several cultivars of lettuce) (Rollin, 1972). The levels of preexisting Pfr in these light-independent seeds may be higher than the threshold for germination (Cone and Kendrick, 1985).

The light requirement frequently depends on the temperature. Grand Rapids lettuce, for example, generally is dormant in darkness only above about 23 °C, so below this value seeds germinate without illumination (Borthwick *et al.*, 1954). Where no dark germination occurs, fluctuating temperatures enhance the light effect; but where some dark germination occurs at constant temperature, fluctuating temperatures may give high germination and appear to replace the light effect (Toole *et al.*, 1955). Derkx and Karssen (1993a) also found that temperature regulates the responsiveness to light in *A. thaliana* seeds. Sensitivity to light is enhanced by either a chilling treatment or a dry after-ripening period in GA-insensitive mutant (*gai*) and GA-deficient mutants (*gai/gal*) seeds of *A. thaliana* (Derkx and Karssen, 1993a), but in some species (*e.g. Betula maximowicziana*) light terminates dormancy only in seeds that have previously been chilled (Bewley and Black, 1985).

Most light-requiring seeds do germinate in the dark when exogenous GAs are applied (Taylorson, 1982). This by-pass of light requirement has led to the suggestion that Pfr (active phytochrome) plays an essential role in the biosynthesis of GAs. In several species light also enhances the stimulation of germination by exogenous GAs whereby the effects

of light and GA are mostly additive. Therefore, it was suggested that Pfr also increases the sensitivity of the seeds to GAs (Bewley, 1980; Taylorson and Hendrichs, 1976).

Since light stimulates the synthesis of GAs as well as the responsiveness to GAs, temperature-induced changes in dormancy may indirectly change the capacities to synthesize GAs and to respond to GAs (Derkx and Karssen, 1993a). Detailed analyses of dose responses to light and GAs demonstrated that dormancy control in the related species *Sisymbrium officinale* was indeed not at the level of the GA receptor, whereas the phytochrome receptor was primarily involved in temperature-induced changes in dormancy (Hilhorst, 1990a; Derkx and Karssen, 1993b). Changes in sensitivity to GAs and light may consist of changes in the number of receptors, changes in the affinity of the receptors and changes in the signal-transduction pathway initiated by GA or phytochrome (Hollenberg, 1985; Firn, 1986; Weyers *et al.*, 1987).

2.5.4 Afterripening

Dormant seeds, when "dry", slowly lose their dormancy by the process of afterripening, perhaps requiring as little time as a few weeks (*e.g.* barley) or as long as 60 months (*e.g. Rumex crispus*) (Cavers, 1974). In *Avena fatua* differences in depth of dormancy between individuals have been used to select pure lines, thus demonstrating a genetic basis to such variation (Naylor and Jana, 1976).

During dry storage, seeds undergo physiological changes which are often reflected in a decline in the level of innate dormancy. Accompanying these afterripening changes, germination requirements usually become less specific. In *Dactylis glomerata* the proportion of individuals which require light and/or alternating temperatures to trigger germination declines during dry storage. After prolonged storage, seeds are capable of maximum germination even in the dark at constant temperatures (Probert *et al.*, 1985).

The loss in dormancy during afterripening is a function of environment as well as time. The efficacy of afterripening also depends on the environmental conditions -- moisture,

temperature, and oxygen (Bewley and Black, 1985). In nature, moisture and temperature are more variable than oxygen. Low temperatures prevent or greatly retard the process. For example, in one seed lot of *Oryza glaberrime* at 11.2% moisture content half of the seeds lost dormancy after 65 days at 30 °C while at 40 °C only about 20 days were required (Ellis *et al.*, 1983). Storage of *Rumex crispus* seeds for 5 years at 2-4 °C, results in no appreciable change in dormancy (Cavers, 1974). Previous reports have indicated that increasing the relative humidity at which seeds are stored may result in an increase in the rate of afterripening (Quail and Carter, 1969; Baskin and Baskin, 1979). These observations are supported by a study on rice (Ellis *et al.*, 1983), which demonstrated that a reduction in moisture content from 11 to 8% resulted in a 2.5-fold reduction in the rate of after-ripening.

Dry-seed water contents generally fall in the range 5% - 15%, according to the species and external conditions. Bewley and Black (1985) pointed that complications set in at moisture contents higher than the normal for dry seeds when substantial loss of viability may ensue and, as moisture contents become higher still, secondary dormancy develops. A minimum water content is also required for afterripening, and if seeds become too dry (*e.g.* 5 % water content), the process is delayed (Bewley and Black, 1985). At very low moisture contents (< 8%) the rate of dry afterripening is minimal; it is greatest in cereals between 11 and 15% moisture content (Roberts, 1962, 1988; Ellis *et al.*, 1983). As the afterripening temperature increases, the seed moisture content must decrease for maximum afterripening (germination) to occur in *Avena fatua* (Foley, 1994)

The mechanisms underlying the process of dormancy relief in dry seeds are largely unknown. Mature dry seeds usually contain low water contents (as mentioned above). Under these conditions metabolic activity is virtually reduced to zero. Only a few enzymes are known to show activity at low water content. Examples are hydrolytic enzymes, *e.g.* phosphatases, but it is not known if and how these enzymes contribute to dormancy relief (Karssen and Laçka, 1986). Trewavas (1988) speculated that a bistable metabolic switch is required to switch between non-dormancy and dormancy. The required metabolic bistability could be produced by an autophosphorylating protein kinase. These kinases have been used to explain the molecular basis of long term memory (Lisman, 1985). The

unphosporylated form of the enzyme would be related to germination because only this form would be able to activate the several transduction paths leading to increased metabolic activity, and the highly autophosphorylated irresponsive form to dormancy. The phosphatases could then, in the dry seed, activate the irresponsive form.

Some chemical reactions are also known to occur in dry systems (Benson, 1990). Unsaturated fatty acids are especially susceptible to oxidation. Armstrong *et al.*, (1982) suggested that drying altered the physical structure of membranes, which then led to the hormone-responsive state. As membranes have often been claimed to be involved in dormancy relief, Hilhorst and Karssen (1992) speculated that 1) membranes are "primed" in this way, perhaps by making them permeable for inhibiting compounds which may leak out upon imbibition; 2) alteration of membrane structure through oxidation results in structural changes of membrane bound or associated proteins. Leopold *et al.*, (1988) reported that the changes involved in afterripening might be related to some oxidative reactions which were inhibited by metabolism. Esashi *et al.*, (1993) indicated that afterripening involves the removal of some protein which imposes the dormant state on the *Xanthium* seed.

There are indications that the residual amount of ABA in tomato seeds is reduced after one year of dry storage (Hilhorst and Karssen, 1992). Afterripening reduces seed ABA content to amounts comparable with those in seeds from ABA-deficient *sitiens* mutant plants (Groot and Karssen, 1992). However, afterripening wild type seeds have a slightly longer lag-time for germination than *sitiens* mutant seeds. It has been reported that a short drying (3 days) of dormant *Helianthus* embryos caused ABA levels to drop substantially, whereas germinability was increased (Bianco *et al.*, 1994). However, subsequent dry storage for 6 weeks did not further reduce ABA content but it did promote germinability. This lack of correlation between germinability and ABA content was ascribed to an increasing sensitivity to GAs during dry storage in *Helianthus* (Bianco *et al.*, 1994), a phenomenon described in several other species (Hilhorst and Karssen, 1992). Consequently, the suppression of the *in situ* ABA synthesis, induced during dry storage, appears necessary for the release of dormancy (Le Page-Degivry and Garello, 1992; Bianco *et al.*, 1994).

As the sensitivity to GA_3 was induced immediately after the start of dry storage in *Avena fatua*, a role for GAs in relief of primary dormancy may be considered (Hilhorst and Karssen, 1992). Whether differences in levels of endogenous GA could explain differences in dormancy in this species has been studied. However, dormant and non-dormant dry seeds contained similar amounts of GA_1 (Metzger, 1983). When the level of GA in the seed was strongly reduced by continuously feeding the motherplant with CCC during seed development, the rate of afterripening was not affected. Therefore, it was suggested that synthesis of GAs plays no roles in dormancy breaking in dry seed (Metzger, 1983). GA-synthesis is often observed after the start of imbibition. After 24 hours imbibition non-dormant (afterripened) seeds of *Avena fatua* contained 60 to 70 times more GA_3 equivalents than dormant seeds (non-afterripened) (Metzger, 1983). Apart from an increased capacity to synthesise GAs, afterripening may also result in the development of a GA-responsive system. Studies on dormancy breaking of *Arabidopsis thaliana* seeds showed that the sensitivity to GAs gradually increased during the period of dry storage. This increase in sensitivity occurred both in wild type and gibberellin-deficient mutant seeds, demonstrating that the release from dormancy is independent of the capacity to synthesise GAs (Karssen and Laçka, 1986). It has been proposed that the mechanisms for GA_3-induced and afterripening-induced germination of dormant embryos are different (Metzger, 1983; Foley *et at.*, 1993). The use of $[^3H]GA_4$ demonstrates that enhanced sensitivity resulting from drying was not caused by changes in gibberellin uptake or by a change in its metabolism (Bianco *et al.*, 1994). Therefore, the increased sensitivity to GA was proposed to be correlated with an activation of GA receptors (Hilhorst and Karssen, 1992). Indeed, drying might alter membrane composition and structure.

2.6 Breaking of dormancy by potassium nitrate

Nitrate has long been known to break dormancy and to stimulate germination of many seeds (Roberts and Smith, 1977; Vincent and Roberts, 1977). In addition, the International Associations for Seed Testing have, since 1954, adopted officially the systematic use of 0.2% KNO_3 in their suggested germination protocols for many species (ISTA, 1985).

Potassium nitrate is encountered by seeds in the natural environment, but it is nevertheless of great interest because it may help us understand the mechanism of dormancy breakage.

Roberts and Smith (1977) assume that nitrate acts as an alternative hydrogen acceptor that re-oxidizes NADPH to NADP, thus stimulating the operation of the pentose phosphate pathway (PPP). The PPP is assumed to be the alternative oxygen requiring process essential for germination (Roberts, 1969; 1973; Roberts and Smith, 1977), which represents a minor alternative respiration pathway in established plants, using NADP as a hydrogen acceptor instead of NAD. The other hypothesis for the mechanism of action of nitrate (Hendricks and Taylorson, 1975) also acknowledges the importance of the pentose phosphate pathway but assumes that reoxidation of NADPH proceeds through peroxidase action. The substrate for this enzyme, H_2O_2, is made available by the inhibition of catalase activity by nitrite, thiourea or hydroxylamine. These compounds are believed to inhibit enzyme activity by direct binding to the catalase heme protein (Hilhorst and Karssen, 1989a).

In both theories a shift in respiratory metabolism is thought to be essential for dormancy-breaking. Adkins *et al.*, (1984) showed that the results of oxygen uptake and germination were obtained 13 days apart in *Avena fatua.* Although stimulation of oxygen uptake was recorded 4 days before visible germination, it is not clear if a causal relationship exists between nitrate action and oxygen uptake. This result was supported by Hilton and Thomas (1986). Thus, it was concluded that the effect of nitrate was on germination and not on oxygen uptake (Hilhorst and Karssen, 1989a).

Whether or not a causal relationship exists between the action of nitrate and respiration, all proposals for the stimulating action of nitrate presume that its effectiveness is the result of its reduction, thereby re-oxidizing NAD(P)H_2 to NAD(P). As early as 1972, Hendricks and Taylorson indicated a role of nitrate in the unreduced state. They were unable to detect nitrate reductase activity and found that sodium tungstate, an inhibitor of the synthesis of active nitrate reductase, did not influence the nitrate induced germination. In seeds of *Sisymbrium officinale* also, no effect of either sodium tungstate or sodium chlorate (competitive inhibitor of nitrate reduction) on the nitrate induced germination was found

(Hilhorst and Karssen, 1989b). Thus, the evidence for the participation of the PPP in dormancy breakage is equivocal. The pentose shunt hypothesis has been criticized extensively (Bewley and Black, 1982). Information is accumulating in the research literature that casts doubt on the validity of the hypothesis that the termination of dormancy requires a switch in respiratory metabolism toward increased PPP activity.

For a number of species a positive interaction between light and nitrate has been observed (Vincent and Roberts, 1977). More detailed studies have shown that the effect of nitrate is dependent on Pfr (Hilton, 1983; Probert *et al.*, 1987; Hilhorst *et al.*, 1986). From studies on seed germination in *Kalanchoë blossfeldiana* it was concluded that nitrate increased the sensitivity of the seed to Pfr (DePetter *et al.*, 1985). In *Sisymbrium officinale*, the germination response is determined by the product of the concentrations of Pfr and nitrate, indicating a multiplicative interaction (Hilhorst and Karssen, 1988). Nitrate direct binding to the phytochrome receptor protein has been suggested (Hilhorst, 1990a; 1990b).

A model that integrates the effects of temperature, light and nitrate on germination and breakage of dormancy has been proposed recently (Hilhorst, 1990a; 1990b). In this model these three factors interact on a common reaction site, the plasma membrane. It has been hypothesized that nitrate has an effect on the phytochrome-receptor X, in that nitrate enhanced the number of active receptors and/or inhibited the inactivation of the receptors (Hilhorst, 1990a). The number of phytochrome receptors present is the net result of synthesis and degradation, and is presumably under temperature control (Hilhorst, 1990b). For many species, it has been shown that increasing temperatures enhance the rate of breaking and reduction of dormancy (Hilhorst *et al.*, 1986). It was concluded by Hilhorst and Karssen (1988) that nitrate might be regarded as a cofactor for phytochrome action. The combined action of R (red light) and nitrate could be replaced by addition of GA_{4+7}, which inhibited by the growth retardant tetcyclacis, an inhibitor of GA biosynthesis. This indicated that the combination of R and nitrate stimulated GA biosynthesis (Hilhorst and Karssen, 1988).

2.7 Quantitative genetic analysis

Plant breeding can be divided into three stages: assembly or creation of a pool of variable germplasm, selection of superior individuals from the pool, and utilization of the selected individuals to create a superior variety. Estimates of genetic variance and heritabilities can be of value in all three stages.

2.7.1 Estimation of genetic variances

Phenotypic expression of an attribute can be partitioned into genetic effects and non-genetic effects, the latter attributable to environment and interaction between genotype and environment (Comstock and Robinson, 1948). The simple model to describe this relationship is:

$$P_i = G_i + E_i + GE_i$$

where P_i is the phenotype of individual measured for character I, G_i is genetic effect potentially inherited for the character, E_i is environmental effect causing variation in the character, and GE_i is the interaction effect of genotype and environment. Thus, total phenotypic variation in the population, σ^2_p, would be:

$$\sigma^2_p = \sigma^2_G + \sigma^2_E + \sigma^2_{GE}$$

where σ^2_G represent genotypic variance, or variance of genetic effect, σ^2_E is variance of environmental effect and σ^2_{GE} is covariance of genotype and environmental effect. If the genotypes are randomly distributed relative to variation in environment, covariances amongst these effects are negligible.

The total genotypic variance (σ^2_G) can also be further partitioned into components describing the type of gene action:

$$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_I.$$

where σ^2_A is the additive genetic variance associated with average effect of alleles at the same locus, σ^2_D is the dominance variance due to interaction of average effect of alleles within locus, and σ^2_I is the epistatic variance due to non-allelic interaction, or epistatic effect.

There are mainly two methods for the breakdown of the phenotypic variation into its components, those based on the generation means and those based the variance component analysis. Typically the estimation of the components is based upon the relative genetic effects deduced from the means of different generations. However, this method lacks in general utility for various reasons. As Sprague (1966) has pointed out, the generation means method is strictly applicable only where gene frequencies are known. While this method may provide information on the existence of different types of gene actions, it provides no measure of their relative importance. Furthermore, the result cannot be related to any ancestral population as the estimate obtained from each pair of inbred parents may be unique in varying degrees. Also, only genetic variation which generates means variability is analysed.

For those reasons, analysis of variance components is much more widely used than that of generation means. This method was formerly introduced by Fisher in 1925 and, since then, developed by various workers (Crump, 1946; Comstock and Robinson, 1948; Mather, 1949). Basically the estimation procedures involve a mating scheme to generate progenies. Using appropriate experimental designs and statistical analysis, variance components can be calculated. To interpret them genetically, the raw components of variance are translated into covariances amongst relatives, which reflect the degree of the relationship amongst individuals in the populations. These covariances of relatives are then translated, in terms of gene models, into genetic and environmental variance components (Falconer, 1989). Translation of the covariances between relatives into additive, dominance and epistatic genetic variances requires a rigorous definition of the genetic population being sampled. For cross-pollinators or for self-pollinators which can be readily crossed where the designs appropriate for cross-pollinators are used, the genetic population is assumed to be at random mating equilibrium. The following additional assumption are usually also necessary: (a) normal diploid and solely Mendelian inheritance; (b) no environmental correlation among relatives; (c) the progenies are not inbred and can be considered as random members of some non-inbred population; and (d) linkage equilibrium (Cockerham, 1963).

Variance or covariance component estimates are subject to large standard errors. If the estimates are to be useful in characterizing populations, or as a basis for estimating expected progress in a selection scheme, adequate sampling is necessary to obtain consistent and meaningful results. Sampling requirements may vary with the magnitude of the genetic variability being estimated.

2.7.2 Heritability and its standard error

The heritability is estimated from the degree of resemblance between relatives (Falconer, 1989). The most important function of the heritability is its predictive role, expressing the reliability of the phenotypic value as a guide to the breeding value. Heritability is used in both a broad sense (h^2_{BS}) and a narrow sense (h^2_{NS}). The broad sense heritability is then defined as the proportion of total genetic variances (σ^2_G) to the phenotypic variance (σ^2_p):

$$h^2_{BS} = \frac{\sigma^2_G}{\sigma^2_p}.$$

The estimate of broad sense heritability provides quantitative information on the relative magnitude of genetic and environmental variation for a given trait in a specific population but is not usually an indication of response to selection which might be made on that population (Duddley and Moll, 1969).

The narrow sense heritability, on the other hand, is the proportion of additive genetic variance (σ^2_A) to the total phenotypic variance (σ^2_p):

$$h^2_{NS} = \frac{\sigma^2_A}{\sigma^2_P}.$$

The estimate of narrow sense heritability is of great interest to plant breeders because the effectiveness of many selection schemes depend on the additive (or average allele) portion of genetic variation (Falconer, 1989).

Since the estimation of heritability is based on partitioning the variance of phenotypes into various genetic and environmental components, for a balanced design, the

estimation of variance components is straightforward. It is usually accomplished by computing the mean squares in the standard analysis of variance, equating these mean squares to their expectations, and solving for the unknown variance components. But most research data is unbalanced, and estimating variance components from such data becomes very complicated. Henderson's (1953) paper has been widely used as a foundation paper dealing with unbalanced data, and based on this paper many methods have been developed. A detailed review of variance component estimation was made by Searle (1971). The narrow sense heritability can also be estimated in terms of the parent-offspring regression techniques (Falconer, 1989).

The heritability estimates can be competed in two forms: "full" heritability, using the full phenotypic variance of the analysis and the "restricted" heritability, using only the single experiment variance components (Gordon *et al.*, 1972).

I. Full heritability $h^2 = \sigma_G^2$ /all variance components;

II. Restricted heritability $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 + \sigma_{er}^2)$.

The restricted type has been more commonly used in the literature (Allard, 1960), and is more appropriate when the phenotypic performance of two genotypes are compared within the one experiment.

The development of accurate measures of precision for heritability estimators has been complicated because the distributions of the estimators were not known (Graybill, 1976; Searle, 1971). The variance of heritability estimated by regression is well known (Kempthorne and Tandon, 1953). The standard error method traditionally has been used to measure the precision of heritability estimates. Since heritability is a statistic involving the ratio of the genetic variance to the total phenotypic variance, its standard error is large but unknown (Jensen and Barr, 1971). However, an approximate variance of the estimated heritability can be obtained by using the approximation to the variance of a ratio (Baker, 1986; Becker, 1967; Gordon *et al.*, 1972; Gordon, 1979; Osborne and Paterson, 1952). But the procedure developed for the purpose is limited since it requires equal numbers of observations in the subclasses, and most biological data does not meet

this requirement. Searle (1958) derived expressions for calculating variance and covariance of variance components for the two-way classification, and they can be used to approximate the standard error of heritability from an unbalanced design. Because of the number of calculations necessary in Searle's methods, an approximate method similar to Searle's two-way procedure was developed by Jensen and Barr (1971).

2.7.3 Heritability of dormancy and germinability

The inheritance of seed dormancy and germinability has been studied for selection criteria against sprouting damage in wheat (Gordon, 1980; Zvomuya, 1996) and barley (Buraas and Skinnes, 1984). Gordon (1980) reported that heritabilities of dormancy and net germination at harvest ripeness were of medium value, 0.41 and 0.43 (narrow sense), respectively. A high heritability of dormancy (h^2_{NS} = 0.89) was observed at harvest ripeness by Zvomuya (1996). The heritability of seed dormancy was high for three and five weeks after yellow ripeness with two records of germination, ranged from 0.69 - 0.80 (narrow sense) (Buraas and Skinnes, 1984).

2.8 Multivariate analysis

Multivariate analysis is the branch of statistics concerned with analyzing multiple measurements on one or more population. It is a useful technique for summarizing, reducing, grouping and analyzing data, which can also be used for discriminant populations on the basis of many associated attributes. Multivariate analysis has been widely used in biological science. Having introduced correlated characters and response including the selection index, it is clear that multivariate methods generally may be of use to the geneticist and breeder. Growth curves have long been the subject of quantitative study. Multivariate methods may also have utility to explore maximum information from a study of growth curves for the biologist.

2.8.1 *MANOVA*

The analysis of variance (ANOVA) which proved to be the most widely used and basically useful approach to study differences among several populations or treatments. However, ANOVA only handles one dependent variable, and the dependent variable is assumed to be normally distributed with the same variance in each population, and the research issues concern the "realness" of the differences among the population means for this variable. If one wants to deal with many characters together, ANOVA cannot do it and the multiple analysis of variance (MANOVA) is needed to solve this kind of problem. In MANOVA one is concerned with the multivariate generalization (vector variable) of the analysis of variance for testing the equality of the mean vectors of several populations.

Since MANOVA deals with the vector variables rather than scalars, it looks at the whole dispersion of the variables, *i.e.*, variance and covariance. Therefore, it is the more suitable method of handling combined variables. It is also a very important tool for genetic studies, because most quantitative genetics information derives from partitioning variance and covariance of phenotypic values, and MANOVA can generate a dispersion matrix for this purpose. The distinctive nature of MANOVA design is that dependent variable is a vector variable. This dependent vector variable is assumed to be multivariate, normal in distribution and with the same dispersion, or variance-covariance matrix, for each population. Equality of dispersions is the MANOVA extension of the assumption of variance homogeneity in ANOVA design. In MANOVA the research issues concern the realness of the differences among the population centroids, or the mean vector (Bose, 1977).

Multivariate analysis of variance was originally developed by Wilks through the generalized likelihood-ratio principle. That approach led to the test statistics.

$$\Lambda = \frac{|W|}{|T|},$$

where W is an error matrix (within-groups); T is a total matrix (hypothesis and error matrix). The general utility of the determinant ratio statistic is based on its transforms of it that distribute approximately as χ^2 and F (Cooley and Lohnes, 1971).

2.8.2 *Discriminant analysis*

When two or more populations have been measured for several characters, special interest attaches to certain linear functions called discriminant functions, by which the populations are best discriminated. Since MANOVA cannot make a comparison of various means, we need to use a discriminant function to get test scores for a means comparison. Therefore, it is of great importance in multivariate analysis, and it has been widely used in social science (Manly, 1986; Cooley and Lohnes, 1971).

The discriminant function will maximize the ratio of the among groups sum of squares ($v'Av$) to the within-groups sum of squares ($v'Wv$), so that among-group differences will be large relative to within-group. The function is defined as follows:

$$y_{ij} = v_j' x_i .$$

where x_i is the best discriminant function of the measurement vector ($x_i = X_i$ - m, m is the grand centroid, or vector of total sample means).

The task for the calculus of partial differentiation with respect to the unknown weights in v is to maximized the ratio of these two quadratic forms:

$$\lambda = \left. \frac{v'Av}{v'Wv} \right|_{\text{maximum}}$$

subject to the restriction

$$v'v = 1 .$$

In order to be able to view the discriminant function as a factor of the test battery, discriminant scores for a standardized function of a standardized test vector are derived as follows:

$$f_{ij} = \theta^{-1/2} y_{ij} = \theta^{-1/2} v_j' x_i$$

where θ is a grand variance of $\mathbf{y}$. Thus, defining $\mathbf{b}_j = \theta^{-1/2} \mathbf{v}_j$. Continuing,

$$x_i = D_{diag}^{1/2} z_i$$
$$f_{ij} = \theta^{1/2} v'_j D_{diag}^{1/2} z_i = c'_j z_j$$

where $\mathbf{D}_{diag}$ is the standard deviations of **x**; when,

$$c_j = D_{diag}^{1/2} v'_j \theta^{1/2}$$

c_j'(standardized canonical coefficients) was reported in MANOVA of SAS results. Thus,

$$f_{ij} = c'_j z_j = c'_j \frac{X - m}{D_{diag}^{1/2}}$$

Several solutions (n) are to be retained from the eigenstructure reported by the MANOVA routine. Thus, **C** is the *p x n* matrix of discriminant factor coefficients.

2.8.3 *Principle component analysis*

The principal component technique is that of summarizing most of the variation in a multivariate system in the number of variables which are explained by component scores. A general linear transformation of the standardized vector variable **z** of the form as follows:

$$z_{ij} = \frac{x_{ij} - \bar{x}_j}{s_j}$$

$$\mathbf{y} = \mathbf{V'z}$$

where **V** is a *p* x *n* coefficients matrix that carries the *p*-element variable *z* into the derived *n*-element variable **y**. Thus, *p* principal components can be computed.

According to Morrison (1990) generally the few first components describe most of the variance in data. The first principal component explains the maximum of variance, and the second principal component explains the maximum of remaining variance and so on. A certain minimum number of components (parsimony) with large and distinct variances should be extracted. Beyond that number components might be computed until some arbitrarily large proportion of the variances has been explained.

The major difficulty with this method is the interpretation of resulting principal components. Interpretation of the meaning of components is on the basis on factor structure matrix which indicates the correlation of each variable with each of the components. Frequently, it is better to summarize the complex in terms of the first components with large and markedly distinct variances, and to include highly specific and unique variate responses which are generally independent in the system (Morrison, 1990).

2.8.4 *Cluster analysis*

Cluster analysis is widely applicable in research to determine clusters of similar objects. It may be worthwhile owing to grouping of similar objects or for data reduction. Many algorithms have been proposed for cluster analysis. Here attention will be restricted Ward's minimum variance clustering method. Ward (1963) described a class of hierarchical clustering methods, including the minimum variance method. Anderberg (1973) showed that Ward's method would appear to be more suitable than other methods of clustering. Ward's method tends to join clusters with a small number of observations and is strongly biased toward producing clusters with roughly the same number of observations.

Word's method follows a series of clustering steps that begin with t clusters, each containing one object, and it ends with one cluster containing all objects. At each step it makes whichever merger of two clusters that will result in the smallest increase in the value of a sum-of squares index, or variance. This means that at each clustering step all possible mergers of two clusters must be tried, computed the value of sum-of-square index for each, and selected that one whose value of sum-of square index is the smallest. Then the next clustering step is followed and repeated the process (Romesburg, 1984).

Determination of useful number is still main difficulty of this analysis. In hierarchical clustering system the number of clusters which may be obtained from n, the number of individual (objects) to one depending on the level at which the hierarchy is cut-off

(Anderberg, 1973). Anderberg (1973) suggested that the normal way is to make a subjective choice according to previous knowledge of the data. Gordon (pers. com.) and Teow (1978) applied a cut-off point based on the F-ratio of amongst cluster sums of square to within cluster sums of squares.

2.9 Quantification of biology

The interest in quantification of various aspects of biology has been aroused in recent decades. The advantages of fitting functions to growth or germination data are manifold and these, together with the rationale of curve fitting, are given by Hunt (1979). These "functional" approaches to plant growth analysis fit cumulative growth data to either polynomial approximations of exponential functions, or to explicitly defined sigmoid models (Venus and Causton, 1979). With determinate growth data, statistically comparable descriptions can be achieved by either method, but practical observations appear to favour the use of sigmoid models. A sigmoid curve may be either symmetric or asymmetric according to whether the point of inflexion is or is not midway between the two asymptotes.

The logistic is the best known sigmoid function, which is symmetric. In plant growth studies, the fact that the function is S-shaped (albeit inflecting rigidly at A/2) has rendered it very popular. The equation of logistic function is as follows (Causton and Venus, 1981):

$$L = A(1 + e^{(\beta - kt)})^{-1}$$

with asymptotes at $f(t) = 0$ and $f(t) = A$. k is a 'rate' parameter - a high value indicating a rapid rise of the function between the two asymptotes, and *vie versa*; and β/k defines the value of t at the point of inflexion. Thus, the parameters β and k are not biologically independent of one another.

The Gompertz function is a non-symmetric sigmoid function. The equation of Gompertz function is as follows (Causton and Venus, 1981):

$$L = A \cdot \exp(-e^{(\beta - kt)})$$

where the parameters have the same general meaning as in the logistic function. But the value of $f(t)$ at the point of inflexion is A/e instead of $A/2$.

The Gompertz function has been found to be more appropriate in biological work than other sigmoid function. Hackett and Rawson (1974) considered that the asymmetry of the Gompertz function was more appropriate to leaf growth data than the symmetry of the logistic in tobacco.

A major disadvantage of the two preceding functions is that each has an unalterable shape (Causton and Venus, 1981). This means that the point of inflexion occurs at a given distance between the two asymptotes regardless of the parameter values. Although change of the latter will alter the position of the upper asymptote, the position of the point of inflexion with respect to the t-axis, and the rate at which the curve rises from one asymptote to the other.

The Richards function has four-parameters. It has the point of inflexion anywhere between 0 and A by the adjustment of only one of its four parameters. The Richards function is based on a simple biological mode embodied in the following equation.

$$L = A(1 \pm e^{(\beta - \kappa t)})^{-1/v}$$

where the parameters have the same general meaning as in the logistic and Gompertz functions. v estimates the inflexion point of the curve, *e.g.,* the shape of the curve. The negative alternative is used when v is negative, and *vice versa* (Causton and Venus, 1981). For $v = 0$, Richards function becomes indeterminate but this value of v represents a limiting situation - the Gompertz function. For $v = 1$, the Richards function reduces to the logistic.

The Richards function is probably the simplest empirical model which is both realistic and sufficiently flexible for growth description in a wide variety of instances. It has been used both as a smoothing device in place of polynomials (Venus and Causton, 1979) and in a situation where the values of the parameters of the fitted functions may be interpreted in a biologically meaningful way (Causton *et al.*, 1978). Causton and Venus's (1981) analysis of the sequential development of area in individual leaves (sunflower) shows that a remarkably high initial relative growth rate (almost 1.0 day^{-1}) in the first leaf decays away almost entirely within a fortnight and, throughout, can be

followed (within tight limits) by this most flexible of common asymptotic function. Voldeng and Blackman (1973) fully exploited the properties of the Richards function in analysis of dry matter production of cultivars and hybrids of *Zea mays* (maize) which span almost the entire life cycle of the plant. Although the Richards function provides a very realistic description of plant growth (Causton and Venus, 1981), it has not been used widely in seed germination studies.

NonLINear regression (NLIN) procedure in SAS computes least squares or weighted least squares estimates of the parameters of a nonlinear model (SAS Institute Inc., 1990). Nonlinear models are more difficult to specify and estimate than linear models. Instead of simply listing regressor variables in SAS, the regression expression, declaring parameter names, supplying starting values, and possibly supplying derivatives of the model with respect to the parameters are also required. In NLIN, there are five iterative methods: steepest-descent or gradient, Newton, modified Gauss-Newton, Marquardt and multivariate secant or false position (DUD) methods. Marquardt's method is equivalent to performing a series of ridge regressions and is useful when the parameter estimates are highly correlated or the objective function is not well approximated by a quadratic (SAS Institute Inc., 1990). The Marquardt method is a compromise between Gauss-Newton and steepest descent. The Marquardt iterative method regresses the residuals onto the model partial derivatives of the model with respect to the parameters, until the estimates converge. The derivatives of Richards function required for the NLIN program are different to those given by Causton and Venus (1981), who used Newton-Raphson iterations and MINRES-based differentials. The required differentials are not available in the literature, and their solution was an important pre-requisite for curve fitting.

Chapter 3. Meadowfoam germplasm

3.1 Introduction

Genetic variability is essential for effective plant breeding. Furthermore, estimates of genetic variability permit credible predictions of population behaviour under the influence of selection and dispersion for many field crops. Variance component analysis, which partitions the phenotypic variance into its genetic and environmental components, is used to estimate the types and extent of gene action. From the results of variance component analysis, estimates of heritability can be made also. Heritability determines the degree of genetic determination of attributes, and may reflect also the resemblance between relatives. The most important use of heritability is its predictive role in estimating genetic advance from selection: in expressing the reliability of the phenotypic value as a guide to the breeding value (*i.e.* the average-allele genotypic expectation).

Phenotypic variation for numerous physiological and morphological characters in the population appears sufficient to warrant a quantitative breeding approach in *Limnanthes* domestication (Brown *et al.*, 1979; Pierce and Jain, 1977; Krebs and Jain, 1985). However, study of genetic variation in agronomic traits should be guided by a knowledge of which of these have the greatest effect on overall crop performance. Thus, potential use of variability for plant breeding programs has not been assessed. This present experiment estimated the variability of genetics and biology in flowering and seeding characters of a diverse gene-pool in meadowfoam (*Limnanthes alba*).

The specific objectives of this experiment were:

1) to evaluate meadowfoam germplasm by investigating date and duration of flowering, in order to explore rate of floral initiation and flower period, investigating seed set and seed shattering, and estimating phenotypic variance components and heritability for these traits; and

2) to establish a method for grouping of phenotypes based on genetic variance components.

3.2 Material and methods

3.2.1 Plant material

The experimental material was drawn from bulk seeds of the open-pollinated composite of "Moginie". This consisted of two generations of bulk random-mating of eight accessions of meadowfoam (Table 3.1). The first six were bulk derivatives of open-pollinated Californian accessions. The last two were mass-selection-bulks of wide diversity, being the first and second cultivars, respectively, of the species.

The experiment was conducted in Massey University Pasture and Crop Research Unit. Rows were spaced in 50 cm apart, while plants within rows were spaced at 25 cm. Two hundred and fifty-nine plants were chosen randomly to form an unbiased sample representing the composite.

3.2.2 Characters studied

As this is a pioneering study, characters chosen gave a strong emphasis to reproductive matters which had shown conspicuous variation, and were potentially of agronomic interest.

Table 3.1 The description of parental lines.

Accession	Name	Type	Origin
G 1	305 Bulk	Topodeme	Davis, California
G 2	308 Bulk	Topodeme	Davis, California
G 3	309 Bulk	Topodeme	Davis, California
G 4	312 Bulk	Topodeme	Davis, California
G 5	322 Bulk	Topodeme	Davis, California
G 6	323 Bulk	Topodeme	Davis, California
G 21	Foamore	Mass selection bulk	Corvallis, Oregon
G 22	Mermaid	Mass selection bulk	Corvallis, Oregon

3.2.2.1 Flowering and seed set

In order to explore the patterns of flowering and seeding in this new crop, number of flowers plant^{-1} and seed set flower^{-1} were counted four times. Number of flowers plant^{-1} was recorded at 7 day intervals, starting 10 days after first-flowering (D1, D2, D3 and D4). At each time, the numbers of fresh and old flowers were recorded. Fresh flowers were the spread during flowering period, whereas old flowers were the flower retention.

$$\text{Total flowers (Dx)} = \text{Fresh flowers (Dx)} + \text{Old flowers (Dx)};$$

$$\text{Proportion of fresh flowers (Dx)} = \frac{\text{No. of fresh flowers (Dx)}}{\text{Total flowers (Dx)}}.$$

(Δ proportion (fresh) = floral initiation rate = rate of "general" flower production.)

Seed set was counted on 10 random flowers per plant at 7 day intervals (S1, S2, S3 and S4), starting at (D1+7) days. Maximum seed-set during seed development (Smax) was noticed. The data of seed set could also assess seed shattering (see 3.2.2.3).

3.2.2.2 Yield and yield components after harvest

After harvest, yield components, *e.g.* number of total flowers (Fh), number of total seeds plant^{-1} (Seeds), weight of 100 seeds for each plant (Seedwt) were recorded, to gain a better understanding of this germplasm. Total seed yield plant^{-1} was also recorded directly (Yld).

Seed set flower^{-1} (Lset) (10 flowers) was also investigated, to assess harvest seed shattering.

3.2.2.3 Seed shattering

In this new crop, seed shattering is one of traits which provide a challenge for its domestication. Both field seed shattering (Fsh) and harvest seed shattering (Hsh) were studied, to determine how seed yield was affected.

$$Fsh(\%) = \frac{Smax - S4}{Smax}$$

$$Hsh(\%) = \frac{Smax - Lset}{Smax}$$

3.3 Data analysis

3.3.1 Multiple regression

Multiple regression analysis of seed yield was done as a function of the most effective factors: fourth total flowers (T4), second, third and fourth seed-set (S2, S3 and S4), field seed shattering (Fsh), harvest seed shattering (Hsh), flowers plant^{-1} after harvest, and 100 seed weight. Multiple regression analysis was effected using PROC REG with the STB (standardized β) option of the SAS statistical package. The results of standardized β of multiple regression showed that fourth total flowers (T4) and second seed-set (S2) were less associated with seed yield (Table 3.2). Therefore, multiple regression analysis of seed yield was rerun, only considered third and fourth seed-set (S3 and S4), field seed shattering (Fsh), harvest seed shattering (Hsh), flowers plant^{-1} after harvest and 100 seed weight, to explore clearly the relationship between seed yield and both seed shattering.

Both results of standardized β of multiple regression showed that harvest seed shattering (Hsh) was highly negatively correlated (0.3138 and 0.3225) to the seed yield (Table 3.2), whereas the field seed shattering was little negatively correlated (0.0879 and 0.0636) to the seed yield. Thus, the shattering variable only considered harvest seed shattering in the second principal analysis (see Section 3.3.2).

3.3.2 Pattern analysis

The principal component technique is that of summarizing most of the variation in a multivariate system in the number of variables which explained by component scores. There are several principal components can be computed. In order to minimize the number of principal components utilized for each attribute (parsimony), it was decided to use only those components which accumulated at least 70% of total variance in this study.

Table 3.2 The standardized ß of multiple regression for seed yield against yield components.

Variable	Regression 1	Regression 2
Total Flowers (T4)	0.0685	----
Seed set (S2)	0.0592	----
Seed set (S3)	0.1194	0.1033
Seed set (S4)	0.1826	0.2152
Field seed shattering	-0.0879	-0.0636
Harvest seed shattering	-0.3138	-0.3225
Flowers	0.7476	0.8112
Seed weight	0.1486	0.1519

N.B. the higher standardized ß (absolute value) indicated the higher correlation between yield and variable.

The patterns of fresh flowers (D1, D2, D3 and D4), total flowers (T1, T2, T3 and T4) and seed set (S1, S2, S3 and S4) were analyzed by principal components (PC) analysis, because of not enough time observations for regression analysis. PC analysis can be used for exploring polynomial relationships and for multivariate outlier detection. The PRINCOMP procedure of the SAS statistical package was used for PC analysis. The analysis was done on standardized data. The un-rotated structure of each analysis was used to interpret the meaning of the components, and were regarded as orthogonal common factors underlying the different flowering and seed set patterns of individual plants.

3.3.3 Clustering phenotypes

According to solving rule (the basis of 70% of the total variation explained), two principal components for fresh flowers, total flowers and seed set in first PC analysis were used to summarise data and detect linear relationships (Table 3.3). Thus, the second PC analysis was conducted for the combination of variables, including 6 principal components and seed set maximum (Smax), harvest seed shattering (Hsh), and measuring data after harvest (Flowers, Seeds, Lset, Seedwt and Yld).

A cluster analysis was done based on the second PC scores, in order to recognize the groups of plant habit which had been defined. According to solving rule, the first four

principal components were used in cluster analysis (Table 3.4). The similarity matrix for clustering consisted of PC scores for each plant was based on Euclidean distance, and the Ward method of clustering was subsequently used. In this method, minimal increase of within-cluster sum-of-squares is the criterion of merging. This is fundamentally acceptable to the concept of defining "natural" clusters. There are several previous reports of the successful application of Ward method for defining credible cluster (Teow, 1978; Cullen, 1981; and Hashjin, 1992). The CLUSTER procedure in SAS was used to find hierarchical clusters of four PC scores. SAS's handling of the post-analysis is not good, and other program was used for this purpose.

After clustering, program CLUMEM (I.L. Gordon, unpublished) was used to reveal the hierarchical relationships (dendrogram). CLUMEM examined the growth in the "error" sum of squares (*i.e.* pooled within-cluster sum of squares) of similarity attribute as clustering progressed. CLUMEM also included the probability of the F-test amongst/within cluster. The least F-probability indicated the greatest significance for clustering and the stage to which that belonged was used as the natural truncation level of the dendrogram (Gordon, in Teow 1978).

Table 3.3 Flowering and seeding behaviours, as observed through PC analysis on fresh flowers, total flowers and seed set.

	Component	Eigenvalue	Percentage of variation	Cumulative percentage
Fresh flowers	1	1.8206	45.52	45.52
	2	1.0517	26.29	71.81
	3	0.8328	20.82	92.63
	4	0.2949	7.37	100.00
Total flowers	1	2.7286	68.21	68.21
	2	0.9634	24.08	92.30
	3	0.2555	6.39	98.68
	4	0.0526	1.32	100.00
Seed set	1	2.7663	69.16	69.16
	2	0.8867	22.17	91.32
	3	0.3179	7.95	99.27
	4	0.0292	0.73	100.00

Table 3.4 General ordination of plant *via* PC analysis over thirteen characters.

Component	Eigenvalue	Percentage of variation	Cumulative percentage
1	4.0044	30.80	30.80
2	2.9146	22.42	53.22
3	1.5851	12.19	65.42
4	1.3757	10.58	76.00
5	1.0013	7.70	83.70
6	0.9051	6.96	90.66
7	0.6207	4.77	95.44
8	0.1915	1.47	96.91
9	0.1704	1.31	98.22
10	0.1106	0.85	99.07
11	0.0767	0.59	99.66
12	0.0308	0.24	99.90
13	0.0132	0.10	100.00

In this study, several truncation levels in the dendrogram were examined. The truncation points were defined at cluster stage 216 (all plants were involved), 193, 160, 124 and 58. The cluster stage 58 was the "most significant" cluster stage, as discussed above. The other truncation levels were arbitrarily chosen. The 248 plants (11 plants contained missing data) were divided into several clusters for each stage. The membership of clusters was used to define "phenotypes". The clusters were summarized thereby to define the growth habit of phenotypes in each cluster. The various clusters defined thereby were subjected to ANOVA, and variance components were estimated. The amongst-cluster component was taken as a "genotype" variance, and the within-cluster component as "environment". Heritabilities were estimated as well.

The experimental unit was the plant within clusters, which also formed the analysis unit in the following models. The statistical analysis was based on an unbalanced completely random design model, as follows:

$$X_{ij} = \mu + \gamma_i + \varepsilon_{ij}$$

where X_{ij} = the ij-th phenotypic variate, μ = population mean, γi = genotype (cluster) effect, ε_{ij} = residual (plant) effect.

The expectations of the Mean Squares for this model is given in Table 3.5. As the number of plants varied considerably amongst phenotypes, a linear method of analysis for unbalanced data was used (Searle, 1987). The least squares means, mean square and coefficient of variance components for each phenotype were analysed using GLM procedure (SAS Institute Inc., 1990). ANOVA was operated for 13 characters, *e.g.* 6 PC scores for fresh flowers, total flowers and seed set and maximum seed set, harvest seed shattering and measuring data after harvest.

3.3.4 Estimation of variances

Variance component estimates were obtained from linear functions of the mean squares as indicated by their expectations (Table 3.5). Standard error of variance components were also computed following Crump (1946) and Satterthwaite (1946), to evaluate the precision of these estimates.

The heritability (h^2) in the broad sense is the ratio of genotypic variance (σ_G^2) to phenotypic variance (σ_p^2) (Falconer, 1989).

$$h^2 = \frac{\sigma_G^2}{\sigma_P^2}.$$

Phenotypic variance (σ_p^2) is composed of genetic variance (σ_G^2) and environmental variance (σ_E^2). For this model,

$$\sigma_p^2 = \sigma_E^2 + \sigma_G^2.$$

The computer program AOVCRD (I.L. Gordon, unpublished) was used to estimate the variances of genotype, phenotype and residual and the heritability, their standard errors as well.

Table 3.5 Sources of variation, degrees of freedom, expectations of mean squares and variance component of CRD analysis.

Source	DF	MS	E(MS)	Variance component
Phenotype	g-1	MS_G	$\sigma^2 + r_i^a\sigma^2$	$(MS_G - MS_e)/r_i$
Residue	g(r-1)	MS_e	σ^2	MS_e

a: Harmonic mean for unbalanced ANOVA (Searle, 1987).

3.3.5 Distribution of data

Data from the 248 plants of meadowfoam for 13 characters were adequately summarised by their means (μ) and variances (σ^2). The information was extended by examining their distribution as well, for a better understanding of this germplasm. Data were sorted into 15 equal-standard-deviate categories under the distribution given for ± 1 to 7 standard deviation (σ) from the mean (0). Then, the data were divided into 5 groups with 3 standard deviation (σ) for each, *e.g.* high value of the variable (H) in areas +5 to +7, medium high of the variable (M+) in areas +2 to +4, medium value of the variable (M) in areas -1 to +1, medium lower value of the variable (M-) in areas -4 to -2 and low value of the variable (L) in areas -7 to -5. Program VAREST (I.L. Gordon, unpublished) was used to calculate the frequency distribution of data for 15 areas and 5 groups and also examined whether the data followed normal distribution.

3.4 Results

3.4.1 Pattern analysis

Principal components analysis was used to score plants according to their patterns of fresh flowers plant^{-1}, total flowers plant^{-1} and seed set flower^{-1} due to not enough observations for regression analysis. There were four measurements for each character, and differences amongst these times (which were "attributes" in the PC analyses) defined differing "patterns" of behaviour plant by plant ("entities") in the character being considered. Separate PC analyses were done for each character (fresh flowers, total flowers and seed set). This pattern analysis was considered superior to regressions (over time) because of few time-nodes, and it avoided the need to find optimum functions.

For the PC analysis, Table 3.3 presents the eigenvalues (variance of PC scores) and cumulative percentage of total variance for each component. The factor structure and standard coefficient of the principal components enable the components to be interpreted, which are given in Table 3.6.

Table 3.6 Factor structure matrix and standard coefficient for fresh, total flowers and seed set.

		Factor structure matrix		Standard coefficient	
	Attributes	Component 1	Component 2	Component 1	Component 2
Fresh flowers	Date 1	-0.52043	0.56383	-0.28585	0.53611
	Date 2	0.82165	0.26248	0.45130	0.24957
	Date 3	0.88368	-0.18705	0.48537	-0.17786
	Date 4	0.30626	0.79367	0.16821	0.75465
Total flowers	Date 1	0.47224	0.85928	0.17307	0.89197
	Date 2	0.89582	0.18015	0.32831	0.18701
	Date 3	0.94327	-0.27233	0.34570	-0.28269
	Date 4	0.90185	-0.34405	0.33052	-0.35714
Seed set	Date 2	0.41131	0.91139	0.14869	1.02790
	Date 3	0.87323	-0.10754	0.31567	-0.12129
	Date 4	0.95615	-0.17322	0.34565	-0.19537
	Date 5	0.95933	-0.12021	0.34680	-0.13558

The number of components to be studied was decided on the basis of 70% of the total variation explained. Therefore, the first two components satisfied this criterion for fresh flowers, total flowers and seed set (Table 3.3). For fresh flowers, Component 1 (FFPC1) accounted for 45.52% of the total variation, but Component 2 (FFPC2) only 26.29% of the variation (Table 3.3).

One of the major aspects of PC analysis is the biological interpretation of the important factors. Date 2 (24 days after flowering) and Date 3 (31 days after flowering) had the highest positive correlation with Component 1 (Table 3.6). They also had the highest positive standard coefficients. Thus, these results indicated that "median flowers" were important for Component 1. There was also a medium contrast with beginning of flowering. Date 1 (10 days after flowering) and Date 4 (38 days after flowering) were highly positively correlated with Component 2, with high positive standard coefficients, which suggested that Component 2 was associated with "flowering extremities" (with an emphasis in late flowers).

For total flowers, Component 1 (TFPC1) and Component 2 (TFPC2) accounted for 68.21% and 24.08% of the total variation (Table 3.3). Component 1 associated with "flower production" since Date 2, Date 3 and Date 4 were highly positive correlations with Component 1 (Table 3.6) and had also high standard coefficients. Date 1 was the highest

positive correlation with Component 2 and had a high standard coefficient. Date 4 also had a highly negative correlation with the component and a negative standard coefficient although they were not as large as that in Date 1. The results suggested that Component 2 was important for "early or/and late flowers".

For seed set, Component 1 (SETPC1) accounted for 69.16% of the total variation, but Component 2 (SETPC2) only 22.17% (Table 3.3). Date 4 and Date 5 (45 days after flowering) were the highest positive correlation with Component 1 although Date 3 was also highly correlated. The standard coefficients for Component 1 were also higher in Date 3, Date 4 and Date 5. The results was regarded as a "median and late seed set" factor. Date 1 was extremely correlated with Component 2 and had the highest standard coefficient. Thus, Component 2 was significant for "early seed set".

Scatter plots of plants against PC1 and PC2 for fresh flowers, total flowers and seed set are given in Figure 3.1-3.3. No marked discontinuity appears for these characters.

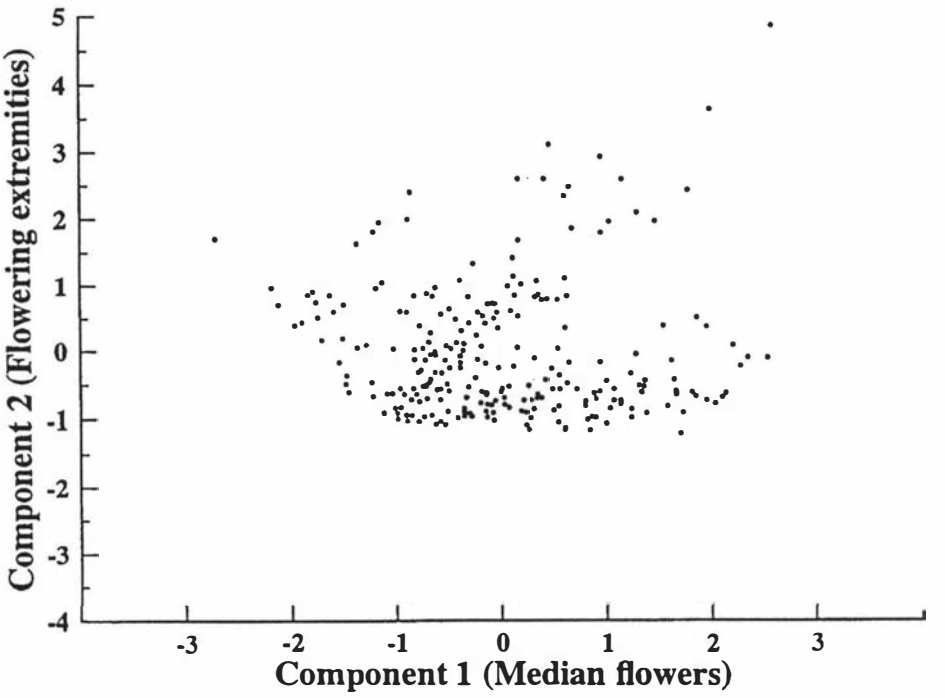

Figure 3.1 Scatterplots of plants for fresh flowers against PC1 and PC2.

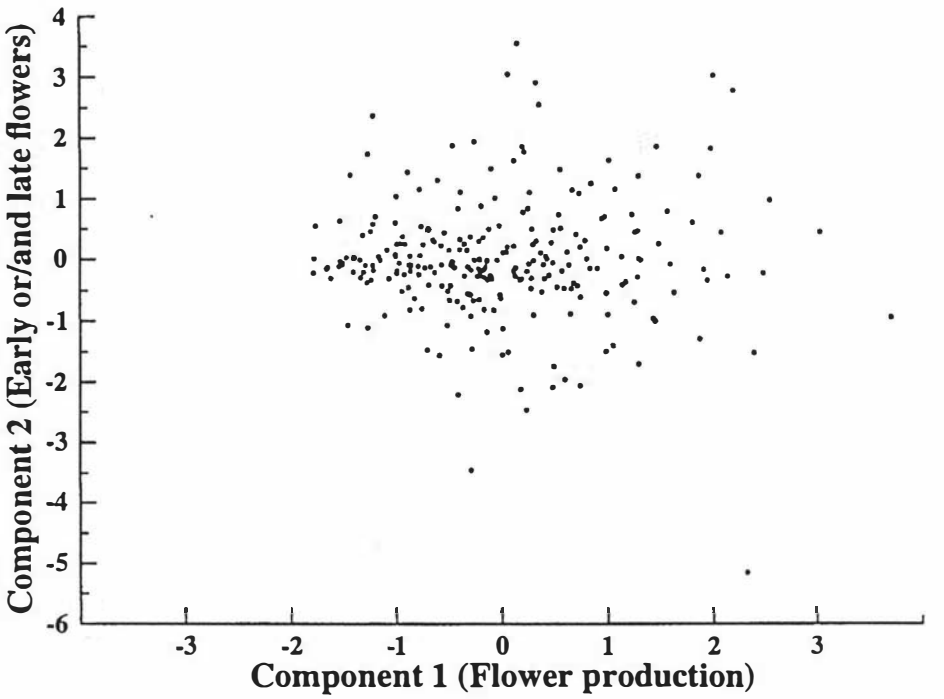

Figure 3.2 Scatterplots of plants for total flowers against PC1 and PC2.

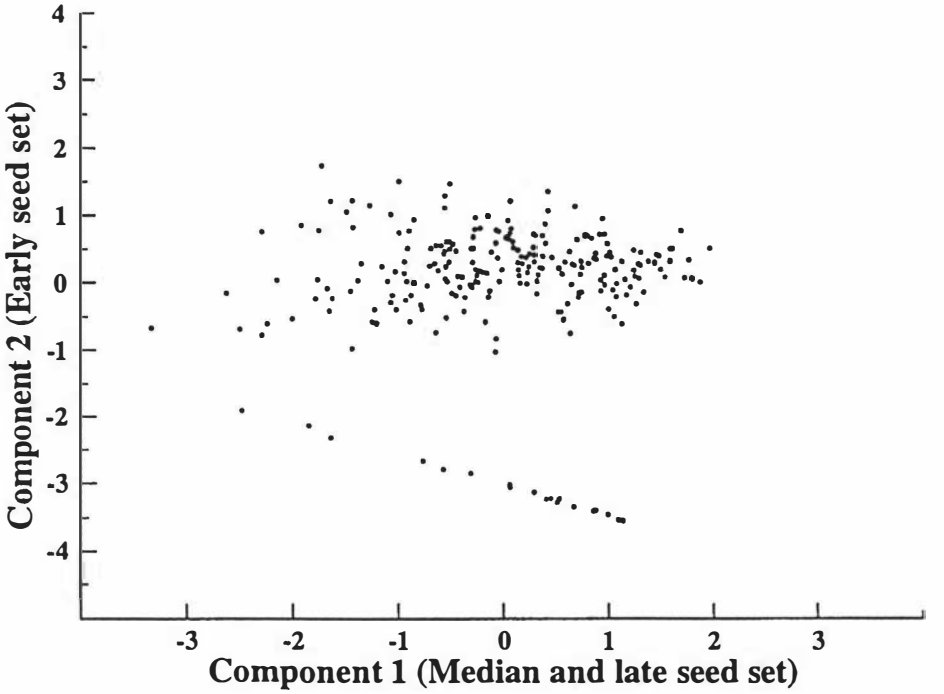

Figure 3.3 Scatterplots of plants for seed set against PC1 and PC2.

3.4.2 Distribution of data

Thirteen characters (*e.g.* PC 1 and 2 for fresh flowers, total flowers and seed set, maximum seed set, harvest shattering, seed set after harvest, flowers plant^{-1}, seeds plant^{-1}, 100 seed weight and seed yield plant^{-1}) did not follow normal distribution (Figure 3.4 - 3.5), except for PC 1 for fresh flower and seed set. The data indicated that no transformation was required, because they did not follow any particular distribution, showing only random perturbations in the data.

Much of the phenotypic variation was found for all characters (Table 3.7). The results for these characters showed that plant with medium level of performance (M) ranged between 35.5% (seed set PC1) to 56.9% (seed set PC2), but only 29.8% in fresh flower PC2. In contrast, only 10% or less plants had either lowest value (L) or highest value (H) for 13 characters. Plants with medium high value (M+) were 20% or less, except in seed set PC1 (29.8%), maximum seed set (31.5%) and seed set after harvest (30.0%). Plants with medium low value (M-) varied in characters. The value was 43.1% for fresh flower PC2, whereas it was only 7.7% for seed set PC2. The medium low value for most characters, however, was around 21.4% to 34.7%.

Table 3.7 Frequency of plants (%) around mean value for 13 characters.

Characters	Lowest (-7 to -5)	Lower (-4 to -2)	Mean area (-1 to +1)	Higher (+2 to +4)	Highest (+5 to +7)
Fresh flowers PC 1	5.6	29.0	36.3	19.8	9.3
Fresh flowers PC 2	0	43.1	29.8	18.5	8.5
Total flowers PC 1	4.0	28.2	39.9	20.2	7.7
Total flowers PC 2	3.6	27.0	48.4	12.1	8.9
Seed set PC 1	8.1	21.8	35.5	29.8	4.8
Seed set PC 2	7.7	7.7	56.9	17.0	0.8
Maximum seed set	10.1	14.9	40.3	31.5	3.2
Harvest shattering	2.8	27.0	47.6	14.9	7.7
Lab seed set	10.1	18.5	35.9	30.0	4.4
Lab flowers plant^{-1}	0.4	34.7	38.3	18.1	8.5
Lab seeds plant^{-1}	2.0	33.1	37.9	16.9	10.1
100 seed weight	2.4	21.4	55.2	19.8	1.2
Seed yield plant^{-1}	1.2	33.9	38.3	17.3	9.3

Mean area was defined within -1 to +1 area in histogram and frequency polygon;
Lowest and Lower were defined within -7 area to -5 and within -4 to -2 area in histogram and frequency polygon;
Highest and Higher were defined within +5 to +7 area and within +2 to +4 area in histogram and frequency polygon.

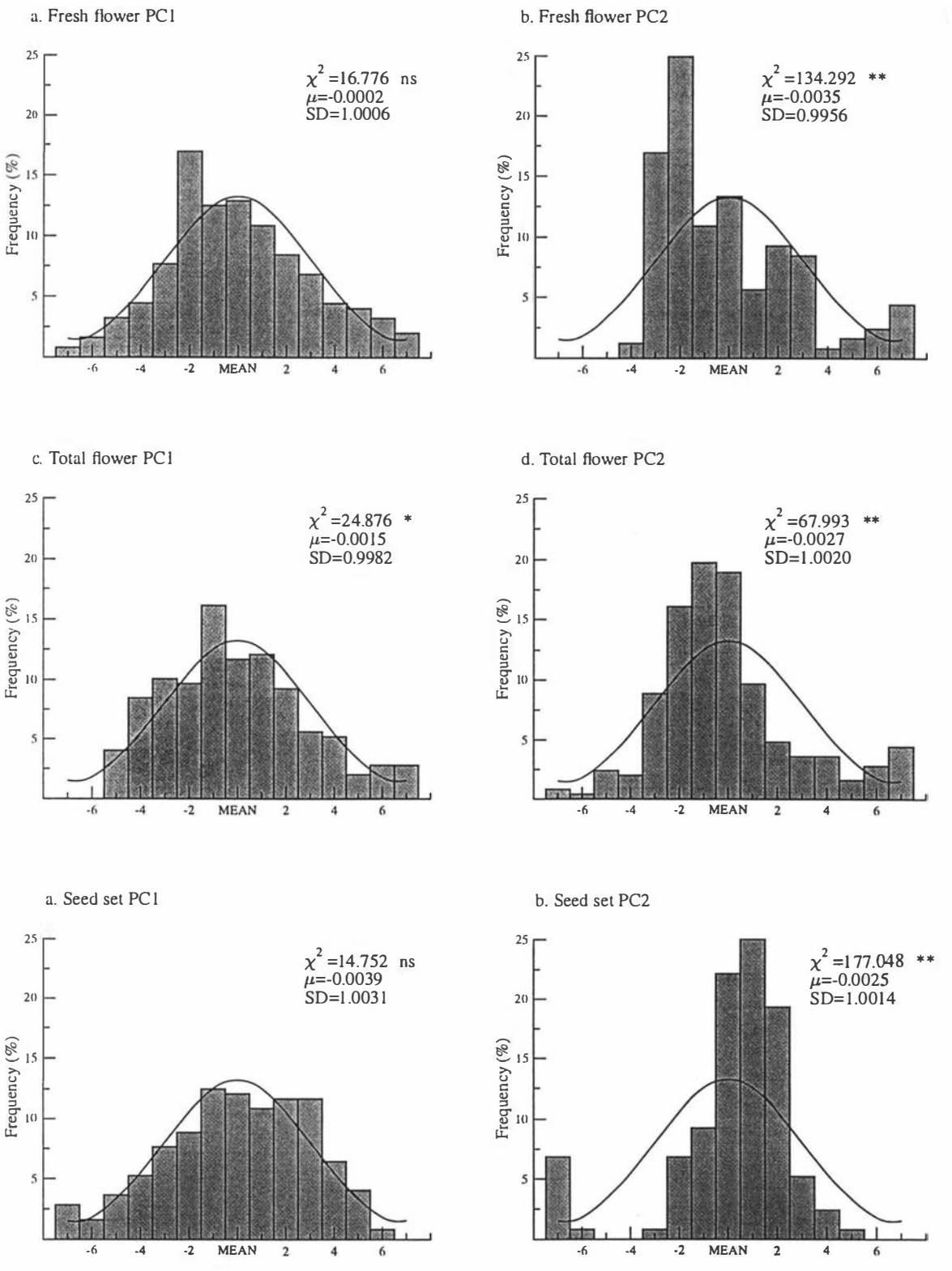

Figure 3.4 Histogram and frequency polygon for PC1 and PC2 of fresh flowers, total flowers and seed set.

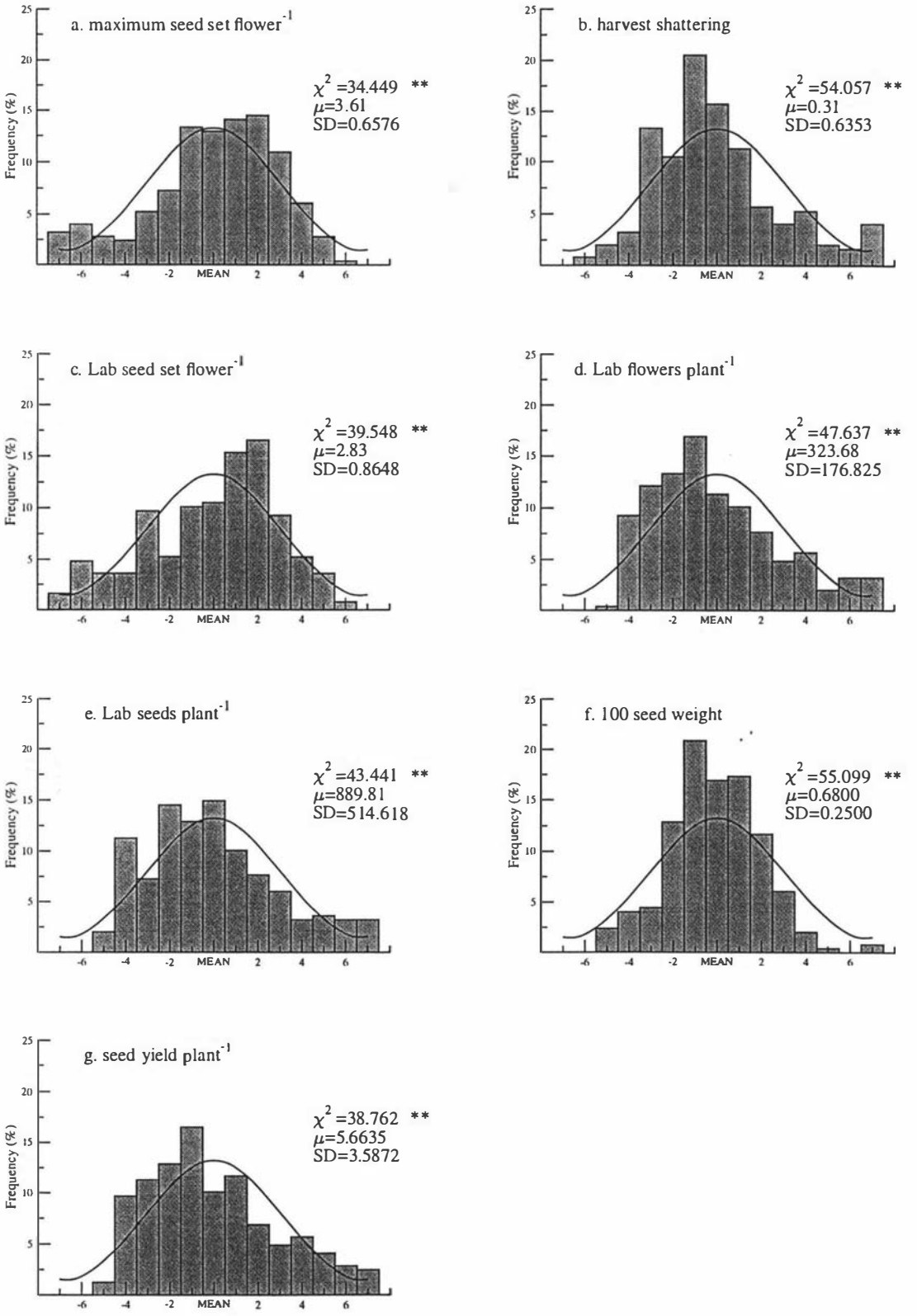

Figure 3.5 Histogram and frequency polygon for 7 characters.

3.4.3 Clustering phenotypes

3.4.3.1 Second principal components analysis

Second PC analysis was conducted in order to obtain a multivariate ordinal score for the individual plants, including 6 first principle scores for fresh flowers, total flowers and seed set, and directly measured characters (seed set flower^{-1}, total flowers plant^{-1}, total seeds plant^{-1}, 100 seed weight and seed yield plant^{-1}), and maximum seed set and harvest seed shattering. This combined PC score aimed to explore the discriminating characters for flower and seed production in this broad meadowfoam germplasm. This diversity of the germplasm could also be evaluated as a result.

For the second PC analysis, Table 3.4 presents the eigenvalues and cumulative percentage for each component. Table 3.8 presents the factor structure matrix and Table 3.9 the standard coefficients. The number of components to be studied was also decided on the basis of 70% of the total variation explained. Therefore, the first four components represented the patterns of relationship of the original 13 variables (Table 3.4).

Component 1 accounted for 30.80% of the total variation, 22.42%, 12.19% and 10.58% for Component 2, Component 3 and Component 4, respectively (Table 3.4). Component 1 was by definition the most important component. The 3rd and 4th components were less important. The 2nd component was middle.

Table 3.8 Factor structure matrix for thirteen characters.

	Component 1	Component 2	Component 3	Component 4
Fresh flower PC1	0.57335	-0.04470	-0.72097	-0.08126
Fresh flower PC2	0.24865	0.63124	0.32872	-0.04203
Total flower PC1	0.67126	0.61818	0.01705	-0.11541
Total flower PC2	-0.43775	0.42604	0.71511	-0.07860
Seed set PC1	0.47898	-0.65624	0.32089	0.39381
Seed set PC2	0.09887	0.16016	0.08601	0.39630
Seed set maximum	0.35137	-0.72152	0.20749	0.42868
Harvest shattering	-0.12627	0.37894	-0.11106	0.83447
Lab seed set	0.44303	-0.73783	0.30666	-0.35042
Lab flowers plant^{-1}	0.78091	0.48249	-0.07688	0.14928
Lab seeds plant^{-1}	0.95627	0.05829	0.08669	-0.08406
100 seed weight	0.19021	0.19524	0.37981	-0.02094
Seed yield plant^{-1}	0.91447	0.10639	0.16744	-0.05460

Table 3.9 Standard coefficient for thirteen characters.

	Component 1	Component 2	Component 3	Component 4
Fresh flower PC1	0.14318	-0.01534	-0.45486	-0.05907
Fresh flower PC2	0.06210	0.21658	0.20739	-0.03055
Total flower PC1	0.16763	0.21209	0.01076	-0.08389
Total flower PC2	-0.10932	0.14617	0.45116	-0.05713
Seed set PC1	0.11961	-0.22515	0.20245	0.28627
Seed set PC2	0.02469	0.05495	0.05426	0.28807
Seed set maximum	0.08775	-0.24755	0.13090	0.31162
Harvest shattering	-0.03153	0.13001	-0.07007	0.60659
Lab seed set	0.11064	-0.25314	0.19347	-0.25472
Lab flowers plant^{-1}	0.19502	0.16554	-0.04850	0.10851
Lab seeds plant^{-1}	0.23881	0.02000	0.05469	-0.06110
100 seed weight	0.04750	0.06698	0.23962	-0.01522
Seed yield plant^{-1}	0.22837	0.03650	0.10564	-0.03969

Component 1 was primarily a measure of seeds plant^{-1} and seed yield plant^{-1} (Table 3.8). TFPC1 (flower production) and flowers plant^{-1} also had high loadings for this component. The standard coefficients for these 4 characters were relatively high (Table 3.9). This suggested that Component 1 associated "yield production".

Component 2 associated positively to FFPC2 (flowering extremities), TFPC1 (flower production) with higher positive standard coefficients (Table 3.8 and 3.9). On the contrary, Component 2 related negatively to SETPC1 (median and late seed set), maximum seed set and seed set after harvest, with negative standard coefficients. The results implied that the more flowers a plant produced resulted in lower seed set due to flowers not presented at optimum time. Thus, Component 2 could be interpreted "seed set problem" which may be caused by pollination problem.

Component 3 was the highest correlations with FFPC1 (median flowers) and TFPC2 (early or/and late flowers) (Table 3.8). TFPC2 had a highly positive standard coefficient, but FFPC1 had a highly negative one (Table 3.9). FFPC2 (flowering extremities), SETPC1 (median and late seed set), seed set after harvest and 100 seed weight also had relatively higher correlations with Component 3 (Table 3.8), with relatively higher standard coefficients (Table 3.9). Therefore, high score of Component 3 was relevant to "extreme flower behaviour but big seeds".

Harvest seed shattering had the highest relationship with Component 4, with the highest standard coefficient (Table 3.8 and 3.9). Seed set during seed development, *e.g.* SETPC1 (median and late seed set), SETPC2 (early seed set) and maximum seed set, associated moderately to Component 4, with moderate standard coefficients. In contrast, seed set after harvest related negatively to this component, with a negative standard coefficient. Thus, Component 4 could be described as a "harvest seed shattering" factor.

3.4.3.2 Clustering and truncation of dengrogram

Following the second PC analysis, the parsimony criteria led to four scores being used for describing meadowfoam seed production. This new data set was still too complex to comprehend, although, in this form, it is the best possible discriminator set for identifying plant properties (phenotypes). Therefore, a cluster analysis was done on the four principal component scores, in order to identify groups of plant properties to assist in interpreting the results. (It will be used subsequently also to partition a "pseudo" genetic variance.) A further issue was a method for defining "natural" cluster-groups *via* truncation level of the dendrogram.

After clustering, the number of clusters was compared for 5 clustering stages in the sequence, *i.e.* stage 216, 193, 160, 124 and 58. The cluster stage 58 was the most significant for amongst-cluster mean-square (compared to within-cluster mean-square), and which was ascertained by the program CLUMEM (I.L. Gordon, unpublished). The other truncation levels were arbitrarily chosen. The number of clusters for each cut-off point are given in Table 3.10.

Table 3.10 Number of clusters and phenotype groups at various cluster stages.

	No. clusters (n)	Clustering plants	Unclustered plants (s)[a]	Phenotype group (n+s)[b]
Stage 216	32	248	0	32
Stage 193	46	239	9	55
Stage 160	70	230	18	88
Stage 124	77	201	47	124
Stage 58	48	106	142	190

a: "singleton" clusters;
b: total of clusters, including "singleton" clusters.

As members of any cluster potentially have similar (multivariate) phenotypes, and as different clusters are potentially divergent in their phenotypes, these cluster definitions (dendrogram cluster-stage grouping) may be interpreted as "genotypic" classifiers: as is done in classical genetics. Therefore, the clusters in Table 3.10 may also be interpreted as genotype classifiers. As well as the F-criterion, the size of estimates of genotypic variance and heritability could be used also to define "efficient" clustering.

The variances of genotypes, residual and phenotypes, the heritability and their standard errors are presented in a series of figures (Figure 3.6- 3.18) for the characters used in the plant ordination, following the various "genotype identifies" defined by the character stages above.

The result for this germplasm was that all criteria of defining optimum clusters (highest genotypic variance, highest phenotypic variance or highest heritability) led to stage 58 (the best from the F-test criterion) was also best for FFPC2 (flowering extremities) (Figure 3.7), TFPC2 (early or/and late flowers) (Figure 3.9) and 100 seed weight (Figure 3.17). While the highest heritability was in stage 124, the highest genotypic variance and phenotypic variance in stage 58 for FFPC1 (median flowers) (Figure 3.6), SETPC1 (median and late seed set) (Figure 3.10), SETPC2 (early seed set) (Figure 3.11), maximum seed set (Figure 3.12), harvest seed shattering (Figure 3.13) and flowers plant^{-1} (Figure 3.15). While the highest phenotypic variance was in stage 58, the highest genotypic variance and the highest heritability were in stage 124 for TFPC1 (flower production) (Figure 3.8), seed set after harvest (Figure 3.14), seeds plant^{-1} (Figure 3.16) and yield plant^{-1} (Figure 3.18).

The genotypic variance, phenotypic variance and the heritability between stage 58 and stage 124 were not significantly different by t-test, except in genotypic variance for seeds plant^{-1} (t=2.770) and phenotypic variance for flowers plant^{-1} (t= 36.8) and seeds plant^{-1} (t=108.9) (Table 3.11). It was decided that the optimum cut-off point was the stage 58 where the amongst-cluster mean squares was most significant relative to the within-cluster mean squares. This resulted in 48 clusters within 106 plants (Table 3.10). The membership

of clusters was defined the same phenotypes. However, meadowfoam is a wild crop with large gamodemes so that another 142 phenotypes were with only one individual plant. Finally, there were 190 phenotypes within 248 plants.

Table 3.11 Significances of differences between genotypic variances and phenotypic variances, and the heritability at stages 58 and 124.

	t-value		
	Genotype	Heritability	Phenotype
Fresh flower PC1	0.0593 ns	0.1759 ns	0.2204 ns
Fresh flower PC2	0.3084 ns	0.2995 ns	0.0735 ns
Total flower PC1	0.0290 ns	0.5064 ns	0.3875 ns
Total flower PC2	0.0408 ns	0.0407 ns	0.0675 ns
Seed set PC1	0.0008 ns	0.3510 ns	0.2441 ns
Seed set PC2	0.0372 ns	0.4439 ns	1.0884 ns
Seed set maximum	0.0260 ns	0.1819 ns	0.1101 ns
Harvest shattering	0.0247 ns	0.1649 ns	0.0960 ns
Lab seed set	0.0236 ns	0.4391 ns	0.1780 ns
Lab flowers plant^{-1}	0.1582 ns	0.3254 ns	36.76 **
Lab seeds plant^{-1}	2.7703 **	0.3515 ns	108.85 **
100 seed weight	0.1631 ns	0.6977 ns	0.0434 ns
Seed yield plant^{-1}	0.0759 ns	0.4744 ns	1.3020 ns

Critical t-value: $t_{(\infty, \alpha=0.01)} = 2.576$; $t_{(\infty, \alpha=0.05)} = 1.960$.
**: Significant differences at the 1% probability level; ns: no significant differences at statistical level.

3.4.4 Quantitative genetic analysis

3.4.4.1 Variance components

Table 3.12 shows the estimates of variance components with their standard errors for 13 characters at the optimum cut-off point of cluster analysis (analysis of 190 genotypes over 248 plants). The variances of genotype and residual for 13 characters were all highly significant.

The relative importance of genetic variability associated with seed yield plant^{-1} was also relatively high (11.896 and 15.915, respectively for variances of genotype and phenotype), which was certainly of practical significance. However, the residual variance was 4.0197, which may have little effect on the precision of estimates of effects contributed by phenotypic clusters.

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).

	Genotype		Phenotype		Residual		Heritability	
	σ^2	S.E.	σ^2	S.E.	σ^2	S.E.	h^2	S.E.
Fresh flower PC1	0.9377**	0.09645	1.1982	0.08977	0.2606**	0.04838	0.783**	0.04159
Fresh flower PC2	0.9113**	0.09375	1.2424	0.08232	0.3311**	0.06148	0.734**	0.04955
Total flower PC1	0.8990**	0.09248	1.2201	0.08161	0.3211**	0.05963	0.737**	0.04903
Total flower PC2	0.9738**	0.10017	1.0923	0.09888	0.1185**	0.02200	0.892**	0.02195
Seed set PC1	0.9486**	0.09759	1.1839	0.09224	0.2353**	0.04369	0.801**	0.03840
Seed set PC2	0.7751**	0.07974	1.7369	0.10305	0.9618**	0.17860	0.446**	0.08219
Seed set maximum	0.4109**	0.04227	0.4984	0.04058	0.0875**	0.01624	0.824**	0.03434
Harvest shattering	0.3848**	0.03959	0.4607	0.03823	0.0759**	0.01409	0.835**	0.03241
Lab seed set	0.7145**	0.07350	0.8491	0.07120	0.1346**	0.02500	0.841**	0.03129
Lab flowers plant^{-1}	29694 **	3054.6	36093.0	2929.0	6399.2**	1188.3	0.823**	0.03466
Lab seeds plant^{-1}	251630**	25884.9	305290	24843.2	53659 **	9964.3	0.824 **	0.03438
100 seed weight	0.0566**	0.00582	0.0813	0.00475	0.0248**	0.00460	0.695**	0.05531
Seed yield plant^{-1}	11.896**	1.22369	15.915	1.09584	4.0197**	0.74645	0.747**	0.04735

**: significance of the difference of the original least square estimates from zero at $p < 0.01$.
S.E.: standard errors.

Apparently most of the variability observed was in flowers plant^{-1} and seeds plant^{-1} after harvest (Table 3.12). The residual variances for those two characters were extremely high, 6399 and 53659, respectively, compared with the others (around 0.02 to 4.02).

3.4.4.2 Heritability

The heritability presented here are broad sense heritability (term of restricted heritability). Heritability estimates of the 13 characters were significantly different from zero (Table 3.12).

The value of heritability varied from character to character (Table 3.12). The heritability estimates were relatively high (h^2>0.800) in TFPC2 (early or/and late flower), SETPC1 (median and late seed set), seed set maximum, harvest seed shattering, seed set after harvest, flowers plant^{-1} and seeds plant^{-1}. However, the heritability was lower in SETPC2 (early seed set), which was only 0.446. For other characters, the heritability was moderate. The results may imply that progress can be expected, though it may be slow in some characters.

The standard errors for heritability (Table 3.12) showed that estimates have reasonably low value, indicating that the heritabilities for these characters were estimated with considerable precision.

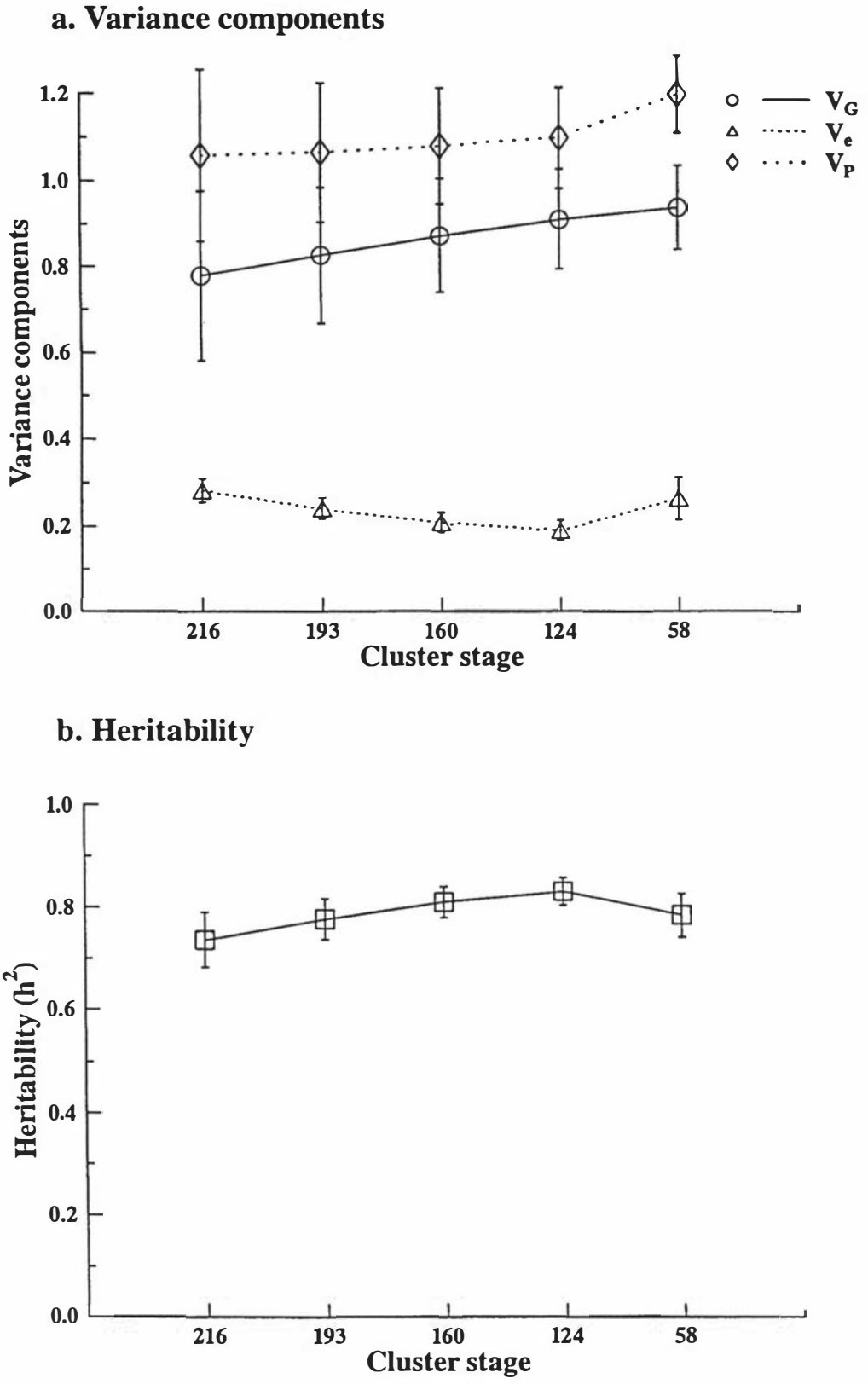

Figure 3.6 Variance components and heritability for FFPC1 (median flowers).

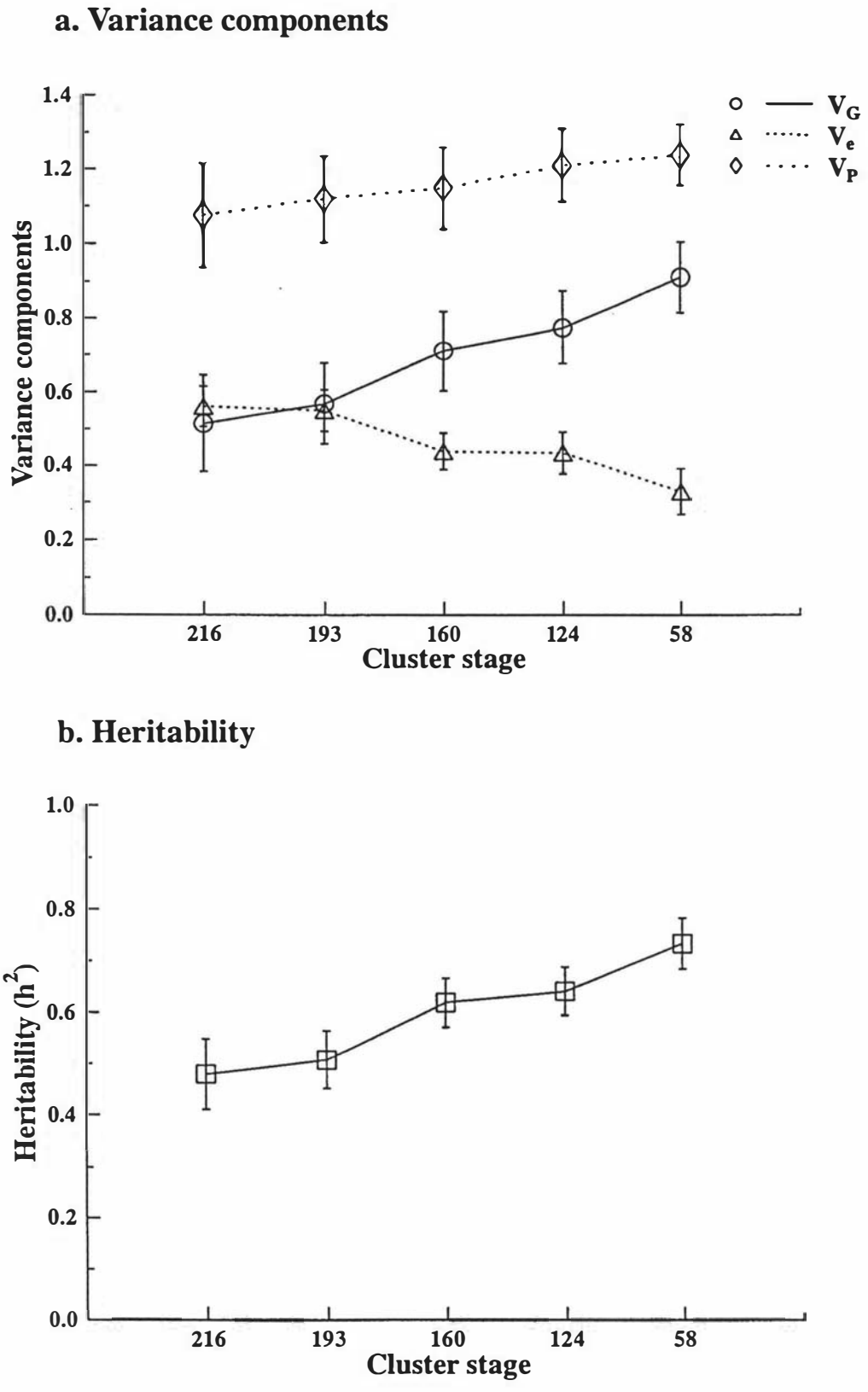

Figure 3.7 Variance components and heritability for FFPC2 (flowering extremities).

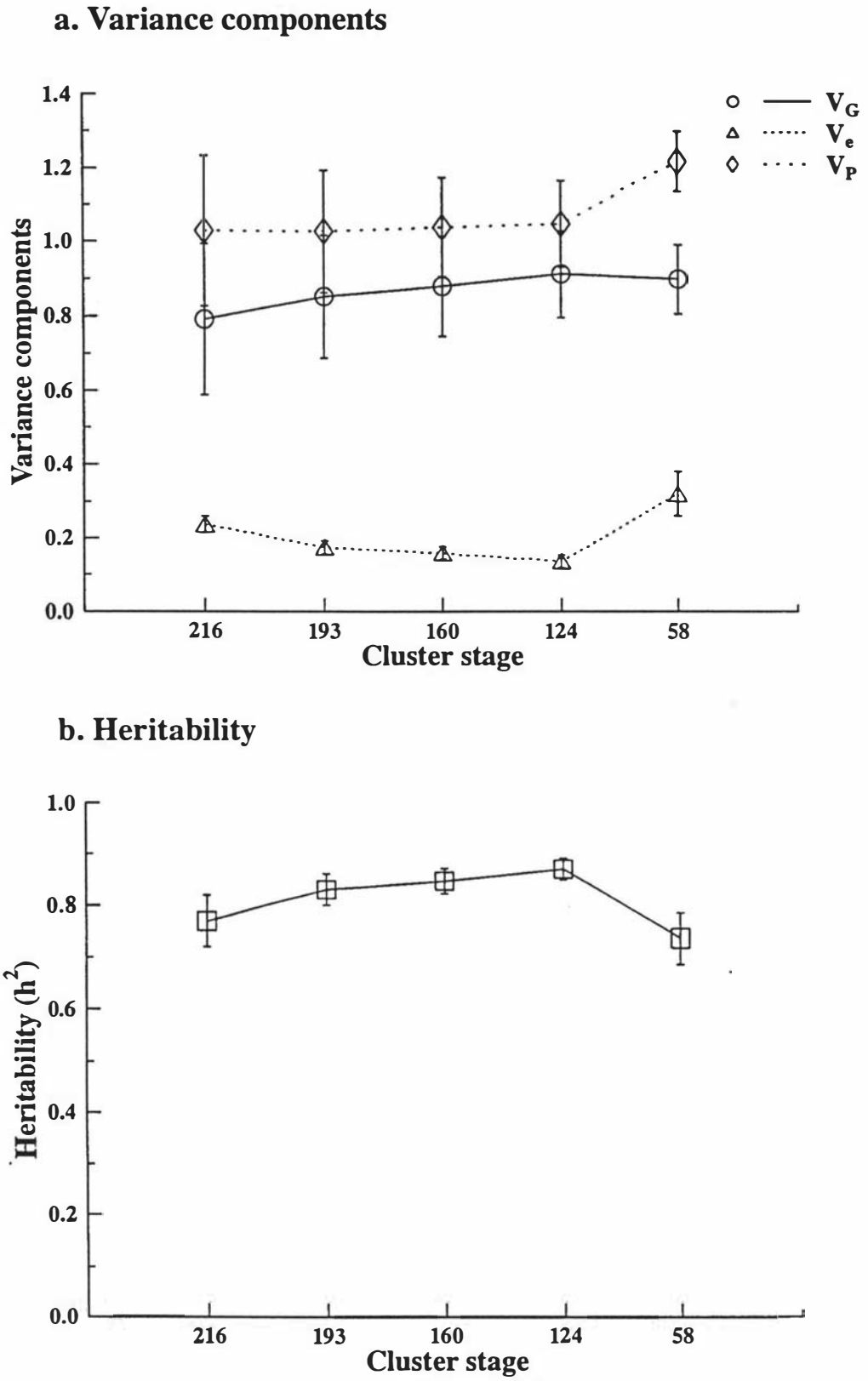

Figure 3.8 Variance components and heritability for TFPC1 (flower production).

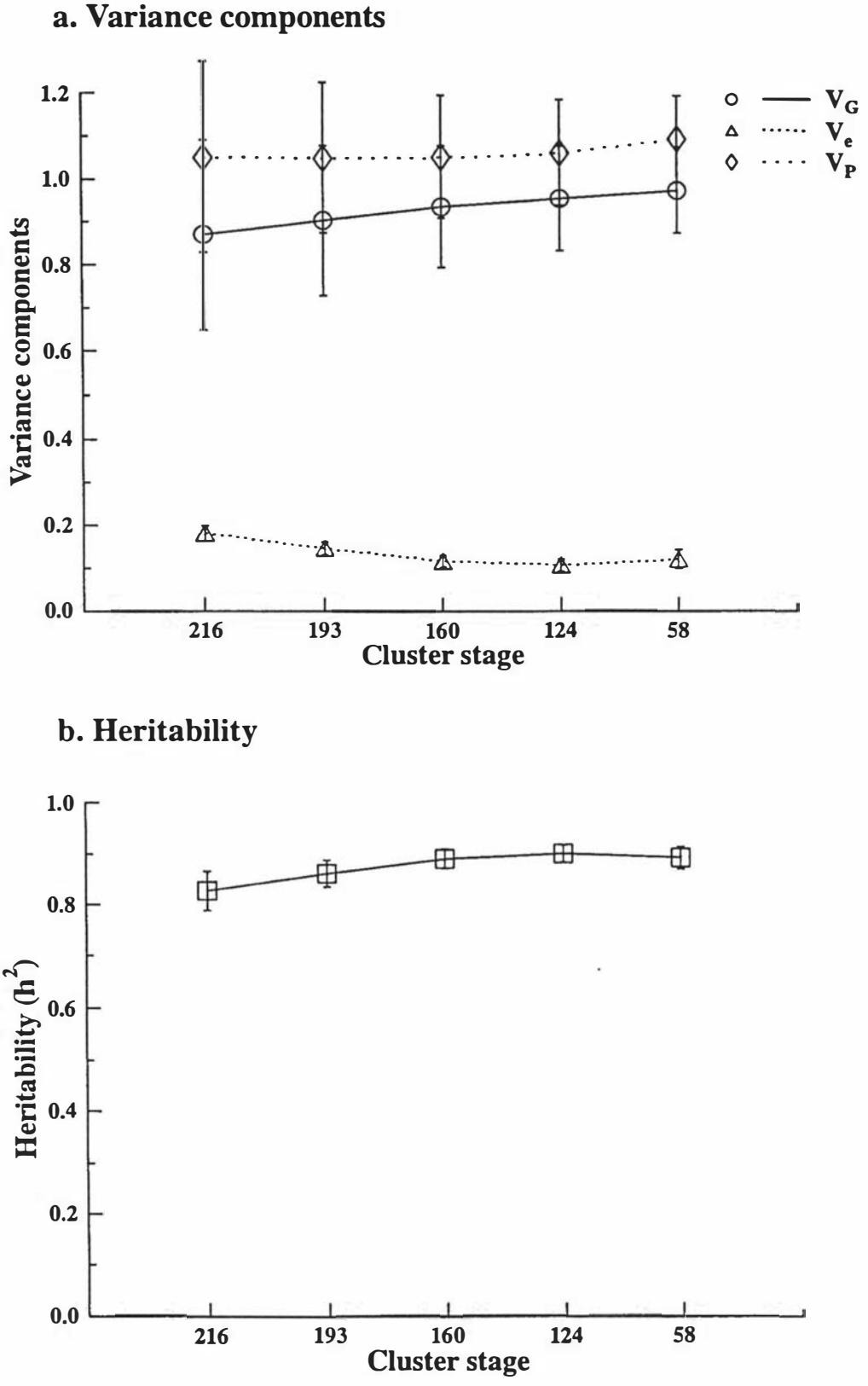

Figure 3.9 Variance components and heritability for TFPC2 (early or/and late flowers).

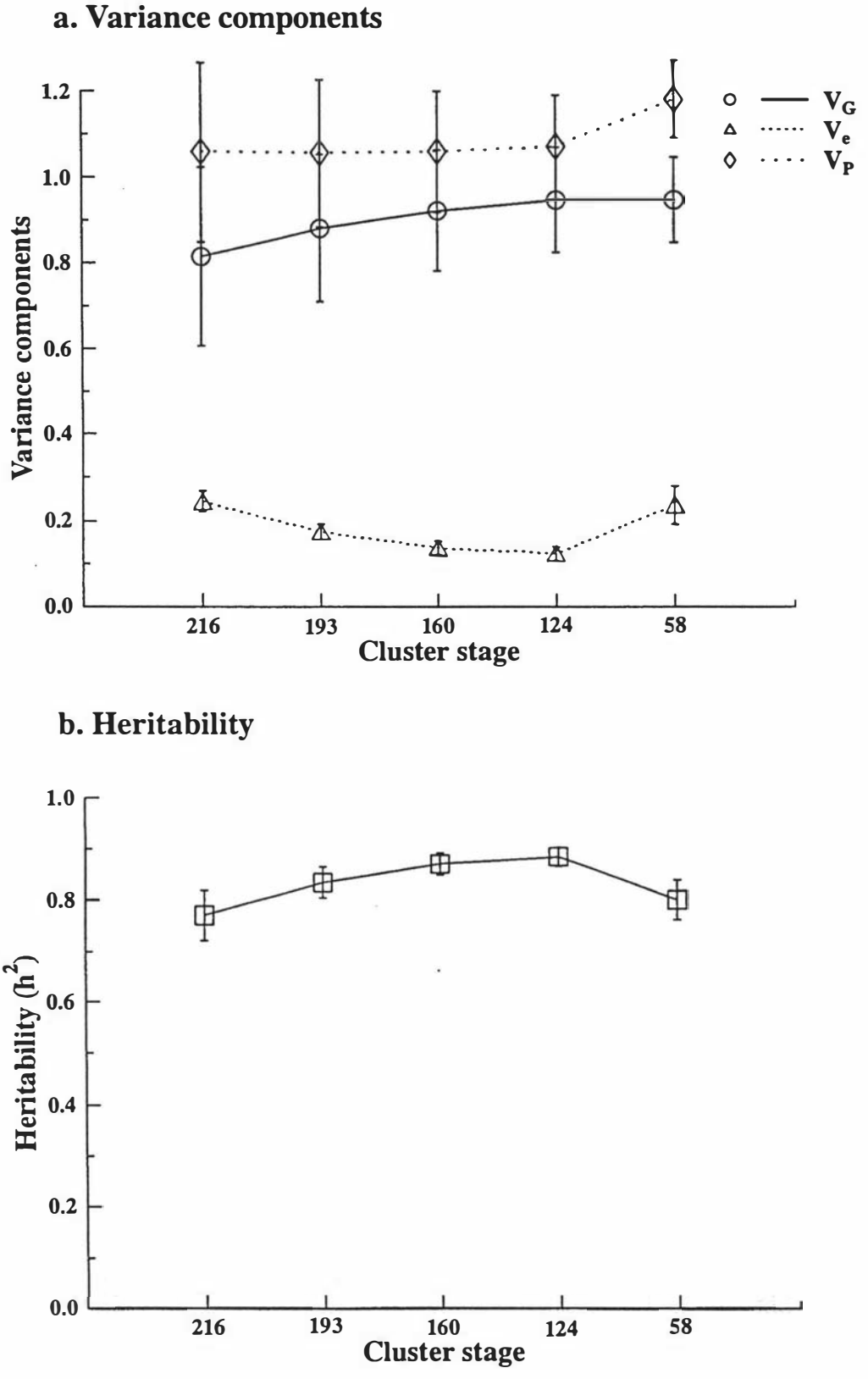

Figure 3.10 Variance components and heritability for SETPC1 (median and late seed set).

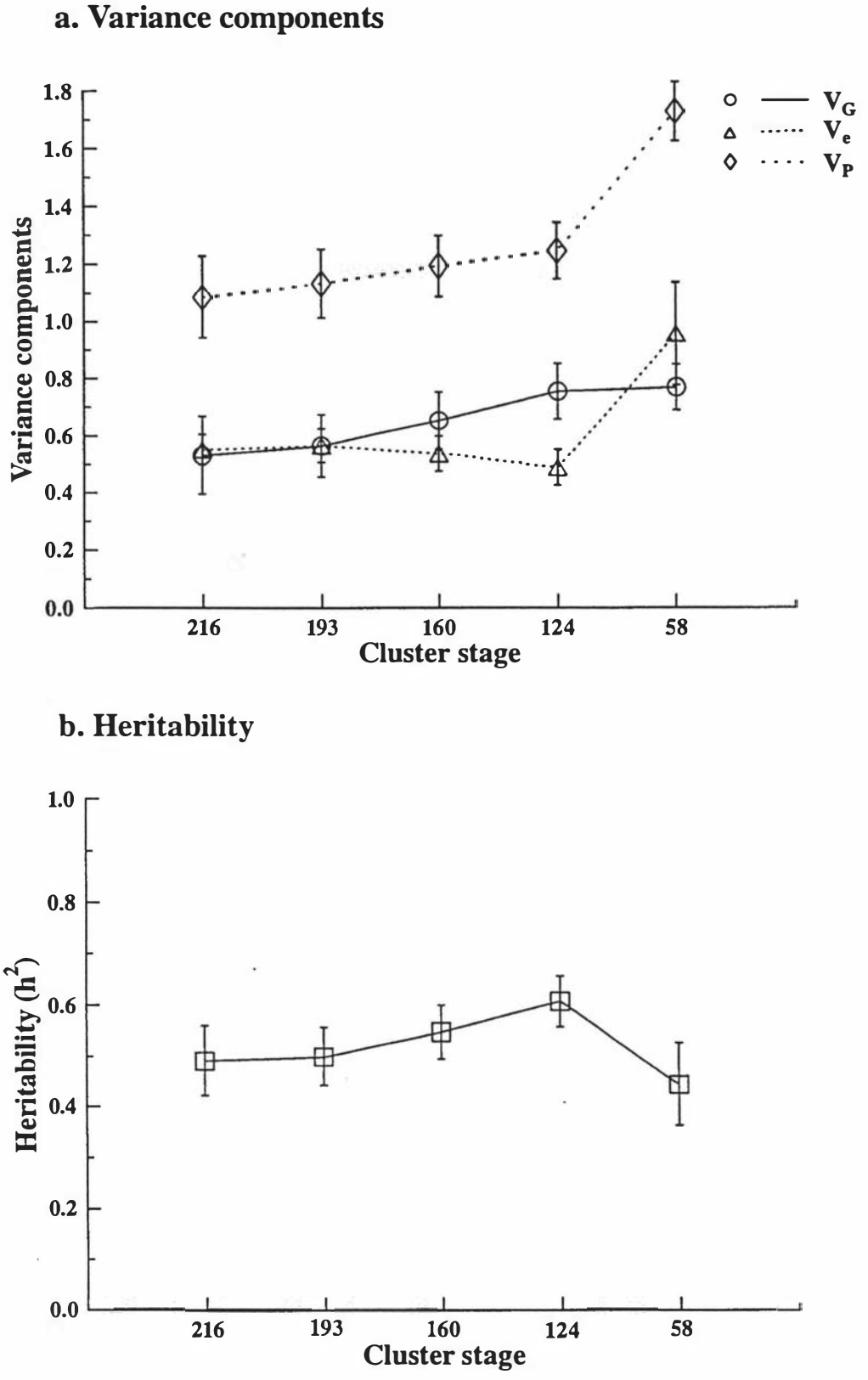

Figure 3.11 Variance components and heritability for SETPC2 (early seed set).

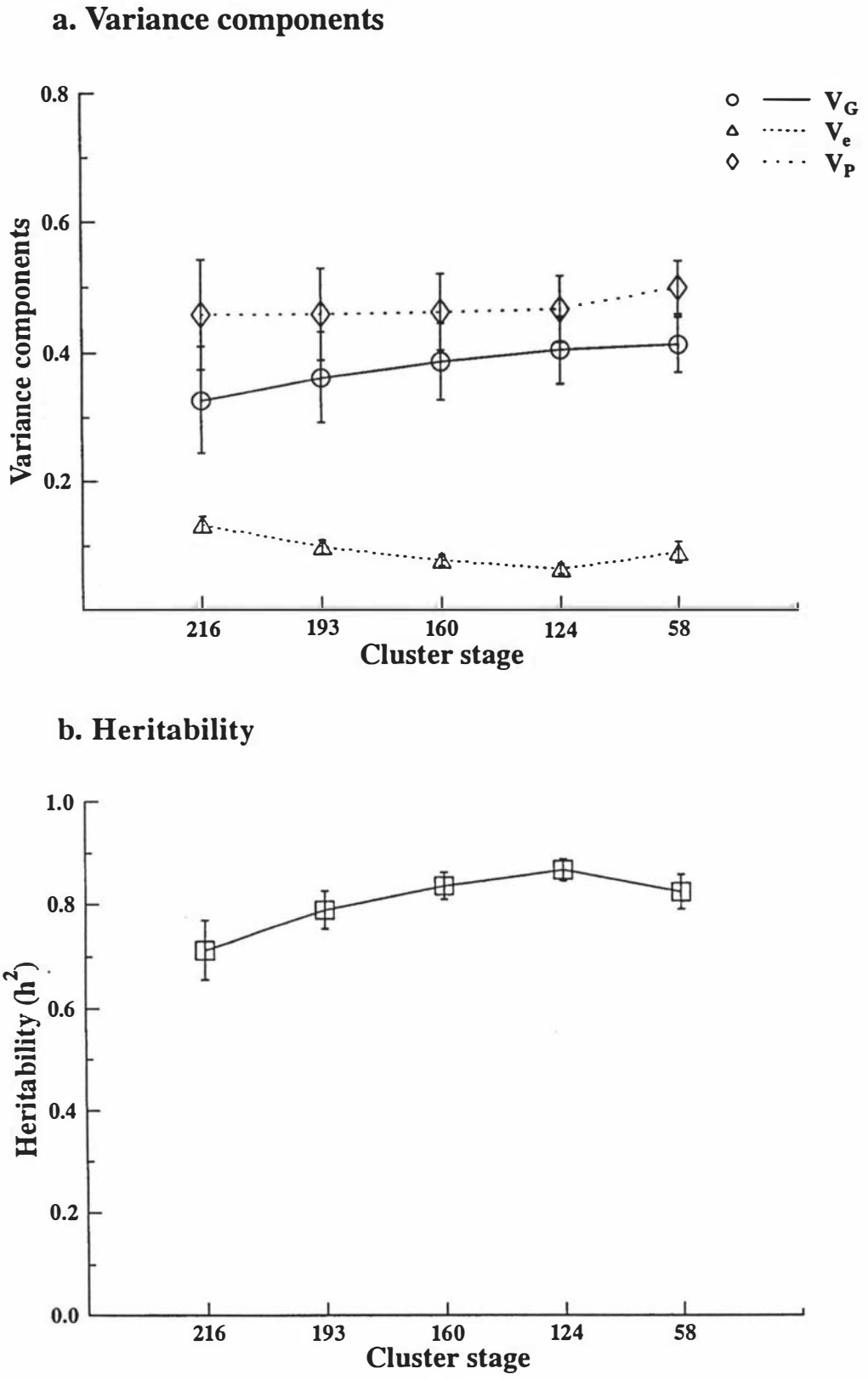

Figure 3.12 Variance components and heritability for maximum seed set (SETMAX).

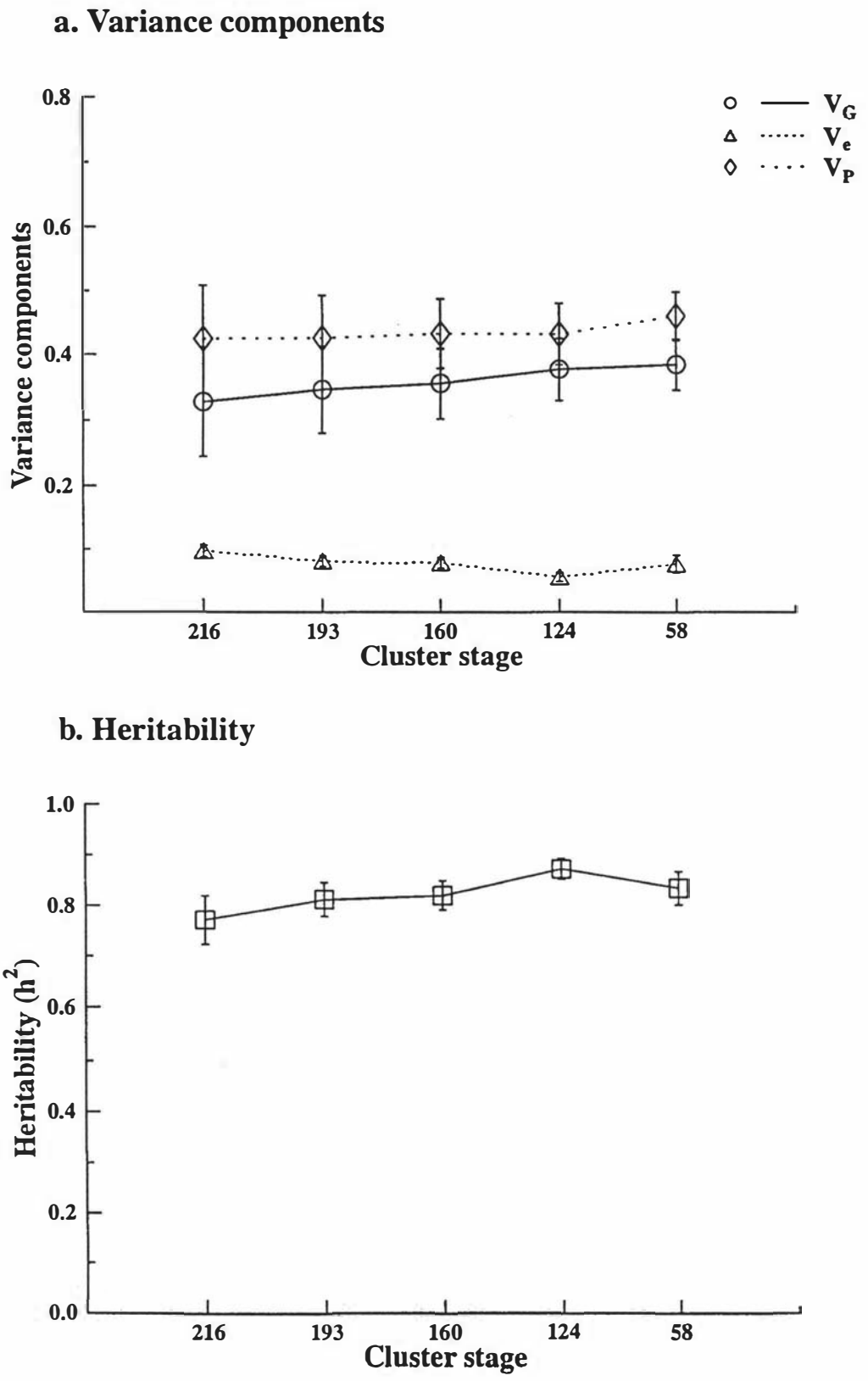

Figure 3.13 Variance components and heritability for harvest seed shattering (HSH).

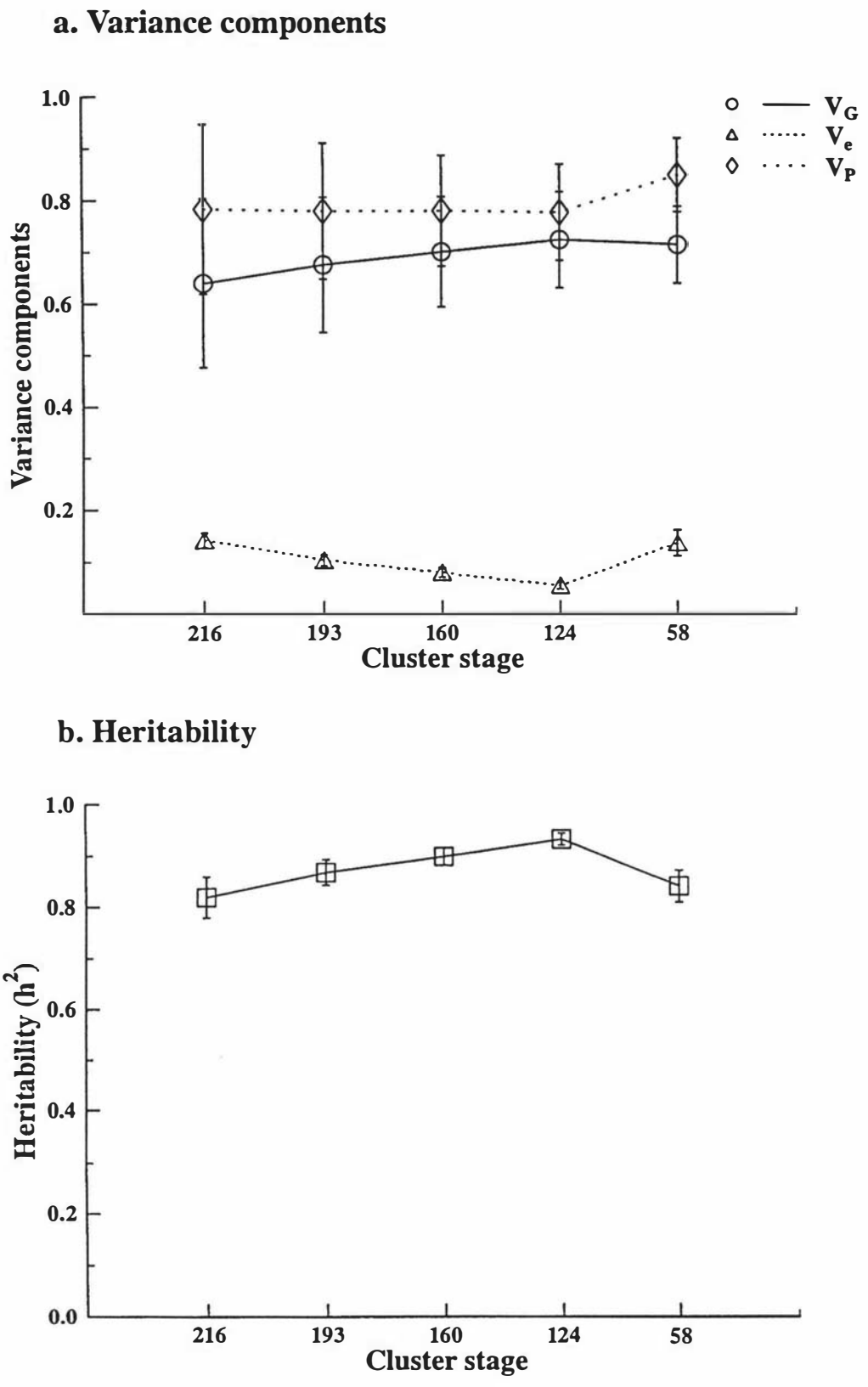

Figure 3.14 Variance components and heritability for seed set after harvest (LSET).

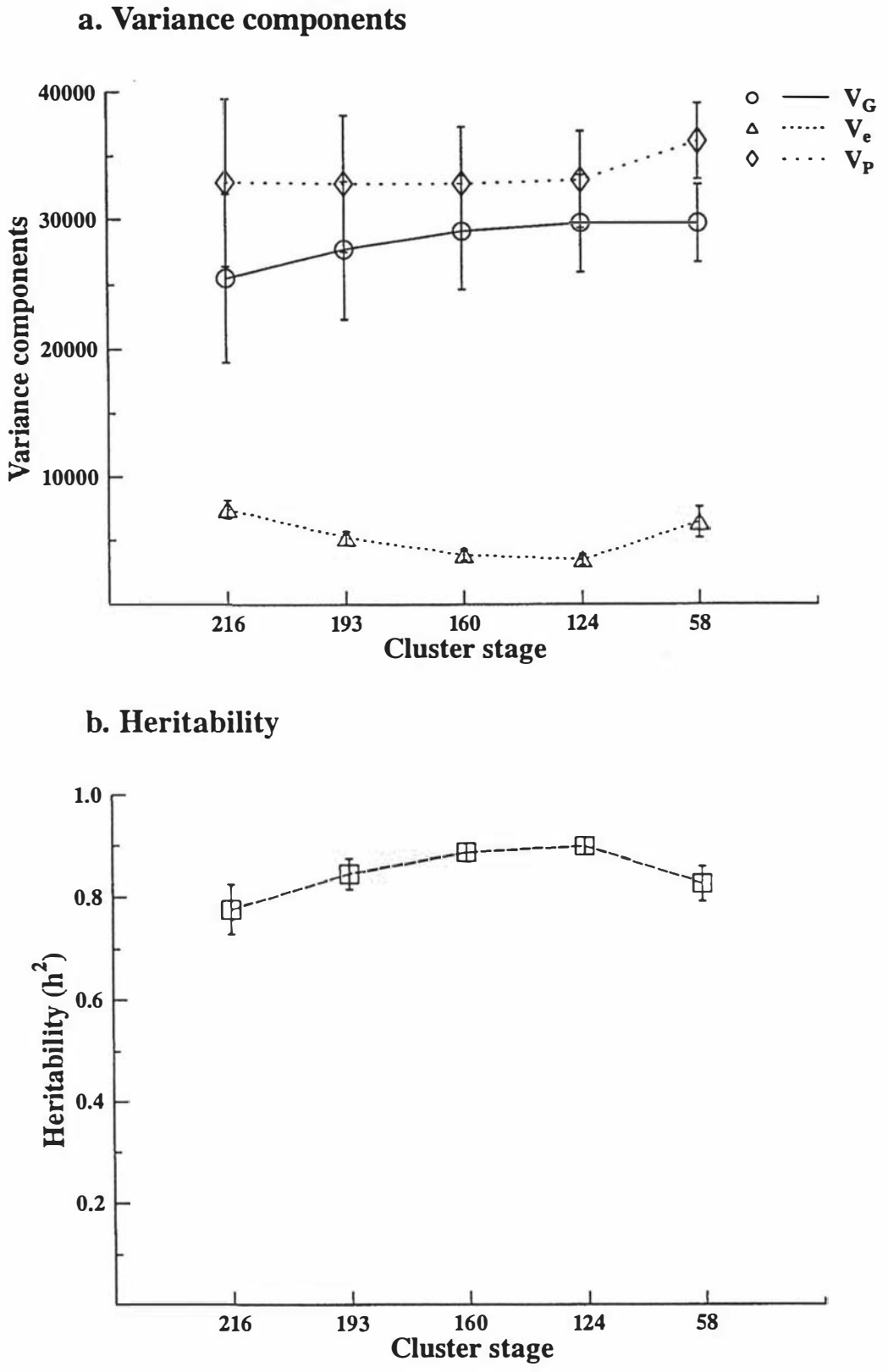

Figure 3.15 Variance components and heritability for flowers plant^{-1} after harvest (FLOWS).

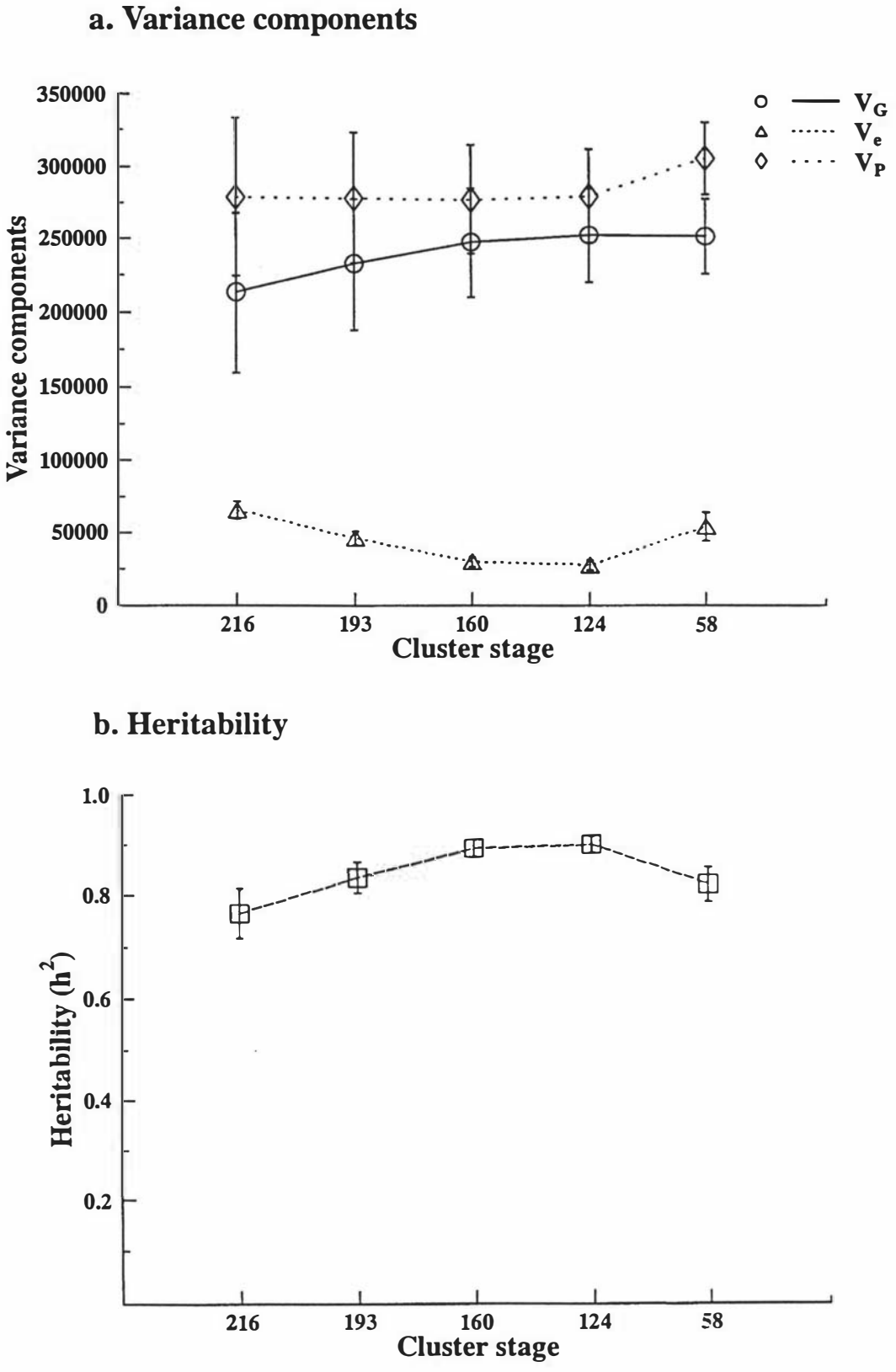

Figure 3.16 Variance components and heritability for seeds plant^{-1} after harvest (SEEDS).

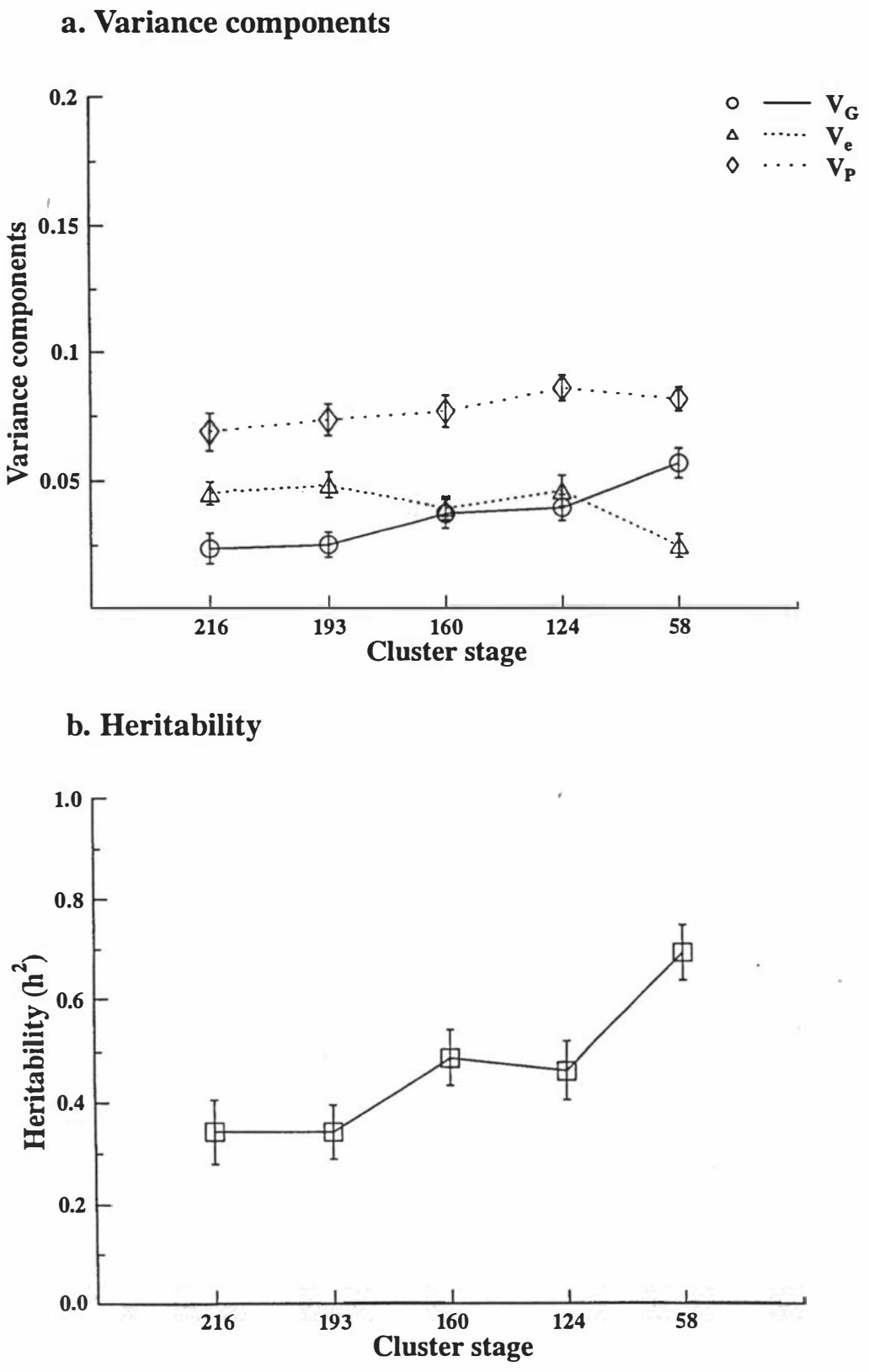

Figure 3.17 Variance components and heritability for 100 seed weight (SEEDWT).

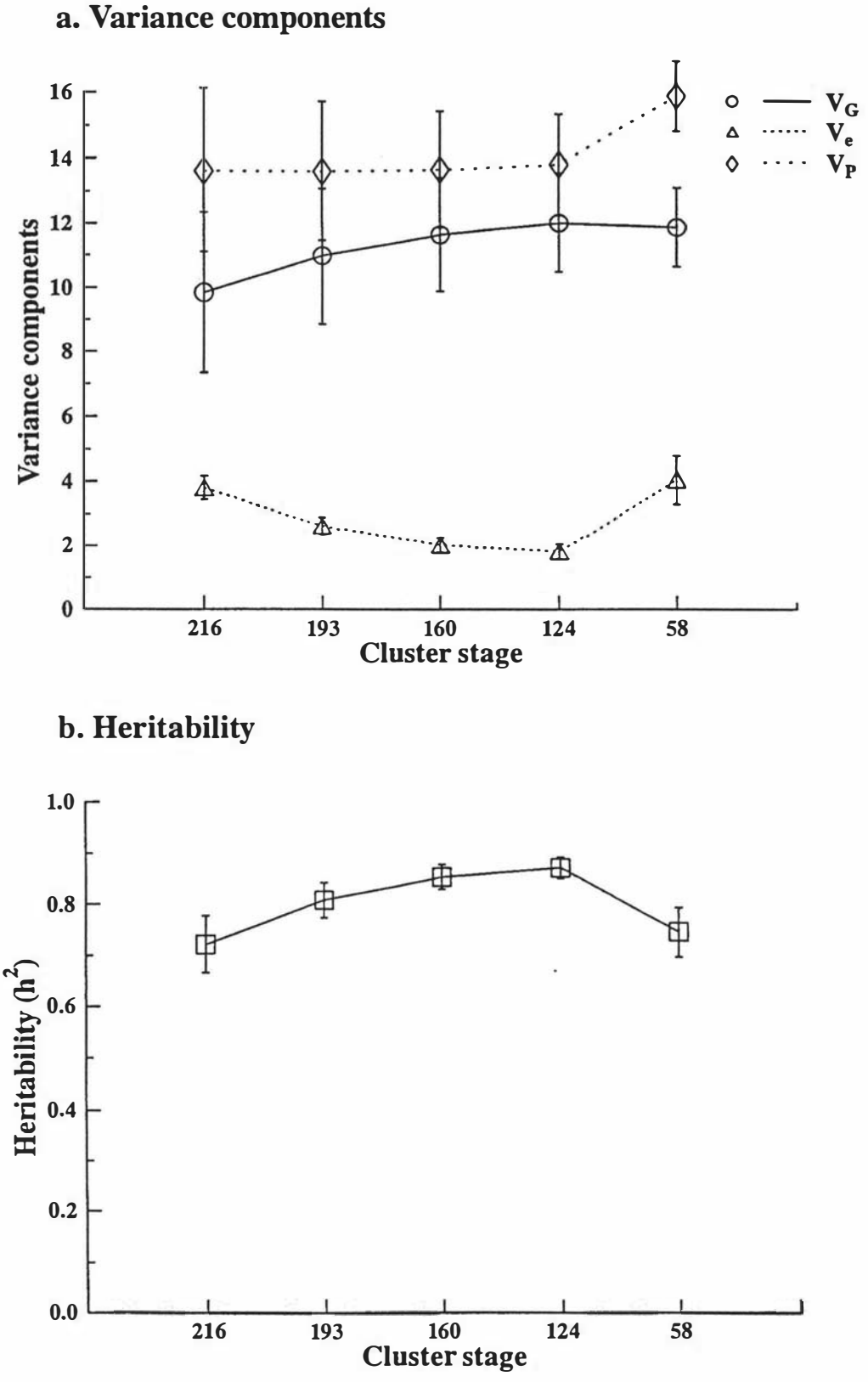

Figure 3.18 Variance components and heritability for seed yield plant^{-1} after harvest (YLD).

3.5 Discussion

3.5.1 Distribution of data

Simple histograms (Figure 3.4 - 3.5) effectively summarised the frequency distribution of the variants within a meadowfoam population with respect to a single character. All the individual plants showed continuous distribution around the mean with no evidence of segregation due to major gene effects.

Measures of spread or variance showed the groups of phenotypic similarities which they separate. The mean was also descriptive statistics, which implied where a distribution of data was positioned or centred. The results indicated that all characters were quite plastic. However, the variability of phenotypic characters described by these distributions is largely under genetic control due to high heritability values for most characters (Table 3.12), and subject to selection. The magnitude and distribution of genetic variances are expected to change with successful selection (Falconer, 1989).

In plant breeding, the usual goal is to combine complementary parental phenotypic virtues. Phenotypes, therefore, should be compared in respect to an aggregate of differences. It was for this reason that the PC analysis and clustering used for pattern analysis in this study were valuable. The results based on a multivariative model indicated that no particular patterns of floral initiation, general flowering, seed set and seed shattering were found (Figure 3.1-3.3), since segregating plants (panmixia) with large gamodemes had very weak group (Table 3.10). The results of this study agreed that a very wide diversity of genotypes is available for use in the domestication and breeding of the new crop (Brown *et al.*, 1979).

3.5.2 Pattern analysis and clustering

3.5.2.1 Principal components analysis

Principal components analysis is the simplest method of the factor analysis, which is variance oriented. The first component indicates usually the general effects and the others may show contrast between variables (Morrison, 1990). The first component always had the largest eigenvalue and explains the largest proportion of total variance (Table 3.3; Table 3.4). Maximum variance does not necessarily mean maximum information, but in the absence of contrary evidence they are regarded as equivalent (Moore, 1975). Therefore, The Component 1 was by definition the most important component.

Principal components analysis is a data reduction and screening technique. The main purpose of PC analysis is to find linear combinations of the measured variables, which may be used to group individuals within a single population. Therefore, PC analysis is a one-population technique, and is a suitable screening technique in single plant selection and in germplasm collection. PC analysis, however, is inferior to the genetic advance of quantitative genetics, because it ignores heritability (h^2) and truncation statistics. It works solely on phenotypic variance (σ_p) ($\Delta G = h^2 i \sigma_p$). Thus, PC analysis is purely phenotypic.

In this study, patterns of fresh flowers, total flowers and seed set were explored by PC analysis based on four observations for each character. This pattern analysis was considered superior to regressions (over time) because of few time-nodes, and it avoided the need to find optimum functions. Moreover, four variables for each character were all reduced to a 2 component model in PC analysis. These data provided a basis for deciding on the number of component to be studied, using a benchmark of 70% of total variation explained (Morrison, 1990).

In second PC analysis, 7 variables together with 6 PC scores were reduced to a 4 component model. Based on these retained factors, the plants were scored so that they could be compared on several characters in combination. Not only the number of comparisons were reduced, but the meaningfulness of the comparisons was enhanced, and

based on these comparisons, the best plants could be selected for different breeding purposes. However, this four component data set was still too complex to comprehend, a cluster analysis following PC analysis would be the best possible discriminator set for identifying plant habit and understanding this germplasm.

3.5.2.2 Cluster analysis

Both cluster analysis and PC analysis have been successfully applied by other workers (Cullen, 1981; Hashjin, 1992; Zeven *et al.*, 1993; Basigalup *et al.*, 1995). This method brought about a reduction in the number of genotypic comparisons to be made which may be valuable for large data sets, and summarized the degree of similarity among genotypes in their response patterns. The results here showed that both cluster analysis and PC analysis maximized the discrimination of genetic differences among plants in this segregating population.

Cluster analysis based on PC scores produces groups of similarly response. Such groupings can also be used to produce by PC analysis, but the components are mathematical artifacts without obvious direct relationship (Lefèbvre and Vekemans, 1995). However, an additional disadvantage of cluster analysis is that a truncation of the cluster dendrogram is made on the basis of previous experience and knowledge about the data set (Anderberg, 1973).

3.5.2.3 Truncation of dendrogram via "natural groups"

In this study, the optimum stage to truncate the dendrogram was based on the maximum genotypic variance (σ_G^2), phenotypic variance (σ_P^2) and heritability (h^2). The maximum variances were either at stage 58 or at stage 124 for those 13 characters (Figure 3.6-3.18). The differences for heritability, and the genotypic and phenotypic variances between stage 58 and stage 124 were not significant, except in variance of genotype for seeds plant^{-1} and variances of phenotype for flowers plant^{-1} and seeds plant^{-1} (Table 3.11). Therefore, the stage 58 was defined the optimum cut-off point since the amongst-cluster mean squares was most significant relative to the within-cluster mean squares. Teow (1978) and Cullen (1981) also used this truncation point (based on the minimum F probability for the ratio of

amongst cluster mean squares to within cluster mean squares). Lin and Butler (1990) has reported that a cutoff point for the dendrogram was decided upon based on the F-ratio of the smallest dissimilarity index and the error estimate from ANOVA; that was, the cycle at which the calculated F-ratio exceeds the tabular F-value would be considered an appropriate cutoff point. Lin and Butler's method emphasized the internal (within group) homogeneity rather than the external (between groups) heterogeneity, thus, it may not be very suitable for grouping natural clusters, such as in taxonomy and our data set. A cutoff point was chosen based on an Euclidean distance (0.7) (Cerutti and Bigler, 1995), and pseudo F and t^2 statistics calculated by SAS during the clustering procedure (Smith *et al.*, 1995). Those methods, however, emphasized neither within group homogeneity nor amongst group heterogeneity.

On the basis of the results of pattern analysis, there were 190 phenotypes within 248 plants. Some phenotypes consisted of only one individual plant (Table 3.10), but the mean cluster-size for the rest was 7.8, 4.5, 2.8 and 2.0, respectively. Heterozygosity level for *L. alba* was found to be in the range of 12% and 27%, whereas percent polymorphic loci varied twofold (between 29 and 57%) among seven populations (Jain, 1978). The average number of alleles per locus in *L. alba* was 1.97 (Brown and Jain, 1979). The population analyzed here could be expected to have considerable levels of heterozygosity, with diversity germplasm. In barley, the average numbers of alleles per locus were 5.15, 2.75 and 1.44, respectively for three stages of domestication, *e.g* wild barley, primitive Middle Eastern landraces and modern Californian cultivars (Allard, 1996). In transition from wild barley to promitive Middle Eastern landraces, numbers of alleles per locus decreased by about one half. During the domestication of maize, frequent (predominant) alleles contribute to adaptedness in many habitats and survive many cycles of selection in cultivation whereas alleles that are present in intermediate frequencies overall appear to be useful in some environments but not in others; rare alleles of allozyme loci appear to be of little value anywhere (Allard, 1996). The data, therefore, provide strong evidence that plant breeding could lead to a reduction in allelic diversity (heterozygosity) during domestication of meadowfoam. The low levels of diversity found in the cultivated species compared with its wild progenitor has been recorded in many other cultivated plants (Doebley, 1992).

3.5.3 Genetic analysis

3.5.3.1 Genetic variances

A high level of significant genotypic effect of all characters (Table 3.12) proved the presence of considerable genetic variation for those attributes. The results suggested that meadowfoam has the genetic capability to respond to selection, but some characters may not evolve independently. Segregation with large variance in a progeny may increase the probability of progeny exceeding a critical value in selection. However, the variance component cannot be separated from σ_G^2 in case of one environment. Thus, σ_G^2 may be overestimated (Wricke and Weber, 1986) (because of the interaction between genetics and environment confounding).

The results showed that sampling errors associated with these estimates of the genetic contribution to phenotype were low (Table 3.12), but the errors in flowers plant^{-1} and seeds plant^{-1} after harvest were sufficiently large to be important in the design of future experiments.

3.5.3.2 Heritability

Heritability is a measure of the degree to which genetics contributes to the variation of a character within a population. The total genotypic variance may be partitioned into additive, dominance and epistatic variance components (Griffing, 1956; Cockerham, 1963; Baker, 1986; Falconer, 1989). Our results cannot distinguish between the contributions of dominance and epistasis to the broad-sense heritabilities because of a bee-pollinator. Thus, the heritability estimates obtained in this study were broad sense.

The standard errors for these heritability estimates (Table 3.12) were of low value, indicating that the heritabilities were estimated with considerable precision. The precision of heritability can probably be achieved by either the appropriate and accurate measurement of characters or sufficient sample size (Hanshe *et al.*, 1966). The imbalanced experimental designs in this study may also improve the precision, because fewer

experimental units are required for degrees of freedom to increase with imbalanced relative to balanced designs (Knapp *et al.*, 1987). It is important that the limitations of estimates of heritability are realised. While a mean is a first degree statistic, variance is a second degree statistic which is inherently less precisely estimated. Heritability, being the ratio of variances, not only shares all biological restrictions for estimating genetic variances, but also shares the higher imprecision of second-degree statistics (Ewing *et al.*, 1987).

The values of heritability varied from character to character in this study (Table 3.12). Most characters had high heritability ($h^2 > 0.800$), but for SETPC2 (early seed set) only 0.446. Chozin (1990) reported that the broad sense heritability estimates of the meadowfoam lines varied in morphological characters (leaf hairiness, clump density, leaf size, budding time and flowering time), ranging from 0.01 to 0.72. He also found that the heritabilities for budding time and flowering time decreased from first self generation to second self generation, while the values for clump density and leaf size increased. Jain (1979) reported that heritability estimates by standard mass selection theory ($h^2=\Delta G/is_p$) ranged from 0.24 to 0.73 for flowering time in meadowfoam. The heritability estimates may imply that progress can be expected using proper selection techniques, though it may be slow in some character with low heritabilities, such as early seed set (SETPC2).

The variations of heritability in these characters were meaningful, because the experimental material was a wide gene pool drawn from 8 accessions (Table 3.1) and had diversity nature characters. These variations appeared clearly to depend much more on genes and therefore, they were potentially under selection. In general, selections directed towards the family or line performances would not be practical. Individual selection may be an effective and efficient method to improve meadowfoam genotypes. The selection combined the highest performing plants from the top performing lines seems to be the most reasonable choice. This is generally expected to be the most efficient selection strategy in any case (Falconer, 1989), and so methods using such combined selection should lead to notable genetic advance in these plants. Such methods include line selection and line breeding (Allard, 1960).

Jain (1979) pointed out that the earliest flowers are probably self-pollinated in meadowfoam, since bees are attracted to a plot only after several flowers had opened. Outcrossing rate, estimated by using two genetic marker loci, varied between 43% and 97% (Jain, 1978). Typically, a plant would flower over a week's period so that intermating among neighbouring plants would not be complete but would occur frequently among and within plants overlapping in flowering duration. Thus, SETPC2 (early seed set) may relate to self-pollination. The result might suggest a potential for developing self-pollination lines in meadowfoam. Genetic control of relative seed set is suggested that successive generations for forced selfing could select for higher natural rates of autogamy for *L. alba*, although no major mutant or high heritability was noted (Jain, 1978). Therefore, evolution toward autogamy would have to be slow.

Chapter 4. Seed germination

4.1 Introduction

The limited research on meadowfoam germination (Toy and Willingham, 1966; 1967; Higgins *et al.*, 1971) has indicated that normal germination occurs at about 5 - 15°C, with a drop towards zero as temperature approaches 20°C. Indication of secondary dormancy (Toy and Willingham, 1967) and primary dormancy (Jolliff *et al.*, 1994) have been found also. Seed losses due to shattering was one of the traits which is a challenge to its domestication. Timing of harvest to minimize seed losses is critical (Jolliff, 1981). Knowledge about the degree of dormancy in different seed colours of meadowfoam could help in the management of harvest time to achieve high seed yield. A major focus of the present work was to define a special germination test which would break dormancy in developing seed, so that seed germinative development could be monitored. This follows an approach first established by Gordon *et al.*, (1979) with wheat.

Most germination studies have analyzed the effects of environmental or genetic factors on the final germination percentage. However, studies on seed dormancy have emphasized a need for the measurement of suitable parameters describing germination in respect to time and germination rate. A regression model for evaluating germinative development would be helpful in understanding the biology of seed germination and the biological significance of environmental responses.

The "functional" approaches to plant growth analysis fit cumulative growth data to either polynomial approximations of exponential functions, or to explicitly defined sigmoid models (Venus and Causton, 1979). With determinate growth data, statistically comparable descriptions can be achieved by either method, but practical observations appear to favour the use of asymptotic sigmoid models. Notable amongst these functions is the Richards function. Special cases of the family include the monomolecular, von Bertalanffy, Mitzerlich, logistic and Gompertz functions (Richards, 1959). Although the Richards function provides a very realistic description of

plant growth (Causton and Venus, 1981), it has not been used widely in seed germination studies.

The objectives of this experiment were: (1) to identify optimum germinating test procedures for dormancy-breaking in this new crop; (2) to investigate effects of seed-colour (putative maturity) on seed germination; (3) to utilise and to evaluate biological applications of quantitative analysis of seed germination in order to obtain maximum information from limited seed material; and (4) to deduce possible mechanisms of seed dormancy of meadowfoam.

As no information was available on meadowfoam after-ripening, the analysis method had to be able to detect low dormancy as well as high. That is, it had to be both precise and robust. This quantitative analysis consisted of multiple (canonical) discriminant analysis and MANOVA of sets of Richards functions.

4.2 Materials and Methods

4.2.1 Experiment design

Dormancy breaking germination methods were investigated using a range of germination conditions and imbibants. The germination conditions were light (continuous white light) and darkness (Factor A), together with two regimes of alternating temperatures (Factor B), namely 10/5°C and 15/10°C (12 hours for each temperature). Combined factorially, these conditions defined 4 separate germinator experiments. The design of each experiment was a factorial combining, 4 imbibants (Factor C) x 2 seed-colour (Factor D) x 2 chilling (Factor E), blocked with 3 replications. The combination of these experiment, thus, was an A x B factorial pooling of a C x D x E factorials blocked in a full layout, with 192 experimental units (EU).

4.2.2 Seed germination and treatments

The experimental material was drawn from four-month-old bulk seed of 250 random plants from the open-pollinated composite of "Moginie" with an unknown residual dormancy level. This composite consisted of a random-mating bulk of eight accessions of meadowfoam, as outlined in Chapter 3, and provided a broad genetic base to investigate mean germinability. The experimental unit consisted of fifty random seeds in a 9 cm petri dish on a Whatman No. 1 filter paper with 5 ml germination imbibants. Various germination imbibants provided four dormancy-breaking treatments (Factor C) as follows: (1) distilled water, (2) 0.2% KNO_3, (3) GA_3 (100 mg l^{-1}) and (4) GA_{4+7} (100 mg l^{-1}). Seed samples were divided into putative "maturity" groups, namely brown and green seeds (Factor D). The seeds were also provided with a further dormancy-breaking treatment: pre-chilling (7 days at 5 °C in darkness) and non-chilling (Factor E).

Seeds were considered as germinated when the embryo had ruptured the testa. The numbers of germinated seeds were counted and removed every day during the first 10 days of incubation, and at two-day intervals thereafter. The test was terminated when there was no germination in remnant seeds for 2 consecutive periods.

Ungerminated seeds were tested in 1% tetrazolium solution for viability (Perry, 1987). Triphenyl tetrazolium chloride is reduced by the terminal oxidase systems in living plant tissue from a colourless solution to a red, water-insoluble formazan compound which is precipitated within living cells. In dead cells, no reaction takes place and they remain colourless. Ungerminated seeds were bisected longitudinally in the narrow side of seeds, exposing the main structures of embryo, which were soaked in 1% 2, 3, 5-triphenyl tetrazolium chloride solution (TTC) over night at room temperature (about 25°C). Seeds were regarded as viable when the embryo was coloured red, whereas dead seed remained unstained. Viable seeds were considered as the total of germinated and stained seeds, *e.g.*, the difference between total seeds used and unstained seeds:

Viable seed = Total seeds - Unstained seeds.

Thus, viability was expressed as the percentage of viable seeds based on total seeds used in the test.

$$\text{Viability}(\%) = \frac{\text{Viable seeds}}{\text{Total seeds}} \text{x} 100\%$$

Germination was expressed as the percentage of germinated seeds based on total viable seeds used in the test.

$$\text{Germination}(\%) = \frac{\text{Germinated seeds}}{\text{Viable seeds}} \text{x} 100\%$$

4.2.3 Data analysis

4.2.3.1 Curve fitting for seed germination

Germination levels of each experimental unit followed a sigmoid pattern when plotted against days (duration of the test). Therefore, regression analyses were used to describe these germination "profiles", using germination percentage as the dependent variable (Y), and time (days) from commencement as the independent variable (X). Because each experimental unit was fitted separately, the statistics of the sigmoid functions could be analyzed as data, according to the experimental model. Subsequently, secondary variables were estimated from the several germination functions, and there also were analyzed.

The most useful "generalized sigmoid" is the Richards function. It includes within its family all of the usual sigmoids (the monomolecular, von Bertalanffy, Mitzerlich, logistic and Gompertz functions). The equation of Richards function is as follows:

$$L = A(1 \pm e^{(\beta - \kappa t)})^{-1/v} \qquad 1$$

where: L: germination percentage at time t (%),
A: upper asymptote of germination (%),
t: time elapsed (days),
v: inflexion point of the curve, ***e.g.,*** the shape of the curve,
ß: the x-axis placement parameter,
k: the rate of change parameter.

The negative alternative is used when v is negative, and ***vice versa*** (Causton and Venus, 1981).

For v = 0 (which defining a Gompertz), the Richards function becomes indeterminate. But the Gompertz can be fitted directly in such cases, the function being:

$$L = A \cdot \exp(-e^{(\beta - \kappa t)}) \qquad 2$$

where the parameters have the same general meaning as in Richards function.

For v = 1, the Richards function reduces to the logistic function:

$$L = A(1 + e^{(\beta - \kappa t)})^{-1} \qquad 3$$

where the parameters also have the same general meaning as in Richards function.

In the form of the curves, either logistic or Gompertz, v is removed from the equations. If A (the upper asymptote) is known, (or observed), then it is not estimated, but supplied. Thus, these forms can be linearized by transforming. However, they may cause a marked influence on the shape as well as the realistic function curve, because of supposition about the values of A and v. For example, Gompertz function, which represents a special case of the Richards function (v = 0), was also rejected due to an inflexible assumption concerning curve shape which did not hold true for all germination curves. The model should not be restrained by assumptions concerning the shape of germination curves. The Richards function, thus, proved to apply describing the germination curves.

Regressions for germination curves were effected using "NLIN" (NonLINear regression) of the SAS statistical package, using least squares method to estimate of the parameters (SAS Institute Inc., 1990). The Marquardt iterative method was operated in NLIN, which regresses the residuals onto the partial derivatives with respect to the parameters, until the estimates converge. The derivatives required for the NLIN program are different to those given by Causton and Venus (1981), who used Newton-Raphson iterations and MINRES-based differentials. The required differentials do not appear to be available in the literature, and their solution was an important pre-requisite for fitting.

Therefore, as part of this work, the first partial derivatives for each parameter of Richards function (v > 0) were solved, as follows:

$$\frac{\partial L}{\partial A} = (1 + e^{(\beta - kt)})^{-1/v}$$

$$\frac{\partial L}{\partial \beta} = -\frac{A}{v} e^{(\beta - kt)} (1 + e^{(\beta - kt)})^{-\frac{1+v}{v}}$$

$$\frac{\partial L}{\partial k} = \frac{At}{v} e^{(\beta - kt)} (1 + e^{(\beta - kt)})^{-\frac{1+v}{v}}$$

$$\frac{\partial L}{\partial v} = Av^{-2} (1 + e^{(\beta - kt)})^{-\frac{1}{v}} \cdot \ln(1 + e^{(\beta - kt)})$$

The first partial derivatives for each parameter of Gompertz function were also solved as follows:

$$\frac{\partial L}{\partial A} = \exp(-e^{(\beta - kt)})$$

$$\frac{\partial L}{\partial \beta} = -A \cdot \exp(-e^{(\beta - kt)}) \cdot e^{(\beta - kt)}$$

$$\frac{\partial L}{\partial k} = At \cdot \exp(-e^{(\beta - kt)}) \cdot e^{(\beta - kt)}$$

Having completed those, the first partial derivatives for each parameter of logistic function were also solved. (They were not used directly for this study, however.) They are as follows:

$$\frac{\partial L}{\partial A} = (1 + e^{(\beta - kt)})^{-1}$$

$$\frac{\partial L}{\partial \beta} = -A \cdot e^{(\beta - kt)} (1 + e^{(\beta - kt)})^{-2}$$

$$\frac{\partial L}{\partial k} = At \cdot e^{(\beta - kt)} (1 + e^{(\beta - kt)})^{-2}$$

The shapes of most of germination curves were similar to that of the Gompertz function, *i.e.* v was close to 0. In that case, the estimated functions would not converge in NLIN using the Richards function model and its derivatives. Such data were re-fitted with NLIN using a direct Gompertz function, together with its derivatives. These alternative fits always converged, and obtained higher η^2 (coefficient of determination). As it was desired to analyze all experimental units collectively in a subsequent MANOVA, the statistic v in

these Gompertz function were set to 0.0001 (close to 0) (Richards, 1959), so that they could all be treated as four attribute Richards functions in the MANOVA.

In order to facilitate physiological "transparency", several secondary data were estimated from the separate sigmoid functions. These were:

(1) time at which seeds reached 5% upper asymptote (Gt5), henceforth referred to as "onset of germination"; and

(2) time at which seeds reached 95% upper asymptote (Gt95), henceforth referred to as "end of germination".

The levels of germination at 5% and 95% of the upper asymptote were chosen as arbitrary low and high levels. They might avoid the problem of x-axis attenuation near the y-axis asymptote, and approximate field perception of the beginning and end of the process (Dennett *et al.*, 1978).

Another two biologically useful variables were also calculated from the fitted statistics, which were defined by Richards (1959). A weighted mean absolute germination rate (R) can be obtained as $Ak/2(v+2)$. It measures the average change of the absolute rate compared against level attained (L). Hence it represents the mean germination-rate within a population in which all size classes are equally abundant. Another useful statistic is $2(v+2)/k$, which represents the time required for the major portion of germination to occur and can be described as the duration of germination (D), although this can only be approximate as it is derived from an asymptotic function.

The derived statistics (R and D) have been usefully employed by Dennett *et al.*, (1978) and Dennett *et al.*, (1979) in the description of leaf growth in *Vicia faba* (broad bean). Here they were used for the first time with respect to germinability.

4.2.3.2 Analysis of variance

The univariate analysis of variance (ANOVA) was conducted for observed the univariate variable, final germination percentage (after 21 days imbibition) and viable seed percentage. A multivariable analysis of variance (MANOVA) was conducted on the four statistics describing the Richards functions, in order to explore the germination profiles. Since the four attributes of the Richards function are correlated, comparison of germination profiles requires the simultaneous analysis of the four statistics, taking into account also their correlation. MANOVA, and canonical discriminant analysis, are the appropriate methods for doing this (Morrison, 1990).

The design consisted of germination tests (light and temperature), which were pooled into a combined analysis. A test for homogeneity of error variances of ANOVA was carried out by using the *Chi-Square* test (Steel and Torrie, 1981). The results of the test showed that error variances were homogenous (Appendix 4.1). Thus, the combined analysis of variances for the four experiments was valid. Based on ANOVA results, error variances for MANOVA were assumed homogenous. The design was a 2 x 2 factorial pooling of a 4 x 2 x 2 factorials blocked in a full layout. The complete model was:

$$Y_{ijklmn} = \mu + \eta_m + \theta_n + \rho_{lmn} + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijklmn} + \textit{ then the rest ...}$$

Where Y_{ijklmn}: the observed value of the variable under consideration of the imbibant i^{th}, in the j^{th} seed-colour, in the k^{th} chilling, in the l^{th} block in the temperature m^{th} in the light n^{th};

μ: the grand mean;
α_i: the effect of the i^{th} imbibant;
β_j: the effect of the j^{th} seed-colour;
γ_k: the effect of the k^{th} chilling;
ρ_{lmn}: the effect of the l^{th} block within the mn^{th} germinator;
η_m: the effect of the m^{th} temperature;
θ_n: the effect of the n^{th} light; and
ε_{ijklmn}: the random error associated with the individual observations.
The rest were combinations for interaction among the treatments.

The expectation of the mean squares for this model are given in Appendix 4.2.

The germination and viability data describe the proportion of seeds which were in one of two conditions (alive or dead), and so followed the Binomial distribution (Steel and Torrie, 1981). These data, therefore, were transformed to arcsin (percentage/100%)$^{1/2}$ prior to statistical analysis. The "days" data were not transformed. The four statistics describing the germination profiles also were not transformed, as they are expected to approximate normal (Morrison, 1990).

The SAS type II sum squares were used for this experiment. The type II sum squares based on the step-down "Fitting Constants Method", in which partially adjusted sum of squares of lower order are used to test for effects of higher order (Speed *et al.*, 1978). Procedure "GLM" of SAS does not give the appropriate complex F-test (Crump, 1946; Satterthwaite, 1946) for some effects, *e.g.*, pooling effects. Therefore, the complex F-test using the appropriate error term (Appendix 4.2) was examined by program THWAITE (I.L. Gordon, unpublished), which implements the Crump and Satterthwaite procedures for the univariate analysis.

4.2.3.3 Multivariate function analysis -- extra issues

4.2.3.3.1 Tests of Wilks' Lambda for special cases

The results of MANOVA from SAS do not give the convert complex Wilks' Lambda F-test, just as they did not for the univariate cases. However, Gordon's THWAITE could not be used here as the multivariate cases have not yet been implanted in that program. Therefore, many multivariate significant tests had to be appropriated. (This problem has not been solved anywhere at this time.) Some tests, involving temperature, light, chilling and seed-colour treatments, had further difficulties, due to low degrees of freedom. So tests of Wilks' Lambda (Λ) for special cases (Cooley and Lohnes, 1971) were applied. Wilks' determinant ratio test statistic is defined as follows:

$$\Lambda = \frac{|W|}{|T|}$$

where W is an error matrix ("within-groups"); T is a total matrix (hypothesis and error matrix).

In the present problem, four attributes (defining each Richards function) were involved (p = 4); also treatments (g) for temperature, light, chilling and seed-colour were equal to 2. Thus, q = g - 1 = 1. Under these conditions, Rao's *F* approximation (of Wilks' Λ) become:

$$F_{n_2}^{n_1} = \left(\frac{1 - \Lambda}{\Lambda}\right)\left(\frac{N - p - 1}{p}\right)$$

Where $n_1 = p$ (degrees of freedom associated with the numerator);
$n_2 = N\text{-}p\text{-}1$ (degrees of freedom associated with the denominator);
N: total number of subjects in all groups.

4.2.3.3.2 Discriminant analysis

As LSD in the univariate analysis of variance (ANOVA), canonical discriminant scores are used in MANOVA to describe the differences between treatments.

The discriminant function will maximize the ratio of the among-groups sum of squares ($v'Av$) to the within-groups sum of squares ($v'Wv$), so that among-group differences will be large relative to within-group. This is solved or the eigenstructure of the matrix $W^{-1}A$, where W is the "error" matrix of Wilks Λ, and A is the corresponding "hypothesis". There are, therefore, several discriminants for each test structure by the MANOVA routine.

Interestingly parsimony, cumulative discriminant power (λ) was checked on 0.70 in this study. Most of the first solution was of possible interest, *e.g.* $\lambda > 0.70$, excepting in imbibant x seed-colour ($\lambda = 0.61$) and temperature x dark x imbibant x seed-colour ($\lambda = 0.68$). On the basis of *a priori* knowledge, imbibant x seed-colour was very interesting although the significance of F-test was only at the 10% probability. Thus, two discriminant solutions were used to calculate discriminant scores. However, the third order interaction was always rather to get information out, so it was less urgent. Moreover, the discriminant power (0.68) was closed to 0.70. Thus, only the first solution was involved in discriminant scores for the third order interaction.

The discriminant solutions (scores) for a standardized function of a standardized test vector are as follows:

$$f_{ij} = c'_j z_j = c'_j \frac{X - m}{D_{diag}^{1/2}}$$

where c_j' (standardized canonical coefficients) was reported in MANOVA of SAS results. Variance of **z** is equal to 1, therefore, t-test for only using the first discriminant solution is:

$$t = \frac{|f_{ij} - f'_{rj}|}{\sqrt{2}}$$

where f_{ij} and f_{ij}' were the discriminate scores of different means.

For imbibant x seed-colour, two discriminant solutions were employed to describe the difference of germination profiles. Matrix of discriminant factor coefficients (C) was 4 (attributes) x 2 (solutions). Thus, two discriminant scores were calculated for each combination. One might test the hypothesis of equal-response mean vectors under two or more attributes by an appropriate Hotelling T^2 statistic, and search for individual mean differences by simultaneous tests (Morrison, 1990). Hotelling T^2 test is defined as follows:

$$T^2 = \frac{n}{2} \Delta' D_{(x)}^{-1} \Delta$$

where n: observations of the sample;
Δ: the treatment-control differences;
$D_{(x)}$: the sample covariance matrix.

A standardised discriminant score with maximum T^2 is available.

$$T^2 = \frac{n}{2} D_{(x)}^{-1} \Delta' \Delta$$

where $D_{(x)}$ is a diagonal matrix. Therefore, an approximate F-value is defined as follows (Morrison, 1990):

$$F_{(p,\, N-p)} = \frac{N - p}{p(N - 1)} T^2$$

where p: attributes of dimensional multinormal population;
N: subjects for obtaining the observation vectors;

The importance of parameters (A, ß, k and v) to discriminant scores was also examined by the interpretation system (I.L. Gordon, pers. comm.) (Table 4.1).

Table 4.1 The interpretation system of the importance of parameters to discriminant scores (I.L. Gordon, pers. comm.).

a. Points system for standardized Canonical coefficients and Canonical structure (between).

Points	Standardized coefficient	Structure
3	5/5* - 4/5	0.99 - 0.80
2	4/5 - 3/5	0.79 - 0.60
1	3/5 - 2/5	0.59 - 0.40
1/2	2/5 - 1/5	0.39 - 0.20
0	1/5 - 0	0.19 - 0

*: the value of maximum one.

b. The function of combining both points of standardized coefficients and Canonical structures.

Standardized coefficient	Structure				
	3	2	1	1/2	0
3	Strange	Strange	Medium	Suppressed	Suppressed
2	Strange	Medium	Medium	Weak	Suppressed
1	Enhanced	Enhanced	Weak	Weak	Suppressed
1/2	Pseudo	Pseudo	Weak	Null	Null
0	Pseudo	Pseudo	Null	Null	Null

4.3 Results

4.3.1 Curve fitting

Germination progress over time ("germination profile") followed a sigmoidal function, as would be expected. The Richards function was fitted, as this is one of the useful general forms of the sigmoid. This was fitted for each of the 192 experimental units separately, thereby making no pre-conception about the effects of light, imbibant, etc.. A sample number of these is given in Figure 4.1. The coefficients of determination (η^2) were very high for all these fits, from 0.9313 (the lowest) to 0.9999 (Table 4.2). The NLIN procedure was highly successful in describing the germination profiles, either as Richards function, or as Gompertz function (when $v \rightarrow 0$) (see 4.2.3.1).

The estimated parameters and their statistics of Richards function for each experimental unit are given in Table 4.2. Most of germination profiles (141 EU) followed the Gompertz function ($v \cong 0$) (Table 4.2). Two germination profiles were considered as logistic functions, for which v was 1.0056 and 0.9081, respectively. Others (39 EU) followed unspecified Richards function ($0<v<1$). Subsequently, MANOVA of these EU profile statistics enabled exploration of the effects of light, imbibant, etc..

4.3.2 Analysis of variance

As well as the four parameter statistics required to describe each Richards function, there were direct observations of final germination level and final seed viability. Univariate ANOVA were done on these, the model of 2 factors pooling of blocked 3 factor factorials. In addition, a MANOVA of the four regression parameters was done, as they should be considered jointly in order to distinguish amongst germination profiles.

The ANOVA results of observation of final germination, seed viability and the MANOVA results of coefficients of Richards function (A, ß, k and v) are presented in Table 4.3. The infinite random F-test in Table 4.3 indicates which effects have been notable in this experiment. The ANOVA results of the four parameters are given in Appendix 4.3.

Comparing both statistical methods for coefficients of Richards function, MANOVA was more powerful to describe the differences amongst germination profiles.

Table 4.2 The observations of final germination and the parameters of Richards function and their standard errors for 192 experimental units.

		Germ (%)	A (%)	A se	ß	ß se	k	k se	v	v se	η^2
10/5°C*Dk*P-ch*Water*B	1	83.3	81.6	0.9555	6.8489	0.4757	1.1450	0.0779	0		0.9981
	2	92.0	90.8	1.2294	8.1297	2.0617	1.2730	0.1895	0.5186	0.3278	0.9987
	3	97.9	91.6	1.6937	5.6860	0.4631	0.8756	0.0710	0		0.9970
10/5°C*Dk*P-ch*Water*G	1	91.7	90.6	1.3534	7.4830	2.5455	1.2650	0.2185	0.3989	0.3791	0.9982
	2	86.0	85.5	0.7292	9.3074	0.4629	1.4313	0.0704	0		0.9991
	3	87.8	87.6	0.8545	6.0688	0.3061	0.9940	0.0494	0		0.9989
10/5°C*Dk*P-ch*KNO_3*B	1	93.6	93.1	0.9364	4.8403	0.2053	0.7888	0.0334	0		0.9990
	2	100.0	96.7	1.0784	7.1139	0.4782	1.0697	0.0771	0		0.9984
	3	100.0	99.5	1.4374	5.4172	0.3282	0.8304	0.0502	0		0.9983
10/5°C*Dk*P-ch*KNO_3 *G	1	95.7	95.6	0.7171	4.3326	0.1268	0.7137	0.0210	0		0.9995
	2	100.0	100.3	2.0734	5.8026	1.9682	0.9294	0.1602	0.5035	0.3618	0.9978
	3	100.0	99.5	1.2920	3.8431	2.8207	0.9241	0.1056	0.1085	0.2234	0.9990
10/5°C*Dk*P-ch*GA_3*B	1	97.7	97.4	1.7360	4.8212	0.4344	0.8803	0.0779	0		0.9951
	2	100.0	98.0	0.4950	8.7244	0.9145	1.4968	0.0982	0.6057	0.1536	0.9997
	3	95.9	94.6	0.6358	10.8207	1.4960	1.8512	0.1756	0.6320	0.0061	0.9994
10/5°C*Dk*P-ch* GA_3*G	1	95.9	92.4	2.9245	5.5032	0.9029	0.9607	0.1551	0		0.9861
	2	95.9	96.7	0.8365	6.4775	0.3186	1.0986	0.0530	0		0.9989
	3	100.0	96.8	0.6791	10.1597	1.4960	1.6498	0.1639	0.6987	0.2479	0.9995
10/5°C*Dk*P-ch* GA_{4+7}*B	1	100.0	98.9	0.9305	5.7090	2.0346	1.2373	0.1486	0.2345	0.2531	0.9990
	2	93.5	89.9	0.8583	5.9301	0.3104	1.0402	0.0535	0		0.9986
	3	97.9	89.7	0.9491	7.1713	0.4830	1.3186	0.0873	0		0.9977
10/5°C*Dk*P-ch* GA_{4+7} *G	1	100.0	97.3	1.1475	7.4610	0.5602	1.3304	0.0978	0		0.9974
	2	100.0	99.0	0.6372	8.5271	1.1495	1.4621	0.1201	0.5733	0.1915	0.9995
	3	98.0	97.5	0.9501	4.8898	0.2218	0.8425	0.0378	0		0.9988
10/5°C*Dk*N-ch*Water*B	1	95.7	93.6	0.8974	6.2394	1.8102	1.2507	0.1386	0.2584	0.2312	0.9991
	2	94.0	90.3	1.5787	5.4982	0.4642	0.9125	0.0761	0		0.9964
	3	93.2	92.8	1.4909	7.1065	0.7140	1.2916	0.1274	0		0.9950
10/5°C*Dk*N-ch*Water*G	1	92.0	90.6	1.9822	7.8506	1.1162	1.4229	0.1985	0		0.9905
	2	100.0	98.1	1.4152	5.9959	0.4726	1.0422	0.0807	0		0.9969
	3	93.9	93.6	2.2540	4.3550	0.3819	0.6879	0.0609	0		0.9955
10/5°C*Dk*N-ch*KNO_3*B	1	93.8	95.8	2.8421	5.1226	0.6914	0.8495	0.1137	0		0.9904
	2	95.8	95.2	1.2001	10.1265	2.4082	1.5701	0.2762	1.0056	0.4551	0.9983
	3	95.9	96.4	4.8133	4.1490	0.7844	0.6871	0.1307	0		0.9784
10/5°C*Dk*N-ch*KNO_3*G	1	100.0	96.8	1.4790	5.7010	1.9202	1.0529	0.1654	0.4310	0.3380	0.9981
	2	95.7	94.0	0.6040	5.6946	1.5356	1.2382	0.0881	0.1517	0.1459	0.9996
	3	100.0	100.3	0.3608	5.7994	0.6142	1.1600	0.0460	0.2730	0.0830	0.9999
10/5°C*Dk*N-ch*GA_3*B	1	97.9	96.0	0.8767	4.9316	0.2246	0.8828	0.0396	0		0.9988
	2	97.9	96.2	0.7219	4.9623	0.1853	0.8849	0.0325	0		0.9992
	3	97.8	93.5	1.0485	5.6389	0.3429	1.0039	0.0600	0		0.9980
10/5°C*Dk*N-ch* GA_3*G	1	98.0	98.1	0.1067	15.0505	0.4005	2.5589	0.0557	0.8455	0.0468	1.0000
	2	97.9	95.7	0.3754	8.8445	0.7331	1.5390	0.0814	0.6263	0.1227	0.9998
	3	100.0	98.1	0.1290	8.7732	0.0808	1.5747	0.0141	0		1.0000
10/5°C*Dk*N-ch* GA_{4+7}*B	1	100.0	94.0	1.7138	6.0923	0.6485	1.1057	0.1155	0		0.9941
	2	100.0	97.0	1.0042	5.5651	0.2956	0.9595	0.0502	0		0.9994
	3	100.0	96.2	2.4889	5.9834	0.8588	1.0549	0.1487	0		0.9896
10/5°C*Dk*N-ch* GA_{4+7}*G	1	100.0	95.9	0.9927	7.4307	0.4990	1.2824	0.0840	0		0.9982
	2	100.0	88.1	1.4680	5.0593	0.4504	0.9341	0.0815	0		0.9953
	3	95.8	95.0	0.7111	10.4508	1.6468	1.8154	0.1904	0.5756	0.2426	0.9993

continued next page

Table 4.2 (continued)

		Germ (%)	A (%)	A se	ß	ß se	k	k se	v	v se	η^2
10/5°C*L*P-ch*Water*B	1	72.9	67.8	1.9906	4.7792	0.6866	0.8587	0.1215	0		0.9878
	1	91.8	88.4	2.1190	3.4690	2.3371	0.7567	0.1489	0.3161	0.4021	0.9961
	3	77.6	78.3	0.6048	4.9165	2.3090	1.2063	0.1108	0.1127	0.1807	0.9993
10/5°C*L*P-ch*Water*G	1	89.6	89.8	1.6342	5.0271	0.3992	0.8202	0.0648	0		0.9967
	2	87.8	87.1	2.0717	5.8040	0.7007	0.9669	0.1150	0		0.9932
	3	81.3	81.3	0.7109	6.1334	0.2892	1.0337	0.0479	0		0.9990
10/5°C*L*P-ch*KNO_3*B	1	93.8	89.0	1.1904	5.2605	0.4559	0.9362	0.0798	0		0.9958
	2	91.8	87.7	1.5374	4.9211	4.6243	1.1880	0.2683	0.1650	0.4821	0.9962
	3	93.9	91.1	0.7808	5.5650	0.2604	1.0006	0.0460	0		0.9988
10/5°C*L*P-ch*KNO_3 *G	1	98.0	95.2	0.3505	6.6463	0.1401	1.1045	0.0228	0		0.9998
	2	96.0	92.0	1.6002	5.8467	0.5213	0.9769	0.0858	0		0.9962
	3	100.0	99.8	1.1840	5.1701	0.2779	0.8513	0.0454	0		0.9985
10/5°C*L*P-ch*GA_3*B	1	87.2	86.3	1.5124	4.8598	0.4753	0.9475	0.0903	0		0.9937
	2	89.8	83.5	0.3390	6.9120	0.1713	1.2386	0.0301	0		0.9997
	3	93.6	85.3	2.0975	5.4513	0.7368	0.9914	0.1314	0		0.9899
10/5°C*L*P-ch* GA_3*G	1	93.8	92.1	0.9594	5.7022	0.3412	1.0466	0.0613	0		0.9981
	2	98.0	93.7	0.8705	7.2387	0.4257	1.2452	0.0715	0		0.9986
	3	100.0	100.7	0.9538	5.2820	0.2294	0.8666	0.0373	0		0.9990
10/5°C*L*P-ch* GA_{4+7}*B	1	93.6	92.3	1.5836	4.2807	0.3838	0.8562	0.0749	0		0.9936
	2	97.8	97.8	2.0705	2.8416	2.3579	0.7199	0.1181	0.2106	0.3191	0.9973
	3	97.9	90.7	1.3182	3.7396	0.2038	0.6505	0.0357	0		0.9978
10/5°C*L*P-ch* GA_{4+7} *G	1	97.9	92.0	1.0912	6.8266	1.7102	1.1200	0.1870	0.8734	0.4054	0.9979
	2	98.0	92.3	1.9030	5.5632	0.6303	1.0042	0.1117	0		0.9929
	3	93.9	85.8	0.5724	7.6461	0.3294	1.3659	0.0576	0		0.9991
10/5°C*L*N-ch*Water*B	1	87.2	87.9	1.5483	4.8140	1.5824	0.8412	0.1192	0.4323	0.2915	0.9983
	2	90.9	83.0	2.8622	5.4779	0.9673	0.9509	0.1653	0		0.9840
	3	81.3	75.4	1.8482	5.9008	0.7943	1.0394	0.1374	0		0.9908
10/5°C*L*N-ch*Water*G	1	86.0	87.0	1.1110	4.6986	0.2851	0.8363	0.0501	0		0.9978
	2	87.5	87.8	1.5071	4.8060	0.3839	0.8351	0.0660	0		0.9963
	3	91.5	88.7	0.9335	4.9736	2.7015	1.1552	0.1293	0.1213	0.2249	0.9989
10/5°C*L*N-ch*KNO_3*B	1	100.0	98.8	0.9480	4.5847	0.1904	0.7803	0.0322	0		0.9990
	2	95.7	91.5	1.3447	5.6729	0.4165	0.9477	0.0686	0		0.9974
	3	95.7	95.3	2.6352	4.2495	0.4592	0.7078	0.0767	0		0.9928
10/5°C*L*N-ch*KNO_3*G	1	92.0	91.2	0.9489	9.8923	2.4394	1.7585	0.2938	0.6656	0.3801	0.9983
	2	95.7	95.0	0.6583	5.9199	0.2107	0.9818	0.0344	0		0.9994
	3	94.0	92.6	0.9823	7.1716	2.1539	1.4006	0.1816	0.2773	0.2720	0.9987
10/5°C*L*N-ch*GA_3*B	1	89.6	86.7	0.8707	6.4574	0.3825	1.1562	0.0672	0		0.9982
	2	85.7	85.8	0.7337	4.8918	1.4927	1.0820	0.1044	0.2510	0.2035	0.9993
	3	91.5	90.8	1.4184	4.8954	0.3432	0.8243	0.0573	0		0.9973
10/5°C*L*N-ch* GA_3*G	1	88.0	86.8	0.7312	7.9488	0.4376	1.4366	0.0775	0		0.9985
	2	87.8	82.8	2.5914	5.0652	4.2561	1.0201	0.3310	0.3427	0.6868	0.9907
	3	92.0	89.5	1.0654	11.1491	0.9502	2.0608	0.1734	0		0.9967
10/5°C*L*N-ch* GA_{4+7}*B	1	93.6	82.4	1.9137	4.7428	0.5741	0.8918	0.1057	0		0.9907
	2	97.7	90.7	2.3721	4.0630	4.0195	0.9350	0.2362	0.2118	0.5114	0.9942
	3	95.7	95.5	1.1380	4.8672	0.2840	0.8674	0.0499	0		0.9980
10/5°C*L*N-ch* GA_{4+7}*G	1	95.7	94.1	1.4483	7.6889	0.7690	1.4142	0.1392	0		0.9950
	2	96.0	86.5	0.6920	6.5382	0.3425	1.2346	0.0633	0		0.9986
	3	100.0	92.4	0.7226	7.8293	1.7529	1.5109	0.1810	0.4399	0.2667	0.9991

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Table 4.2 (continued)

		Germ (%)	A (%)	A se	ß	ß se	k	k se	v	v se	η^2
15/10°C*Dk*P-ch*Water*B	1	88.6	91.1	2.6996	1.8787	0.1901	0.3975	0.0399	0		0.9912
	2	85.7	81.9	2.4008	3.4668	3.7964	0.7331	0.3067	0.9081	1.5557	0.9849
	3	93.5	90.9	1.5182	3.3108	0.3494	0.7710	0.0758	0		0.9894
15/10°C*Dk*P-ch*Water*G	1	98.0	96.1	1.0882	4.3975	0.4220	1.0870	0.0972	0		0.9908
	2	93.6	92.5	0.8395	2.8682	0.1773	0.7027	0.0391	0		0.9965
	3	83.3	78.4	2.0219	2.4508	0.5698	0.6765	0.1296	0		0.9607
15/10°C*Dk*P-ch*KNO_3*B	1	100.0	101.3	2.3575	6.9742	3.4539	0.9399	0.3614	3.2077	2.0807	0.9912
	2	95.9	94.8	0.9907	5.0162	1.3338	0.9828	0.1279	0.6977	0.3442	0.9983
	3	95.7	93.8	1.3010	3.4264	0.3441	0.8316	0.0766	0		0.9903
15/10°C*Dk*P-ch*KNO_3 *G	1	97.9	95.7	1.5245	4.8138	0.5131	1.0875	0.1118	0		0.9887
	2	97.8	94.0	1.7965	3.2343	0.3462	0.7226	0.0732	0		0.9890
	3	95.2	93.4	2.0925	6.8646	5.3736	1.1435	0.6414	2.8992	3.3084	0.9784
15/10°C*Dk*P-ch*GA_3*B	1	93.3	93.0	1.7217	2.6516	0.2624	0.6062	0.0558	0		0.9911
	2	95.5	91.6	1.7469	3.1788	0.2822	0.6596	0.0569	0		0.9927
	3	93.6	91.4	1.4371	3.7854	0.3565	0.8448	0.0759	0		0.9913
15/10°C*Dk*P-ch* GA_3*G	1	98.0	93.9	1.0667	3.2782	0.2162	0.7409	0.0461	0		0.9956
	2	96.0	90.1	0.8310	3.2914	0.3394	0.9087	0.0794	0		0.9912
	3	93.6	93.6	0.3952	3.7089	0.1371	0.9431	0.0315	0		0.9987
15/10°C*Dk*P-ch* GA_{4+7}*B	1	96.0	95.7	2.3845	2.6496	2.0707	0.5446	0.1380	0.8171	0.8436	0.9952
	2	97.9	93.1	1.2402	1.8212	0.1865	0.5166	0.0421	0		0.9932
	3	97.8	96.5	0.7068	2.5302	1.5049	0.8147	0.0795	0.2668	0.2698	0.9990
15/10°C*Dk*P-ch* GA_{4+7} *G	1	95.9	94.6	0.9654	2.7173	0.2131	0.6992	0.0477	0		0.9948
	2	98.0	94.1	1.9845	2.3717	0.2885	0.5713	0.0625	0		0.9876
	3	100.0	94.0	1.2335	6.6133	0.8673	1.6414	0.2086	0		0.9838
15/10°C*Dk*N-ch*Water*B	1	78.7	75.1	1.2962	5.1742	0.7303	1.2493	0.1686	0		0.9798
	2	83.7	82.7	2.4125	6.7469	5.5753	1.1269	0.6507	2.1311	2.6414	0.9756
	3	89.6	81.3	1.7552	2.8390	0.3402	0.6487	0.0725	0		0.9867
15/10°C*Dk*N-ch*Water*G	1	95.7	93.1	1.1868	4.0599	0.4171	0.9973	0.0950	0		0.9895
	2	88.0	85.5	1.9959	2.5952	0.5415	0.7055	0.1231	0		0.9668
	3	93.6	92.7	1.7894	3.5510	0.4142	0.8040	0.0888	0		0.9868
15/10°C*Dk*N-ch*KNO_3*B	1	100.0	99.1	0.6866	4.4315	0.2782	1.1141	0.0647	0		0.9961
	2	98.0	96.6	1.8323	3.0999	0.4160	0.7585	0.0922	0		0.9836
	3	93.6	90.2	0.9473	4.4389	0.3238	1.0285	0.0716	0		0.9946
15/10°C*Dk*N-ch*KNO_3*G	1	100.0	100.5	0.3912	4.2085	0.1236	1.0091	0.0278	0		0.9991
	2	93.9	93.0	1.5312	3.5090	0.3844	0.8249	0.0842	0		0.9884
	3	100.0	102.3	2.3889	2.8244	0.3563	0.6395	0.0755	0		0.9853
15/10°C*Dk*N-ch*GA_3*B	1	87.2	83.9	2.9683	3.3420	0.6417	0.7326	0.1343	0		0.9665
	2	95.9	94.8	1.6459	2.8899	0.3365	0.7033	0.0740	0		0.9878
	3	97.9	94.7	2.5717	3.0160	0.4887	0.6993	0.1052	0		0.9760
15/10°C*Dk*N-ch* GA_3*G	1	95.7	89.5	0.9980	4.7042	0.4340	1.1480	0.0999	0		0.9913
	2	98.0	99.2	0.8966	2.5494	1.4522	0.6528	0.0950	0.7764	0.6301	0.9983
	3	95.8	93.2	1.0331	4.1049	0.3746	1.0136	0.0856	0		0.9918
15/10°C*Dk*N-ch* GA_{4+7}*B	1	98.0	97.0	1.4553	3.3532	0.3254	0.7857	0.0709	0		0.9909
	2	97.9	93.9	1.2889	3.1905	0.3089	0.7761	0.0684	0		0.9912
	3	95.5	95.8	0.9290	2.9021	0.1550	0.6584	0.0329	0		0.9973
15/10°C*Dk*N-ch* GA_{4+7}*G	1	98.0	96.8	1.8181	2.6724	0.3932	0.6940	0.0882	0		0.9822
	2	96.0	91.2	2.6895	3.7993	1.0758	0.9871	0.2498	0		0.9313
	3	97.9	96.1	2.1327	2.6284	0.3194	0.6074	0.0682	0		0.9869

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Table 4.2 (continued)

		Germ (%)	A (%)	A se	ß	ß se	k	k se	v	v se	η^2
15/10°C*L*P-ch*Water*B	1	70.8	73.1	2.0661	2.1821	0.2601	0.4790	0.0545	0		0.9876
	2	85.4	82.1	1.6152	2.8341	0.4528	0.7356	0.1021	0		0.9784
	3	80.9	81.9	1.0146	2.2078	0.1389	0.5172	0.0298	0		0.9966
15/10°C*L*P-ch*Water*G	1	77.3	77.5	1.0961	3.1941	0.2980	0.7596	0.0652	0		0.9918
	2	79.6	78.0	0.3912	4.1231	0.1782	1.0307	0.0410	0		0.9981
	3	78.7	78.9	0.6439	3.2023	0.1741	0.7639	0.0382	0		0.9971
15/10°C*L*P-ch*KNO_3*B	1	91.3	86.5	1.1587	4.7936	0.4413	1.0971	0.0972	0		0.9915
	2	98.0	95.0	0.9444	3.7481	0.2096	0.8153	0.0438	0		0.9969
	3	98.0	94.9	1.4210	3.0592	0.2331	0.6655	0.0484	0		0.9944
15/10°C*L*P-ch*KNO_3*G	1	98.0	96.2	2.5227	3.4276	0.5645	0.7921	0.1223	0		0.9745
	2	95.6	95.1	1.2191	2.6235	2.2462	0.8901	0.1212	0.1817	0.2807	0.9978
	3	100.0	100.8	0.8827	2.7665	0.1584	0.6733	0.0347	0		0.9970
15/10°C*L*P-ch*GA_3*B	1	91.5	86.7	2.1006	2.7311	0.3897	0.6426	0.0840	0		0.9818
	2	85.4	78.1	1.7806	2.9944	0.3005	0.6158	0.0604	0		0.9906
	3	95.9	92.3	2.4372	2.8159	0.3967	0.6350	0.0839	0		0.9815
15/10°C*L*P-ch* GA_3*G	1	97.8	95.6	1.3685	3.5072	0.3041	0.7962	0.0652	0		0.9926
	2	93.9	90.6	1.7938	2.6325	0.3587	0.6579	0.0792	0		0.9843
	3	89.8	89.1	1.3171	3.9227	0.3414	0.8631	0.0721	0		0.9927
15/10°C*L*P-ch* GA_{4+7}*B	1	95.8	88.3	3.4809	2.6824	0.4781	0.5736	0.0983	0		0.9718
	2	93.2	86.1	2.9440	1.9416	0.2123	0.3965	0.0439	0		0.9894
	3	85.7	81.6	3.2700	2.6116	0.3356	0.4869	0.0642	0		0.9849
15/10°C*L*P-ch* GA_{4+7} *G	1	97.8	94.5	1.3530	3.1799	1.8370	0.8693	0.1263	0.2837	0.3034	0.9976
	2	97.9	95.1	0.5748	3.8172	0.1912	0.9522	0.0436	0		0.9976
	3	100.0	99.8	1.4568	3.1476	0.2318	0.6780	0.0479	0		0.9948
15/10°C*L*N-ch*Water*B	1	79.5	80.2	1.9155	1.7236	0.1679	0.4000	0.0364	0		0.9926
	2	89.1	87.3	1.0931	4.5570	0.4331	1.0866	0.0980	0		0.9908
	3	78.7	78.2	1.4742	2.6085	0.2955	0.6241	0.0641	0		0.9888
15/10°C*L*N-ch*Water*G	1	84.0	83.9	1.8061	2.7885	0.3402	0.6443	0.0728	0		0.9865
	2	84.0	83.2	0.7032	3.8899	0.1800	0.8280	0.0371	0		0.9980
	3	85.7	83.8	1.8699	3.8749	0.9994	1.0513	0.2363	0		0.9455
15/10°C*L*N-ch*KNO_3*B	1	95.8	92.3	1.7199	4.2169	0.4735	0.9249	0.1001	0		0.9880
	2	100.0	96.5	1.6286	3.6975	0.3949	0.8468	0.0855	0		0.9885
	3	95.8	93.7	3.9237	2.2858	0.4498	0.5161	0.0950	0		0.9677
15/10°C*L*N-ch*KNO_3*G	1	97.9	95.3	2.0278	3.0447	0.9009	1.2908	0.1860	0		0.9843
	2	95.8	95.3	1.8555	5.4935	2.6655	1.1527	0.3178	0.8912	0.7320	0.9923
	3	95.9	93.8	0.9225	3.8664	0.2261	0.8567	0.0480	0		0.9966
15/10°C*L*N-ch*GA_3*B	1	91.7	90.0	1.0703	3.4091	1.2384	0.7690	0.0509	0		0.9952
	2	94.0	84.4	1.2687	2.9372	0.2745	0.6946	0.0596	0		0.9919
	3	93.9	87.0	1.8771	3.0206	0.3685	0.6863	0.0785	0		0.9862
15/10°C*L*N-ch* GA_3*G	1	89.4	85.5	1.5360	4.0226	0.4703	0.9199	0.1023	0		0.9865
	2	87.2	84.5	0.9991	4.0762	0.2752	0.8768	0.0572	0		0.9957
	3	93.6	89.6	4.2917	2.3924	0.5205	0.5273	0.1088	0		0.9600
15/10°C*L*N-ch* GA_{4+7}*B	1	100.0	95.5	3.1658	2.7620	0.4499	0.6062	0.0938	0		0.9761
	2	95.7	93.4	1.3542	3.1170	0.2446	0.6922	0.0515	0		0.9941
	3	97.7	84.8	2.5681	3.6170	0.8374	0.8867	0.1883	0		0.9507
15/10°C*L*N-ch* GA_{4+7}*G	1	98.0	91.8	1.8022	3.0996	0.3602	0.7130	0.0773	0		0.9873
	2	89.4	84.6	1.2420	3.8342	0.3220	0.8361	0.0675	0		0.9932
	3	95.9	95.2	1.4432	1.7927	0.1423	0.4493	0.0313	0		0.9923

D: dark; L: light; B: brown seeds; G: green seeds.
Germ: germination.

Table 4.3 Significance in analysis of variance for seed germination.

Source	df	Anova				Manova
		Germination		Viability		A, ß, κ & ν
		MS	F-test	MS	F-test	F-test
T	1	0.0663	ns	0.0376	ns	**
L	1	0.3864	(*)	0.0001	ns	**
T*L	1	0.0383	ns	0.0004	ns	**
Blk(T*L)	8	0.0044	ns	0.0084	ns	ns
Ch	1	0.0015	ns	0.0039	ns	**
I	3	0.4619	**	0.0009	ns	**
Col	1	0.0570	(*)	0.2088	ns	**
Ch*I	3	0.0119	ns	0.0024	ns	ns
Ch*Col	1	0.0176	ns	0.0496	ns	ns
I*Col	3	0.0003	ns	0.0013	ns	(*)
Ch*I*Col	3	0.0126	ns	0.0270	ns	ns
Ch*T	1	0.0001	ns	0.0053	ns	ns
Ch*L	1	0.0017	ns	0.0017	ns	ns
Ch*T*L	1	0.0087	ns	0.0012	ns	ns
I*T	3	0.0105	ns	0.0035	ns	*
I*L	3	0.0128	ns	0.0155	ns	(*)
I*T*L	3	0.0054	ns	0.0106	ns	ns
Col*T	1	0.0008	ns	0.0265	ns	**
Col*L	1	0.0001	ns	0.0068	ns	ns
Col*T*L	1	0.0077	ns	0.0370	ns	ns
Ch*I*T	3	0.0112	ns	0.0024	ns	ns
Ch*I*L	3	0.0055	ns	0.0049	ns	ns
Ch*I*T*L	3	0.0103	ns	0.0019	ns	ns
Ch*Col*T	1	0.0007	ns	0.0055	ns	**
Ch*Col*L	1	0.0674	(*)	0.0164	*	*
Ch*Col*T*L	1	0.0001	ns	0.0001	ns	ns
I*Col*T	3	0.0033	ns	0.0125	ns	ns
I*Col*L	3	0.0019	ns	0.0020	ns	ns
I*Col*T*L	3	0.0071	ns	0.0092	ns	**
Ch*I*Col*T	3	0.0041	ns	0.0105	ns	ns
Ch*I*Col*L	3	0.0112	ns	0.0019	ns	ns
Ch*I*Col*T*L	3	0.0177	(*)	0.0046	ns	ns
Residual	120	0.0080		0.0086		
CV (%)		6.63		6.63		
Mean ($\bar{x}$)		1.3532 (95.3%)		1.3859 (96.6%)		
SE $\bar{x}$		0.0065		0.0067		

T: temperature; L: light; Ch: chill; I: Imbibant; Col: seed-colour; Blk: block.
**: significance at the 1% probability level; *: significance at the 5% probability level; (*): significance at the 10% probability level.
ns: no significance at the statistics level.

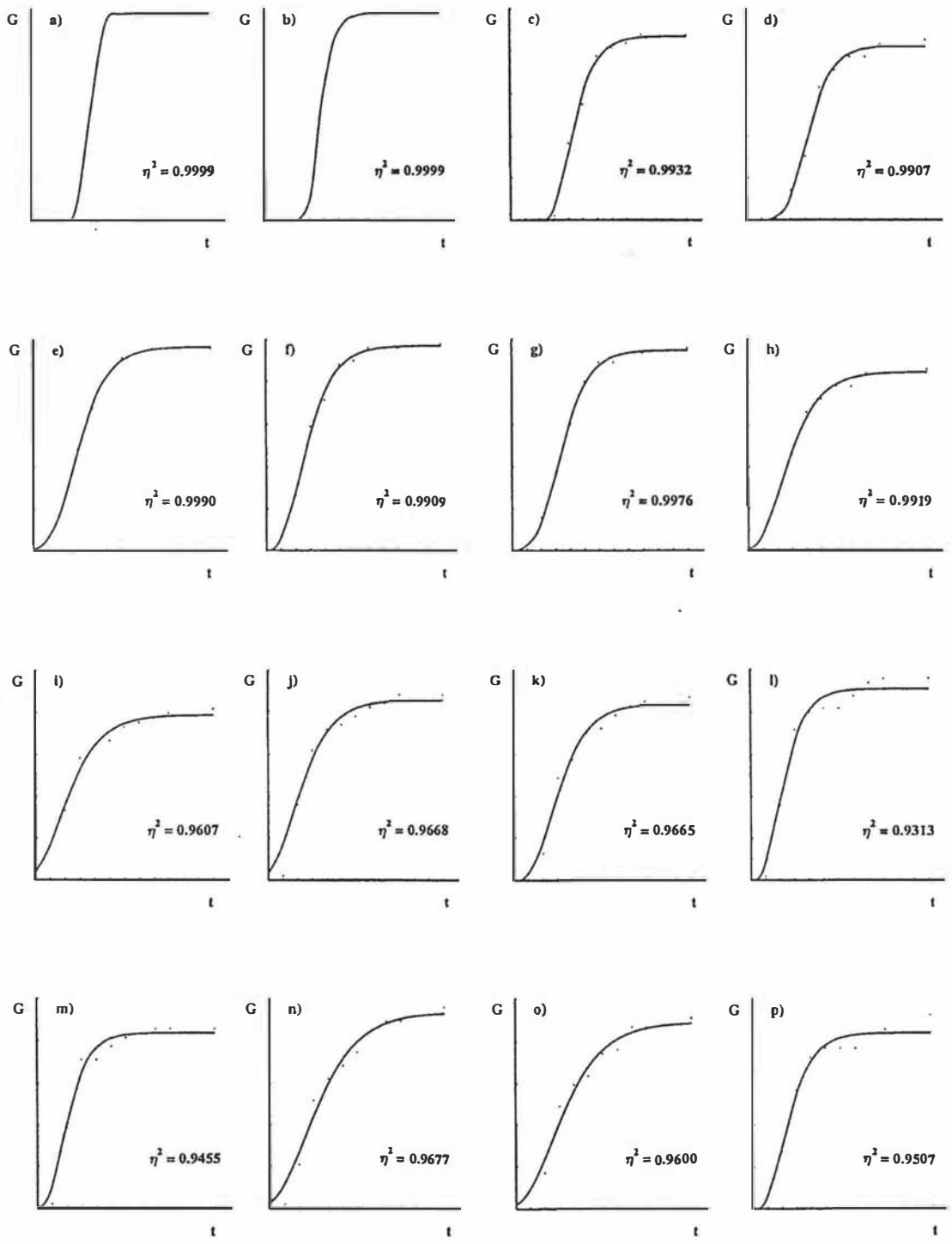


Figure 4.1 The fitted curves of seed germination fitting in Richards function.

4.3.2.1 Viability

In general, the seed viability was the same in every treatment, the one exception being 5% significance for light x pre-chilling x seed-colour interaction. The mean level of viability was 1.3859 (96.6%) ± 0.0067 (Table 4.3). The interaction combination, together with their LSD are given in Table 4.4. The percentage of viable seeds was higher in green seeds than in brown seeds, regardless of light and chilling, but not in light with pre-chilling. Pre-chilling gave low seed viability only in green seeds in light. No differences between light and darkness in viable seeds were found for all combinations. This might be explained by a fungal contamination effect.

Table 4.4 The effect of interaction between light, chilling and seed colour on seed viability.

Chilling	Seed colour	Viability	
		Dark	Light
Non-chilling	Green	1.4387 (98.3) a	1.4403 (98.3) a
	Brown	1.3472 (95.1) d	1.3356 (94.6) d
Pre-chilling	Green	1.4100 (97.4) abc	1.3866 (96.7) bcd
	Brown	1.3459 (95.0) d	1.3831 (96.5) cd
F-test		*	
SE $\bar{x}$		0.0189	

Means within each column which have a different letter are significantly different by t-test at the 5% probability level.
Values in parentheses are the transformation values of the least square means (%).

4.3.2.2 Final germination

Final germination was the germination measured on the last harvest (after 21 days imbibition). The F-test (Table 4.3) indicated a weak main effect ($P<0.10$) from light and seed-colour ("maturity"?) treatments, but the outstanding major effect ($P<0.01$) was from the different imbibants. Some inconsistencies amongst treatment combinations were indicated by weak significances ($P<0.10$) for light x chilling x seed-colour, and the fourth-order interaction for everything combined. The higher probability level was used because the variability in the data might obscure real differences.

The final germination mean-square for imbibants was significant ($F_{4,20}=7.584$, $P<0.01$) (Table 4.5). GA_{4+7} and KNO_3 resulted in high germination, 98.0% and 97.8%, respectively, whereas water only reached 88.0%. GA_3 presented intermediate germination (95.0%). The final germination was higher ($F_{2,4}= 6.698$, $P<0.10$) in darkness (97.1%) than in light (93.3%); and green seeds resulted higher ($F_{4,5} = 4.071$, $P<0.10$) germination (Table 4.7). Overall, temperature effects were not significant ($F_{3,3} = 1.699$), the means being 96.1% for 10/5°C and 94.5% for 15/10°C; and pre-chilling (mean = 95.5%) was similar to lack of chilling (mean = 95.2%, $F_{3,7} = 2.260$) (Table 4.6).

A light x seed-colour x chilling interaction ($F_{2,3} = 7.574$, $P<0.10$) showed that non-chilled green seeds in darkness gave relatively higher final germination compared with other combinations (Table 4.7).

The fourth-order interaction (Table 4.8) ($F_{3,120} = 2.201$, $P<0.10$) showed that GA_{4+7} and KNO_3 gave high final germination in darkness at 10/5 °C for green seeds, regardless of chilling treatments, which were greater than 99.5%. The highest germination (100%), however, was found in brown seeds in GA_{4+7} (non-chilling) in darkness at 10/5 °C. Water gave low final germination in light, which was around 78.1% - 88.4%, regardless of temperature, seed-colour and chilling treatment. Pre-chilling green seeds in KNO_3 were less depressed by light (in both temperatures) than they were in other imbibants. A similar result was found in GA_{4+7} for pre-chilled green seeds, but only at 15/10 °C.

Table 4.5 Main effect of imbibants on final germination of seeds.

Imbibants	Germination
$GA_{4/7}$	1.4273 (98.0) a
KNO_3	1.4226 (97.8) a
GA_3	1.3458 (95.0) b
water	1.2172 (88.0) c
F-test	**
$SE\bar{x}$	0.0129

Means within each column which have a different letter are significantly different by t-test at the 5% probability level.
Values in parentheses are the de-transformation values of means (%).

Table 4.6 Main effects of temperature and chilling on final germination.

Temperature (°C)	Germination	Chilling	Germination
10/5	1.3718 (96.1)	Pre-chilling	1.3560 (95.5)
15/10	1.3346 (94.5)	Non-chilling	1.3504 (95.2)
F-test	ns	F-test	ns
$SE\bar{x}$	0.0092	$SE\bar{x}$	0.0092

Values in parentheses are the de-transformation values of means (%).
ns: no significance at the statistics level.

Table 4.7 Main effects of light and seed-colour as well as interaction amongst light, chilling and seed-colour treatments on final germination.

Seed-colour	Chilling	Germination		
		Dark	Light	Mean
Green	Non-chilling	1.4311 (98.1) a	1.2963 (92.7) de	
	Pre-chilling	1.4012 (97.2) ab	1.3532 (95.3) bc	1.3705 (96.0)
Brown	Non-chilling	1.3767 (96.3) b	1.3202 (93.9) cd	
	Pre-chilling	1.3834 (96.5) ab	1.2638 (90.9) e	1.3360 (94.6)
Mean		1.3981 (97.1)	1.3084 (93.3)	
F-test	Light	(*)	$SE\bar{x}$	0.0092
	Seed-colour	(*)		0.0092
	L*Col*Chilling	(*)		0.0183

Means within each column which have a different letter are significantly different by t-test at the 10% probability level.
Values in parentheses are the transformation values of the least square means (%).
(*): significance at the 10% probability level.

Table 4.8 Final germination of seed at various temperature, light, chilling, imbabant and seed-colour.

Imbibants	Chilling	Seed-colour	Dark		Light	
			10/5 °C	15/10 °C	10/5 °C	15/10 °C
$GA_{4/7}$	Non-chilling	Green	1.4750 (99.6) abc	1.4079 (97.4) abc	1.4341 (98.1) ab	1.3450 (95.0) bcd
		Brown	1.5708 (100.0) a	1.4038 (97.2) abc	1.3652 (95.8) abcd	1.4504 (98.6) ab
	Pre-chilling	Green	1.5235 (99.8) ab	1.4555 (98.7) a	1.3918 (96.8) abc	1.4727 (99.0) a
		Brown	1.4364 (98.2) bcd	1.4056 (97.3) abc	1.3874 (96.7) abc	1.2848 (92.0) cde
KNO_3	Non-chilling	Green	1.5012 (99.5) abc	1.4876 (99.3) a	1.3231 (94.0) bcde	1.3856 (96.6) abc
		Brown	1.3501 (95.2) de	1.4382 (98.3) ab	1.4315 (98.1) ab	1.4332 (98.1) ab
	Pre-chilling	Green	1.5012 (99.5) abc	1.3991 (97.1) abc	1.4564 (98.7) a	1.4531 (98.6) ab
		Brown	1.4855 (99.3) abc	1.4332 (98.1) ab	1.3069 (93.2) cdef	1.3764 (96.3) abc
GA_3	Non-chilling	Green	1.4750 (99.1) abc	1.3851 (96.6) abc	1.2384 (89.4) efg	1.2530 (90.2) def
		Brown	1.4242 (97.9) bcd	1.3324 (94.4) bcd	1.2335 (89.1) efg	1.3077 (93.2) cd
	Pre-chilling	Green	1.4349 (98.2) bcd	1.3711 (96.1) abc	1.4396 (98.3) ab	1.3296 (94.3) bcd
		Brown	1.4521 (98.6) abcd	1.3270 (94.2) bcd	1.2552 (90.4) def	1.2735 (91.4) cde
Water	Non-chilling	Green	1.3920 (96.8) cde	1.2980 (92.7) cd	1.2239 (88.4) efg	1.1672 (84.6) efg
		Brown	1.3307 (94.4) def	1.1629 (84.3) e	1.1976 (86.7) fg	1.1421 (82.7) fg
	Pre-chilling	Green	1.2266 (88.6) f	1.2979 (92.7) cd	1.1933 (86.4) fg	1.0891 (78.5) g
		Brown	1.2864 (92.1) ef	1.2408 (89.5) de	1.1271 (81.6) g	1.0990 (79.4) g

SE $\bar{x}$ = 0.0518.
Means within each column which have a different letter are significnatly different by t-test at the 10% probability level.
Values in parentheses are the de-transformation values of the least square means (%).

4.3.3.3 Germination profiles

The MANOVA significance tests (Rao's F-approximation of Wilks'Λ (Cooley and Lohnes, 1971)) showed that all main effects on germination profiles were highly significant ($P<0.01$), except for chilling treatments (Table 4.3). Several meaningful interactions were also found (Table 4.3).

The mean absolute growth rate (R) measures the average growth of the absolute rate compared against level attained (L). The duration of germination represents the time required for the major portion of growth to occur. The two variables, which were calculated from the derived parameters, were applied to explain the differences amongst treatments (Table 4.9).

The onset of germination (Gt5) was defined in terms of the time required to reach 5% of upper asymptote of germination. The end of germination (Gt95) was defined in terms of the time required to reach 95% of upper asymptote of germination. The numbers of days to reach 5% and 95% upper asymptote were estimated and appear in Table 4.10.

As LSD in ANOVA, canonical (multiple) discriminant scores are used subsequently to describe the differences between treatments, which are presented within figures. In this study, only one discriminant solution was involved, except in imbibant x seed-colour in which first two discriminant solutions were used. The importance of the parameters to discriminant scores examined by the interpretation system (I.L. Gordon, pers. comm.) is presented in Table 4.11.

4.3.3.3.1 Main effects

The discriminant scores indicated that each of the germination profiles for the four imbibants (GA_{4+7}, GA_3, KNO_3 and water) were markedly different ($p<0.01$) (Table 4.3; Figure 4.2). The onset of germination (Gt5) and the end of germination (Gt95) in KNO_3 were the earliest among GA_{4+7}, GA_3 and water (Table 4.10). While the duration of

germination (D) was similar for four imbibants, the mean absolute germination rate (R) was higher in KNO_3 and GA_{4+7} than in GA_3 and water (Table 4.9). These (KNO_3 and GA_{4+7}) also obtained a high asymptote (A). Reasonably, the discriminant scores of MANOVA were strongly influenced by asymptote (A) compared with ß, k and ν (Table 4.11).

Temperature markedly influenced ($P<0.01$) the germination profiles (Figure 4.3). The onset of germination (Gt5) was faster at 15/10°C (5.2 days) than at 10/5°C (6.2 days), but the end of germination (Gt95) was earlier 0.5 days at 10/5°C than at 15/10°C (Table 4.10). Thus, 15/10°C prolonged the duration of germination (D) and decreased the mean absolute germination rate (R) (Table 4.9).

Light significantly ($P<0.01$) affected the germination profiles (Table 4.3; Figure 4.4). The onset (Gt5) and the end (Gt95) of germination were earlier in darkness than in light (Table 4.10). The mean absolute germination rate (R) was considerably higher in darkness than in light (Table 4.9). However, the duration of germination (D) was similar for the two light conditions (Table 4.9). Thus, darkness resulted in a high upper asymptote.

Response of germination to seed-colour was detected as well ($P<0.01$) (Table 4.3; Figure 4.6). The onset of germination (Gt5) varied little between green seeds (5.7 days) and brown seeds (5.9 days), whereas the end of germination was earlier in green seeds (9.9 days) than in brown seeds (10.8 days) (Table 4.10). The mean absolute germination rate (R) was higher in green seeds (Table 4.9); the duration of germination (D) in green seeds was 0.8 days shorter than in brown seeds. The upper asymptote of germination was considerably higher in green seeds than in brown seeds.

However, the main functions between pre-chilling and non-chilling were not significantly different (Table 4.3; Figure 4.5).

Table 4.9 Parameter combinations of Richards' function for seed germination.

Source	Item	R (% d $^{-1}$)	D (days)	Source	Item	R (% d $^{-1}$)	D (days)
Temperature	10/5 °C	23.90	3.8	Light	Dark	21.20	4.4
**	15/10 °C	16.82	5.4	**	Light	19.46	4.6
Imbibant	GA_3	21.56	4.4	Seed-colour	Green	22.26	4.1
**	GA_{4+7}	21.14	4.4	**	Brown	18.45	4.9
	KNO_3	20.09	4.5	Chilling	Non-chilling	21.33	4.3
	water	18.57	4.6	ns	Pre-chilling	19.36	4.7
Temp x Light	10/5 °C x Dark	25.44	3.7	Temp x Light	10/5 °C x Dk x G x GA_3	29.89	3.0
**	10/5 °C x Light	22.38	4.0	x Imbib x Col	10/5 °C x Dk x B x GA_3	25.36	3.8
	15/10 °C x Dark	17.16	5.4	**	10/5 °C x Dk x G x GA_{4+7}	27.83	3.4
	15/10 °C x Dark	16.48	5.4		10/5 °C x Dk x B x GA_{4+7}	25.88	3.6
Temp x Imbib	10/5 °C x GA_3	26.91	3.4		10/5 °C x Dk x G x KNO_3	21.84	4.5
*	10/5 °C x GA_{4+7}	24.50	3.8		10/5 °C x Dk x B x KNO_3	21.41	4.5
	10/5 °C x KNO_3	22.54	4.2		10/5 °C x Dk x G x water	25.11	3.6
	10/5 °C x water	21.60	4.0		10/5 °C x Dk x B x water	21.42	4.2
	15/10 °C x GA_3	16.93	5.3		10/5 °C x L x G x GA_3	28.28	3.2
	15/10 °C x GA_{4+7}	16.40	5.7		10/5 °C x L x B x GA_3	22.00	3.9
	15/10 °C x KNO_3	18.45	5.2		10/5 °C x L x G x GA_{4+7}	26.00	3.5
	15/10 °C x water	15.44	5.4		10/5 °C x L x B x GA_{4+7}	18.13	5.0
Temp x Col	10/5 °C x Green	25.91	3.6		10/5 °C x L x G x KNO_3	25.77	3.7
**	10/5 °C x Brown	21.88	4.2		10/5 °C x L x B x KNO_3	21.08	4.4
	15/10 °C x Green	18.55	4.9		10/5 °C x L x G x water	20.26	4.3
	15/10 °C x Brown	15.17	5.9		10/5 °C x L x B x water	17.61	4.6
Temp x Chill x Col	10/5 °C x N-ch x G	28.00	3.3		15/10 °C x Dk x G x GA_3	19.73	4.7
**	10/5 °C x N-ch x B	21.46	4.3		15/10 °C x Dk x B x GA_3	16.20	5.7
	10/5 °C x P-ch x G	23.73	3.9		15/10 °C x Dk x G x GA_{4+7}	20.47	4.6
	10/5 °C x P-ch x B	22.29	4.0		15/10 °C x Dk x B x GA_{4+7}	14.92	6.4
	15/10 °C x N-ch x G	18.66	4.9		15/10 °C x Dk x G x KNO_3	17.57	5.5
	15/10 °C x N-ch x B	16.98	5.3		15/10 °C x Dk x B x KNO_3	17.06	5.6
	15/10 °C x P-ch x G	18.44	5.0		15/10 °C x Dk x G x water	18.59	4.8
	15/10 °C x P-ch x B	13.49	6.6		15/10 °C x Dk x B x water	13.73	6.1
Light x Chill x Col	Dark x N-ch x G	24.23	3.9		15/10 °C x L x G x GA_3	17.24	5.2
*	Dark x N-ch x B	20.48	4.5		15/10 °C x L x B x GA_3	14.56	5.9
	Dark x P-ch x G	21.61	4.3		15/10 °C x L x G x GA_{4+7}	17.12	5.5
	Dark x P-ch x B	18.72	5.0		15/10 °C x L x B x GA_{4+7}	13.39	6.6
	Light x N-ch x G	22.67	3.9		15/10 °C x L x G x KNO_3	20.78	4.6
	Light x N-ch x B	17.91	4.9		15/10 °C x L x B x KNO_3	18.89	4.9
	Light x P-ch x G	20.49	4.5		15/10 °C x L x G x water	17.11	4.7
	Light x P-ch x B	16.75	5.1		15/10 °C x L x B x water	12.88	6.2

**: significance at the 1% probability level; *: significance at the 5% probability level.
Temp: temperature; L: light; Dk: dark; Ch: chilling; Col: seed colour; G: green; B: brown; N-ch: non-chilling; P-ch: pre-chilling.
R: mean absolute germination rate [Ak/2(v+2)]; D: duration of germination [2(v+2)/k].

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.

Source	Item	Gt5	Gt95	Source	Item	Gt5	Gt95
Temperature	10/5 °C	6.2	10.1	Light	Dark	5.3	9.7
**	15/10 °C	5.2	10.6	**	Light	6.7	11.3
Imbibant	GA_3	5.1	9.5	Seed-colour	Green	5.7	9.9
**	GA_{4+7}	6.1	10.5	**	Brown	5.9	10.8
	KNO_3	6.1	10.7	Chilling	Non-chilling	5.9	10.2
	water	6.3	10.9	ns	Pre-chilling	5.7	10.4
Temp x Light	10/5 °C x Dark	6.1	9.8	Temp x Light	10/5 °C x Dk x G x GA_3	5.4	8.4
**	10/5 °C x Light	6.5	10.5	x Imbib x Col	10/5 °C x Dk x B x GA_3	5.8	9.6
	15/10 °C x Dark	4.3	9.6	**	10/5 °C x Dk x G x GA_{4+7}	5.9	9.3
	15/10 °C x Dark	7.5	12.9		10/5 °C x Dk x B x GA_{4+7}	7.3	11.0
Temp x Imbib	10/5 °C x GA_3	6.0	9.4		10/5 °C x Dk x G x KNO_3	5.1	9.5
*	10/5 °C x GA_{4+7}	6.1	9.9		10/5 °C x Dk x B x KNO_3	6.8	11.3
	10/5 °C x KNO_3	6.2	10.5		10/5 °C x Dk x G x water	7.3	11.0
	10/5 °C x water	6.8	10.9		10/5 °C x Dk x B x water	7.2	11.5
	15/10 °C x GA_3	7.3	12.7		10/5 °C x L x G x GA_3	6.8	10.1
	15/10 °C x GA_{4+7}	6.5	12.2		10/5 °C x L x B x GA_3	7.3	11.3
	15/10 °C x KNO_3	3.9	9.0		10/5 °C x L x G x GA_{4+7}	5.6	9.0
	15/10 °C x water	5.3	10.7		10/5 °C x L x B x GA_{4+7}	6.8	11.8
Temp x Col	10/5 °C x Green	6.0	9.6		10/5 °C x L x G x KNO_3	6.2	9.8
**	10/5 °C x Brown	6.6	10.7		10/5 °C x L x B x KNO_3	8.1	12.5
	15/10 °C x Green	5.3	10.3		10/5 °C x L x G x water	8.5	12.9
	15/10 °C x Brown	5.1	11.0		10/5 °C x L x B x water	5.9	10.4
Temp x Chill x Col	10/5 °C x N-ch x G	5.6	8.9		15/10 °C x Dk x G x GA_3	4.8	9.6
**	10/5 °C x N-ch x B	6.8	11.1		15/10 °C x Dk x B x GA_3	2.9	8.6
	10/5 °C x P-ch x G	6.5	10.5		15/10 °C x Dk x G x GA_{4+7}	2.7	7.4
	10/5 °C x P-ch x B	6.3	10.4		15/10 °C x Dk x B x GA_{4+7}	4.5	10.9
	15/10 °C x N-ch x G	5.9	10.8		15/10 °C x Dk x G x KNO_3	3.4	8.8
	15/10 °C x N-ch x B	5.9	11.2		15/10 °C x Dk x B x KNO_3	2.9	8.4
	15/10 °C x P-ch x G	4.9	9.9		15/10 °C x Dk x G x water	2.7	7.6
	15/10 °C x P-ch x B	4.5	11.0		15/10 °C x Dk x B x water	3.2	9.2
Light x Chill x Col	Dark x N-ch x G	5.5	9.4		15/10 °C x L x G x GA_3	3.0	8.3
*	Dark x N-ch x B	5.8	10.3		15/10 °C x L x B x GA_3	2.8	8.8
	Dark x P-ch x G	5.2	9.6		15/10 °C x L x G x GA_{4+7}	6.7	12.2
	Dark x P-ch x B	4.9	9.8		15/10 °C x L x B x GA_{4+7}	2.8	9.5
	Light x N-ch x G	5.7	9.7		15/10 °C x L x G x KNO_3	4.1	8.7
	Light x N-ch x B	7.6	12.6		15/10 °C x L x B x KNO_3	3.1	8.1
	Light x P-ch x G	6.9	11.4		15/10 °C x L x G x water	2.9	7.7
	Light x P-ch x B	7.7	12.9		15/10 °C x L x B x water	2.5	8.8

Temp: temperature; L: light; Dk: dark; Ch: chilling; Col: seed colour; G: green; B: brown; N-ch: non-chilling; P-ch: pre-chilling.
Gt_5: time to reach 5% of upper asymptote of germination (A) (days).
Gt_{95}: time to reach 95% of upper asymptote of germination (A) (days).

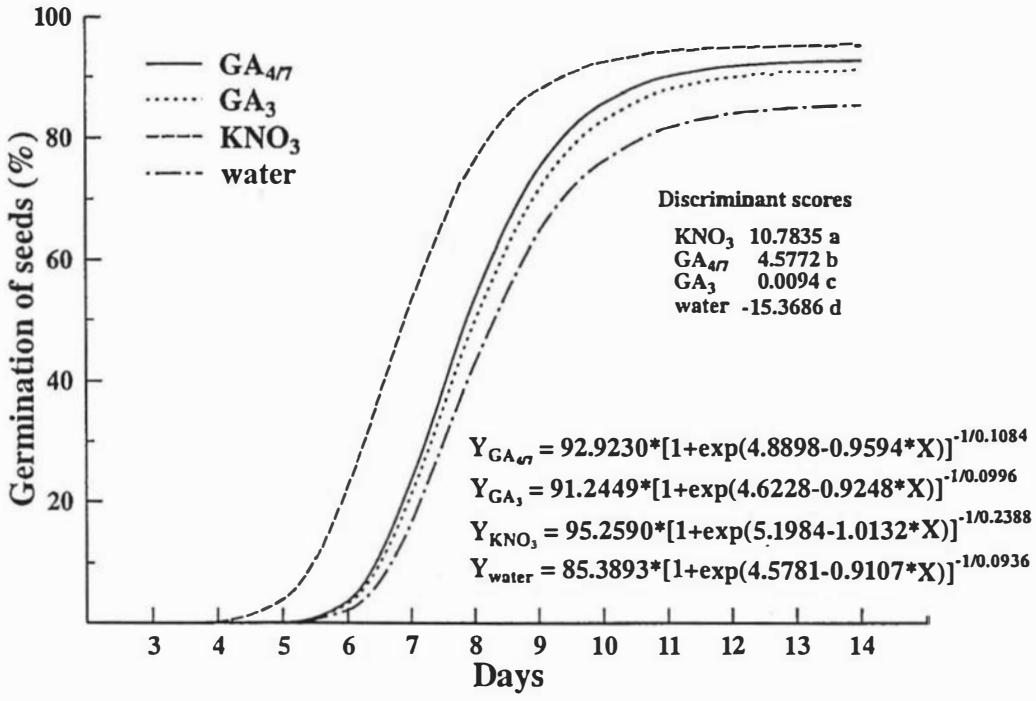


Figure 4.2 The mean Richards functions of seed germination for four imbibants.

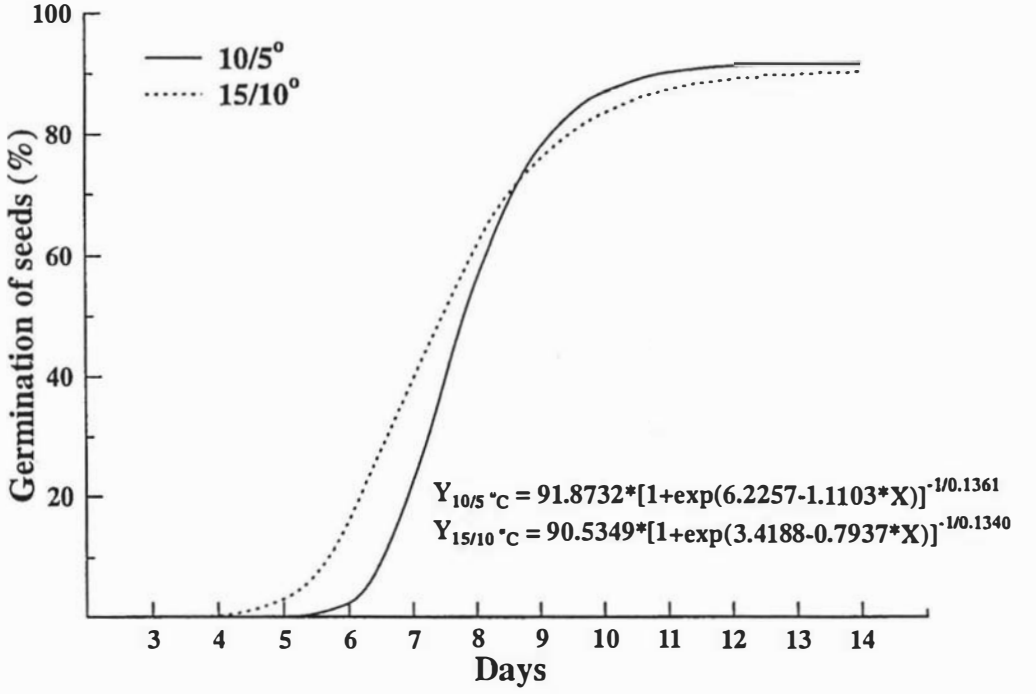


Figure 4.3 The mean Richards functions of seed germination for two temperature treatments.

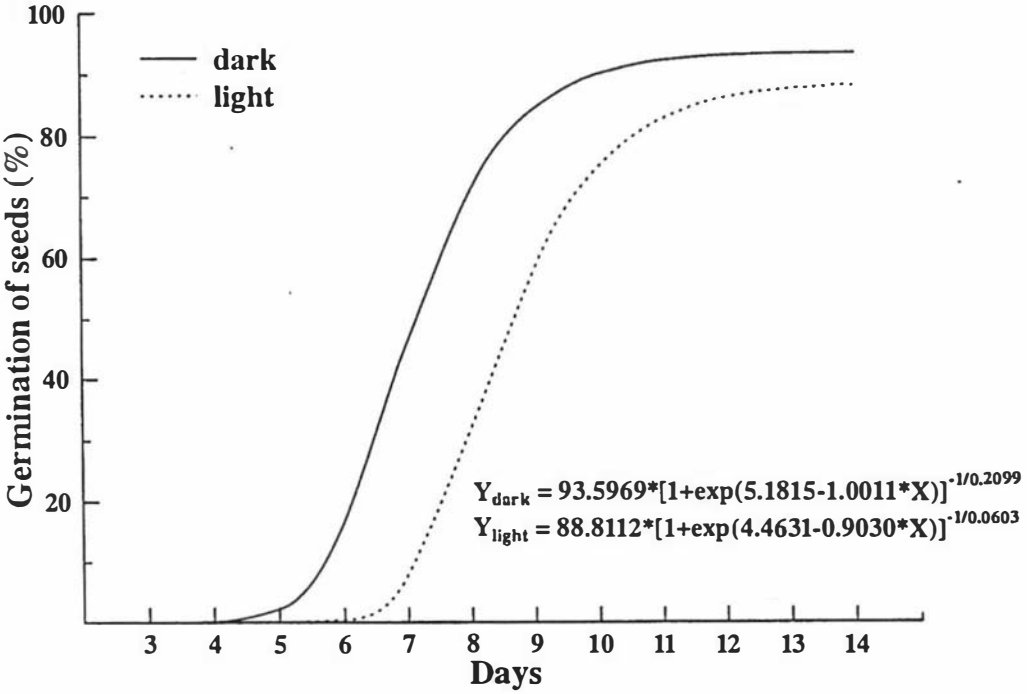


Figure 4.4 The mean Richards functions of seed germination for darkness and light conditions.

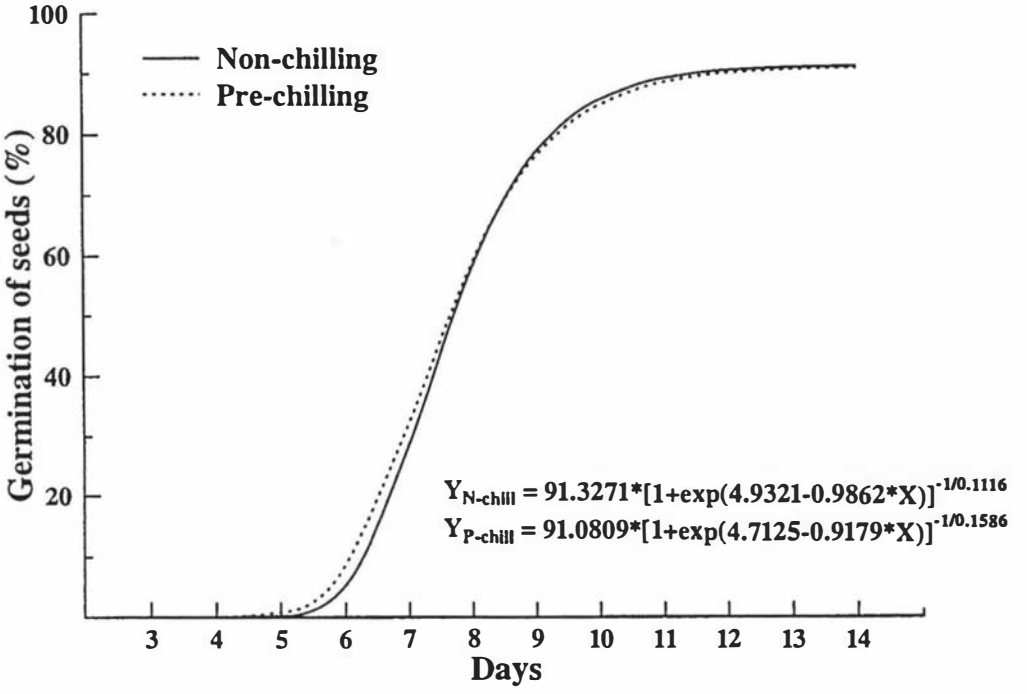


Figure 4.5 The mean Richards functions of seed germination for two chilling treatments.

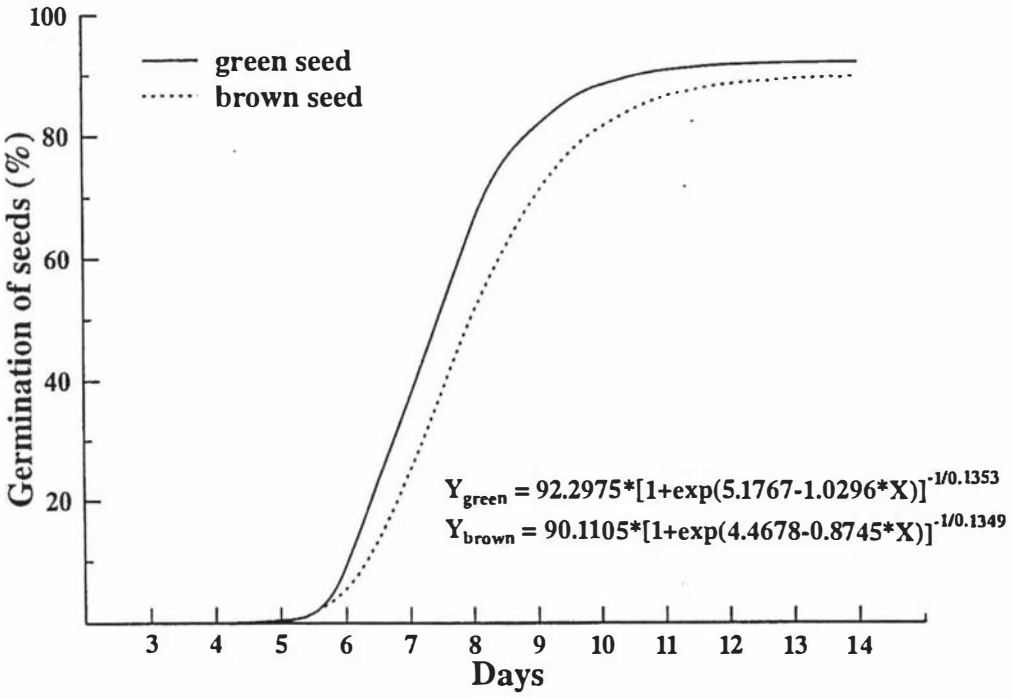


Figure 4.6 The mean Richards functions of seed germination for green and brown seed.

4.3.3.3.2 First-order interaction

A significant interaction ($P<0.01$) between temperature and light was found for germination profiles (Table 4.3; Figure 4.7). The statistics, ß and k, strongly influenced the discriminant scores (Table 4.11). At 15/10°C, the onset of germination (Gt5) and the end of germination were earlier in darkness than in light, whereas they were varied little at 10/5°C (Table 4.10). As noted earlier, 10/5 °C in darkness resulted in a high mean absolute germination rate (R) and short duration of germination (D) (Table 4.9). Light had a greater slowing effect in the cooler temperature regime.

A similar differential effect was found for temperature and seed-colour ($P<0.01$) (Table 4.3; Figure 4.8). Parameters, again ß and k, strongly influenced the discriminant scores (Table 4.11). The onset of germination (Gt5) was faster in green seeds (6.0 days) than in brown seeds (6.6 days) at 10/5°C (Table 4.10); but not at 15/5°C (around 5.1 - 5.3 days). The end of germination (Gt95) was earlier in green seeds than in brown seeds, regardless of temperature (Table 4.10). Brown seeds slowed down more in the cooler germination than the warmer, as shown by both R and D (Table 4.9).

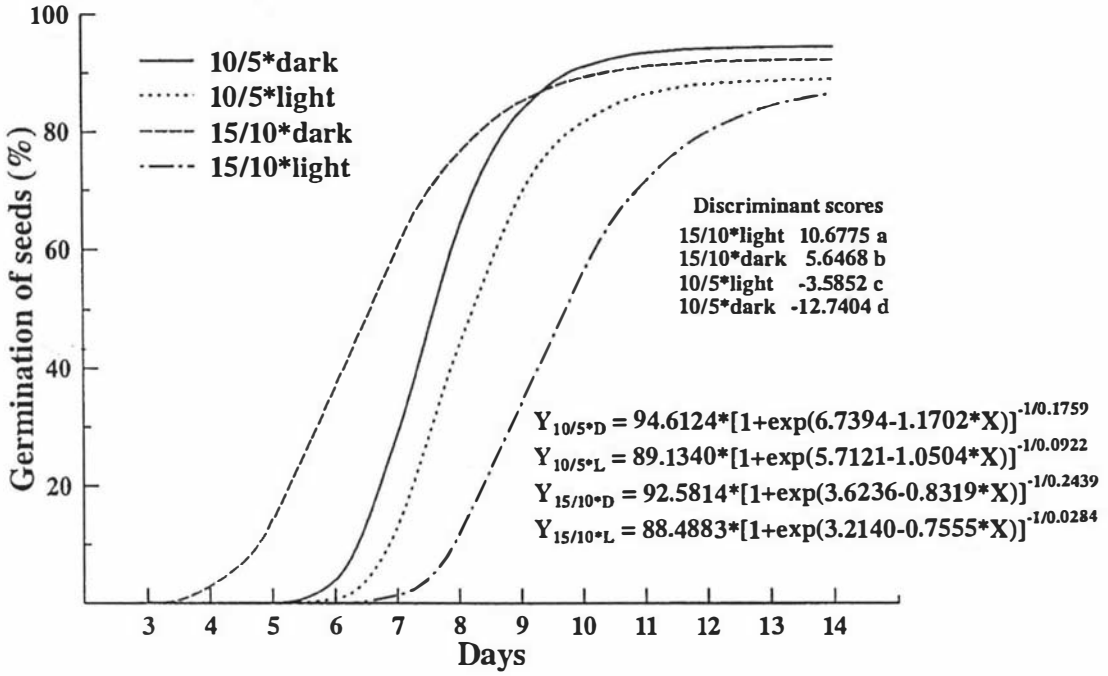


Figure 4.7 The mean Richards functions of seed germination for interaction between temperature and light.

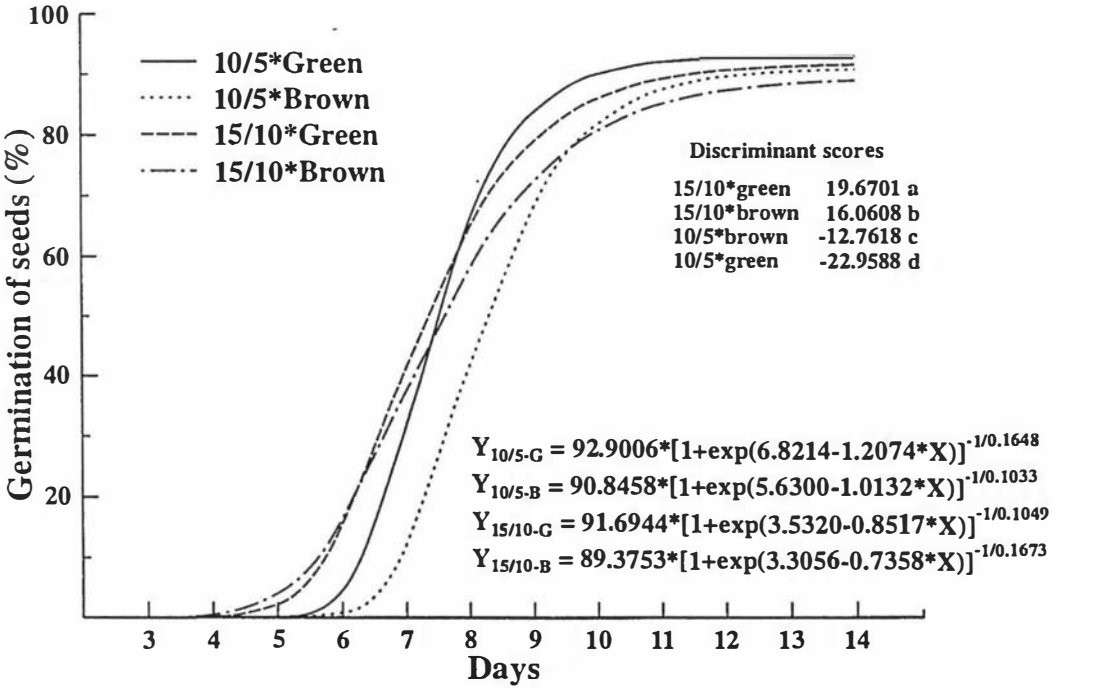


Figure 4.8 The mean Richards functions of seed germination for interaction between temperature and seed colour.

Another interaction ($P<0.05$) affecting germination profiles involved combinations of temperature and imbibant (Table 4.3; Figure 4.9). The discriminant scores were strongly influenced by parameter k, and weakly by A (Table 4.11). The germination profiles in KNO_3 for both temperatures were the same (Figure 4.9), because the upper asymptote (A) and k in both functions were similar, although the onset and the end of germination, the mean absolute germination rate (R) and the duration of germination (D) varied (Table 4.9; Table 4.10). These results, therefore, indicated that the discriminant power of discriminant scores operated well, summarizing several effects simultaneously. In GA_{4+7}, GA_3 and water, the cooler regime (10/5°C) resulted in higher mean absolute germination rate (R) and short germination duration (D) (Table 4.9). The onset of germination and the end of germination for GA_{4+7} and GA_3 were earlier at 10/5°C than at 15/10°C (Table 4.10). In water, the onset of germination was faster at 15/10°C, and the end of germination varied little. At 10/5°C, germination profile of $GA_{4/7}$, GA_3 and KNO_3 formed an overlapped series, and were all different to the profile of water (Figure 4.9). At 15/10°C, however, the germination profiles of the four imbibants fell into three groups, with the two gibberellins being together in one of these groups.

There was a weak interaction between light and imbibant ($P<0.10$) for germination profiles (Table 4.3). Parameters, A and ß, strongly affected on the discriminant scores; and the importance of parameter v was enhanced in discriminant scores (Table 4.11). The germination profiles between darkness and light obviously differed in GA_{4+7}, GA_3 and water, but not in KNO_3 (Figure 4.10). In darkness, the germination profiles for KNO_3, GA_{4+7} and GA_3 were similar, in which the upper asymptote of germination (A) varied little (Table 4.9 and Table 4.10). In light, however, the germination profiles for four imbibants differed much, which reflected in the onset of germination (Gt5) (Table 4.10) and the upper asymptote of germination (Figure 4.10).

A weak interaction ($P<0.10$) between imbibant and seed-colour (Table 4.3; Figure 4.11) was detected as well. Two discriminant solutions were used here, because first discriminant power (λ) was 0.6191, less than 0.70 (Table 4.12). Parameter ß greatly influenced on the discriminant score I, but K did weakly (Table 4.11). Thus, the discriminant score I was mainly associated with the rate of germination. The discriminant

score II mainly related to the shape of the germination curve, which was strongly affected by parameters A and k, with a moderate influence of ß; and the importance of parameter of v was enhanced. However, Hotelling T^2 test made two discriminant scores jointly describe the difference of germination profiles (Appendix 4.4). The germination profiles between green and brown seeds in KNO_3 were similar (Figure 4.12). The onset of germination (Gt5) and the end of germination (Gt95) were earlier in green seeds than in brown seeds for GA_{4+7} and GA_3 (Table 4.10); the results were opposite in water. In green seeds, the germination profiles of KNO_3, GA_3 and GA_{4+7} formed an overlapping series, and were all different to the profile of water. The onset germination was earliest in KNO_3 (4.7 days) and latest in water (7.9 days) (Table 4.10). The asymptote was KNO_3 > GA_3 > water (Figure 4.11), whereas the mean absolute germination rate (R) was highest in GA_3 (24.42). However, the duration of germination in GA_3 was too short, only 3.8 days (Table 4.9). In brown seeds, the germination profiles of the four imbibants fell into three groups, with KNO_3 and GA_3 being one of these groups (Figure 4.12). KNO_3 and GA_3 had high mean absolute germination rate (R) and short duration of germination (Table 4.9). However, the asymptote in GA_{4+7} was as high as that in KNO_3 and GA_3 (Figure 4.11), as GA_{4+7} had relative long duration of germination (5.1 days) and relative high absolute germination rate (17.99) (Table 4.9).

Table 4.11 The importance of parameters of Richards' function contributed to discriminant scores of MANOVA.

Items		A	ß	k	v
Imbibant		Strong	Null	Null	Null
Temp*Light		Pseudo	Strong	Strong	Pseudo
Temp*Imbibant		Weak	Pseudo	Strong	Pseudo
Light*Imbibant		Strong	Strong	Pseudo	Enhanced
Temp*Colour		Pseudo	Strong	Strong	Pseudo
Imbibant*Colour	Solution I	Null	Strong	Weak	Null
	Solution II	Strong	Medium	Strong	Enhanced
Temp*Chill*Colour		Pseudo	Strong	Strong	Pseudo
Light*Chill*Colour		Strong	Strong	Strong	Pseudo
Temp*Light*Imbibant*Colour		Pseudo	Strong	Medium	Null

Temp: temperature; Colour: seed-colour.

Table 4.12 Two discriminant scores for combination of imbibant and seed-colour.

Combination	Discriminant power	Discriminant solution I 0.6191	II 0.3537
GA_3 x green seed		-6.4980	0.3608
GA_3 x brown seed		0.4684	-0.1034
GA_{4+7} x green seed		-2.5651	-1.4811
GA_{4+7} x brown seed		5.6278	-2.7551
KNO_3 x green seed		0.9225	-3.0529
KNO_3 x brown seed		-2.1494	-3.5846
water x green seed		0.6564	3.1926
water x brown seed		3.5495	7.4281

Discriminant solution I (DSI): Rate of germination
Discriminant solution II (DSII): shape of germination curve

4.3.3.3.3 Second-order interaction

The second order interaction involving temperature, chilling and seed colour was strongly significant ($P<0.01$) (Table 4.3; Figure 4.13). Parameters, ß and k, highly determinative on the discriminant scores. The onset of germination (Gt5) was earlier at 15/10°C than at 10/5°C, but not in non-chilled green seeds (Table 4.10). Differences between chilling treatments were only found in green seeds at 10/5°C and in brown seeds at 15/10°C. The germination profiles differed between seed-colour at 10/5°C, regardless of chilling treatments. Similar results were found at 15/10°C in pre-chilling. Non-chilled green seeds germinating at 10/5°C resulted in an early end to germination (Table 4.10), high mean absolute germination rate (R) and short duration of germination (D) (Table 4.9).

There was a clear interaction ($P<0.05$) among light, chilling and seed-colour for germination profile (Table 4.3). Parameters, A, ß and k, influenced strongly on the discriminant scores. The effects of chilling on germination profiles were only found in light for both seed colours (Figure 4.14). Green seeds in non-chilling in darkness gave an early end of germination (Gt95) (Table 4.10), high mean absolute germination rate (R), short duration of germination (D) (Table 4.9), and had a high upper asymptote of germination (Figure 4.14) as well.

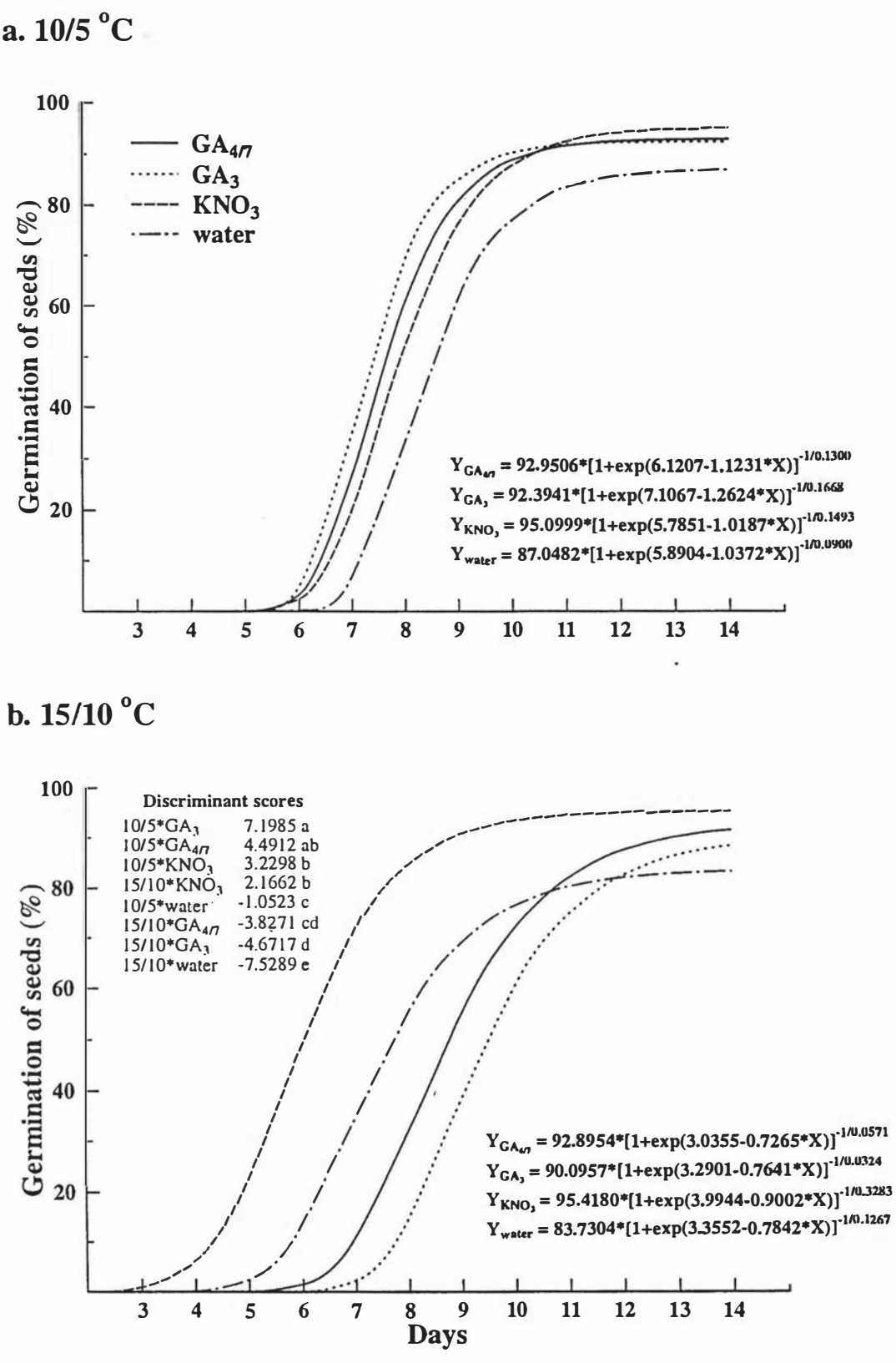
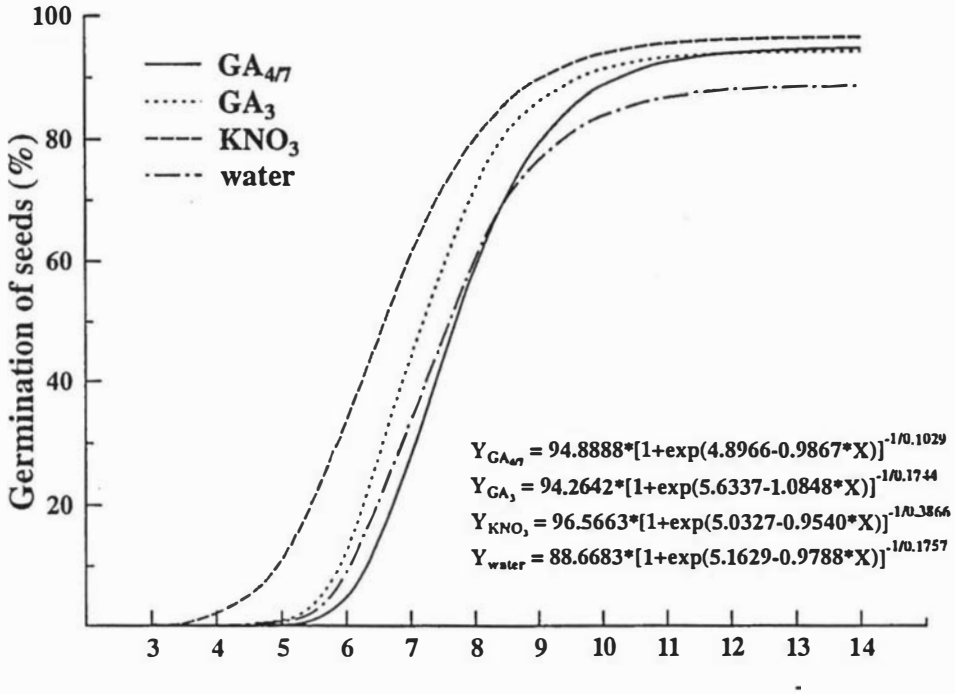


Figure 4.9 The mean Richards functions of seed germination for interaction between temperature and imbibant.

a. Darkness



b. Light

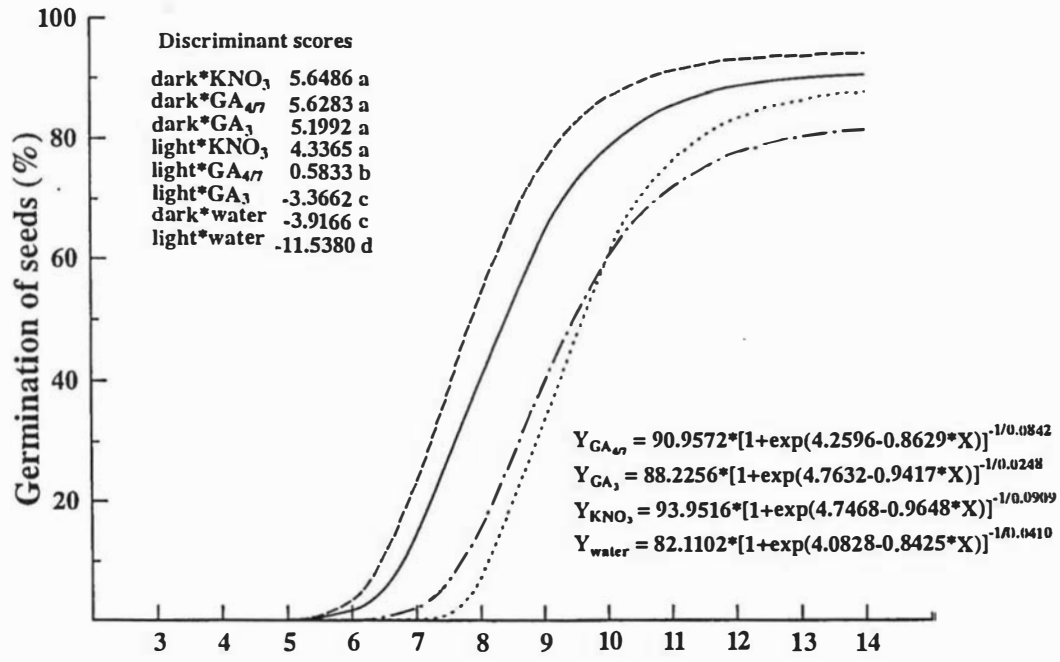
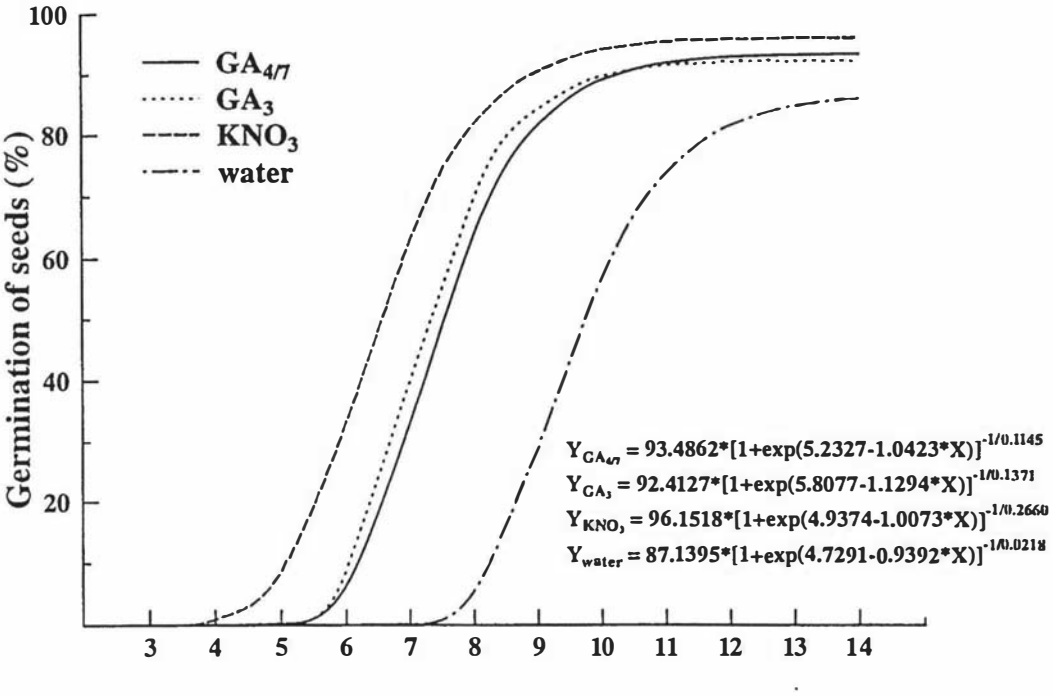


Figure 4.10 The mean Richards functions of seed germination for interaction between darkness and imbibant.

a. Green seeds



b. Brown seeds

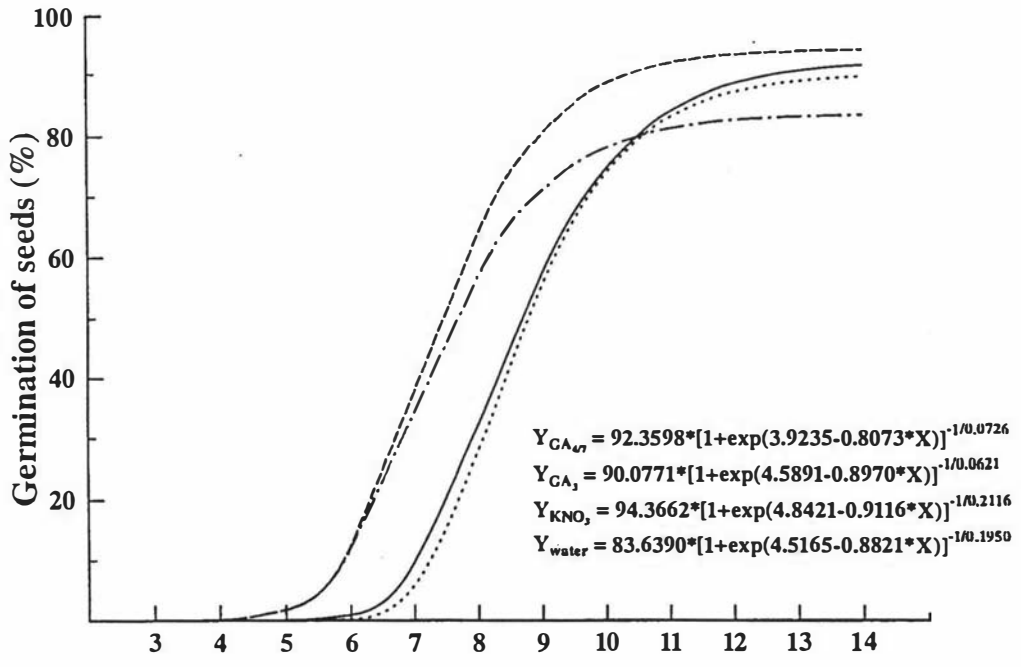


Figure 4.11 The mean Richards functions of seed germination for interaction between imbibant and seed colour.

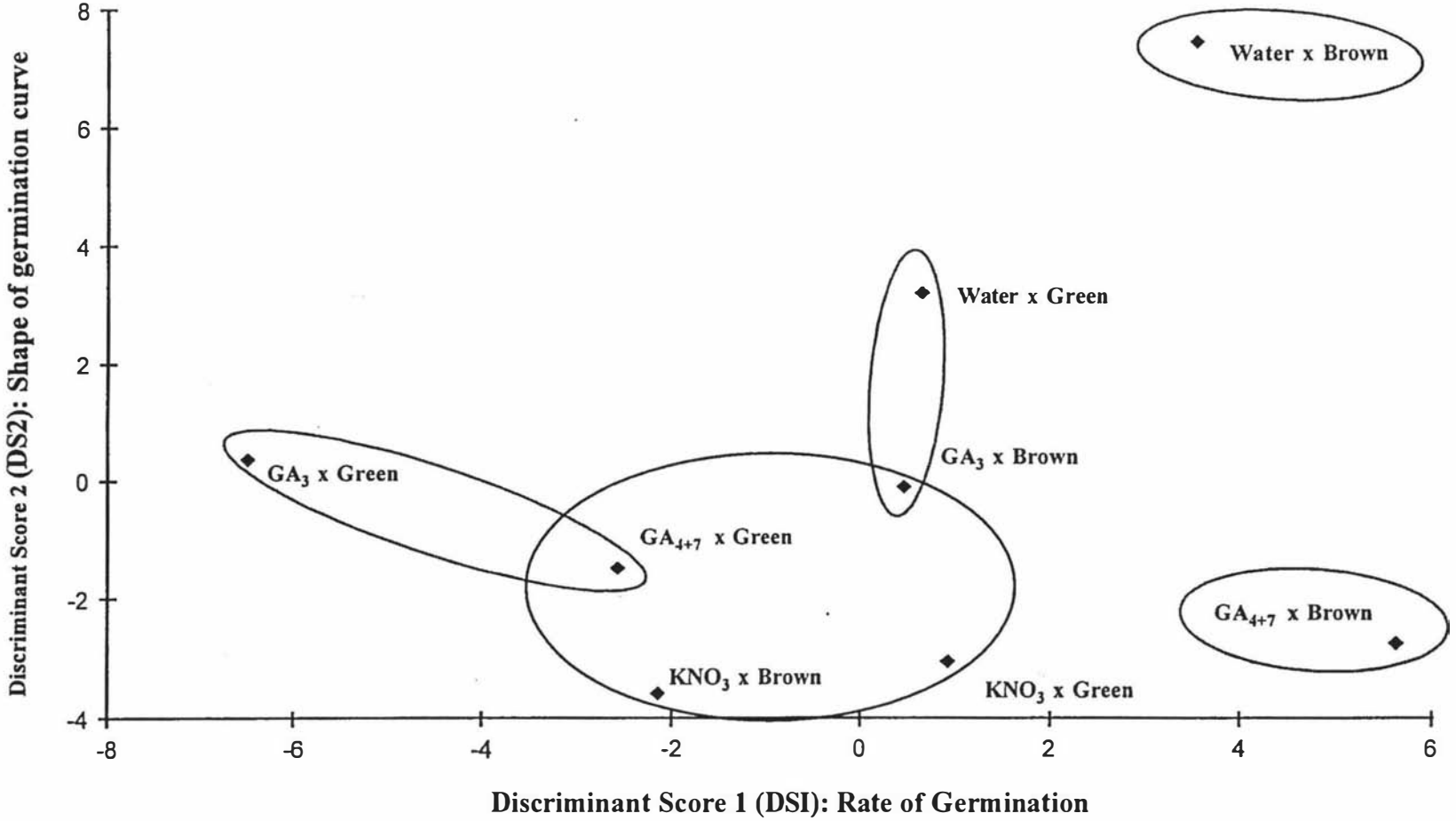
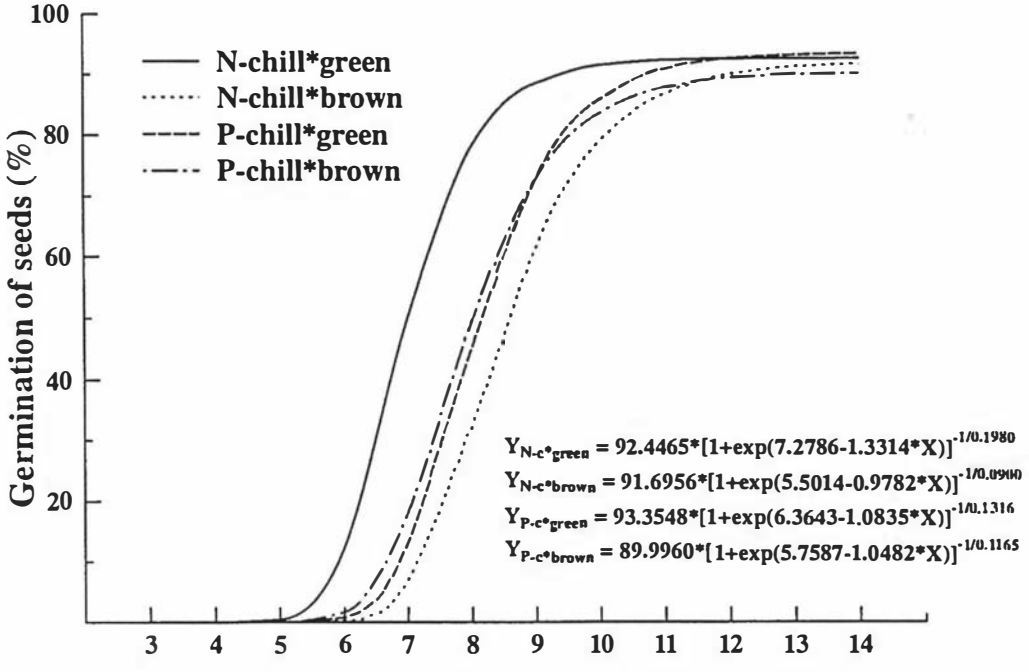


Figure 4.12 The results of Hotelling T^2 for imbibant x seed-colour in discriminant plane (No significant differences within a circle).

a. 10/5 °C



b. 15/10 °C

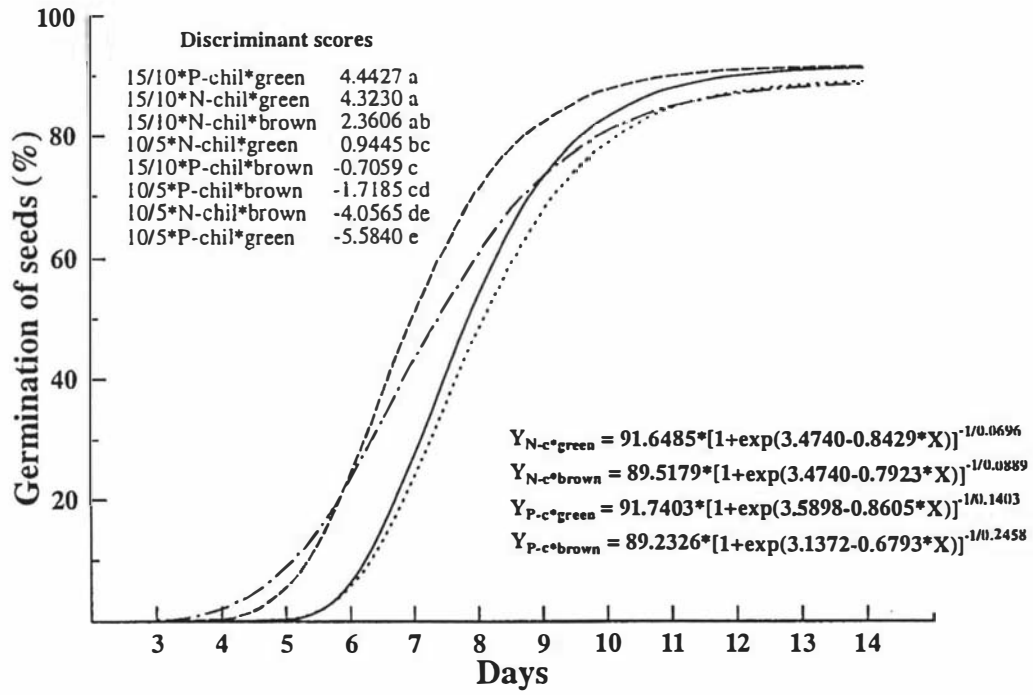
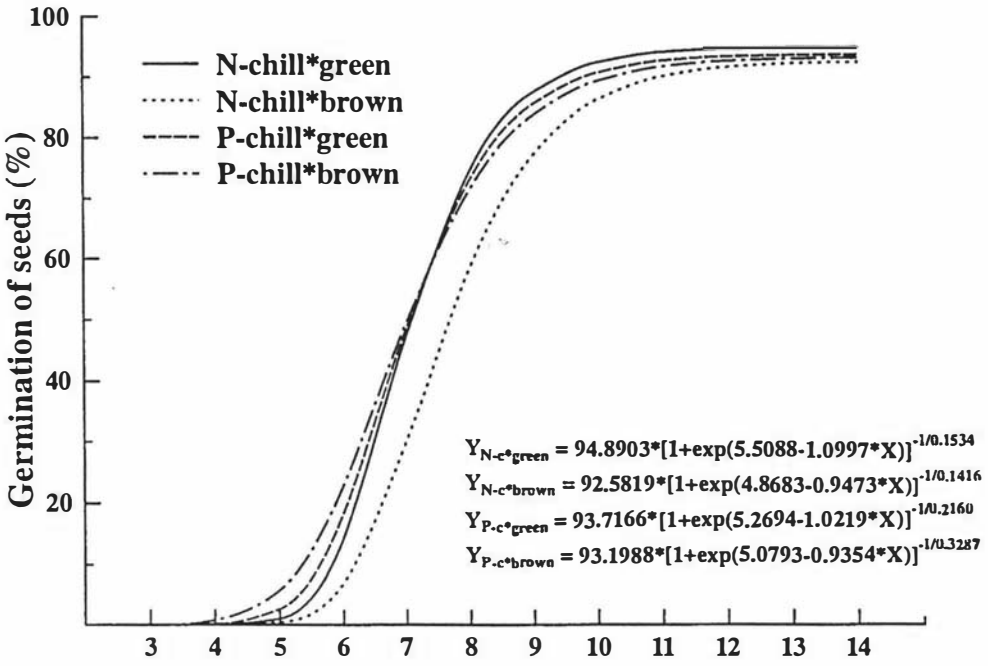


Figure 4.13 The mean Richards functions of seed germination for interaction between temperature, chilling and seed colour.

4.3.3.3.4 Third-order interaction

There was also a significant ($P<0.01$) third-order interaction amongst temperature, light, imbibant and seed-colour (Table 4.3). Parameter ß strongly influenced the discriminant scores, with a moderate influence of k (Table 4.11). Difference between seed colours for germination profiles was seen at 10/5°C, except for GA_3 in light and for water in both light and darkness (Figure 4.15a and 4.15b); at 15/10°C, it was only detected for water in darkness and for GA_{4+7} and KNO_3 in light. The onset of germination was earlier in green seeds than in brown seeds (Table 4.10). GA_{4+7} and KNO_3 provided high mean absolute germination rate (R), short durations of germination (D) in darkness at 10/5°C, regardless of seed colour (Table 4.9), which resulted in a high upper asymptote of germination (A). A similar result was found in GA_3, but only for brown seed. With green seed, GA_3 in darkness at 10/5°C gave the highest mean absolute germination rate (29.89) amongst other combinations, but it had relative short duration (3.0 days). Thus, it resulted in a low asymptote. However, water gave low mean absolute germination rate (R) and low asymptote (A), regardless of temperature, darkness and seed colour.

a. Darkness



b. Light

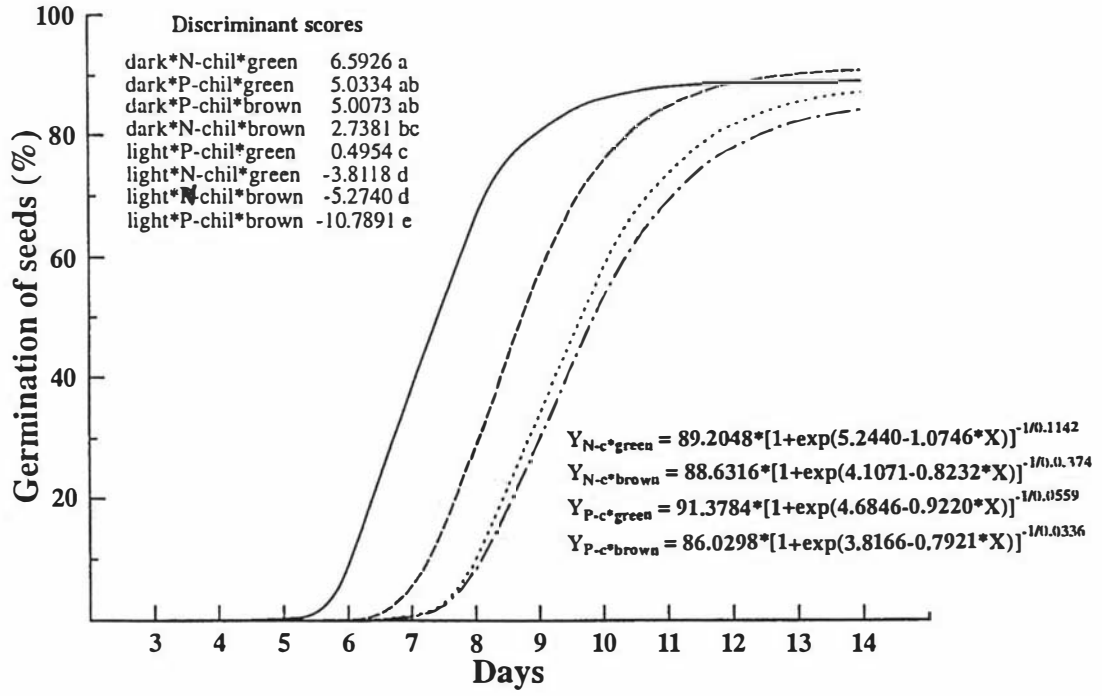


Figure 4.14 The mean Richards functions of seed germination for interaction between darkness, chilling and seed colour.

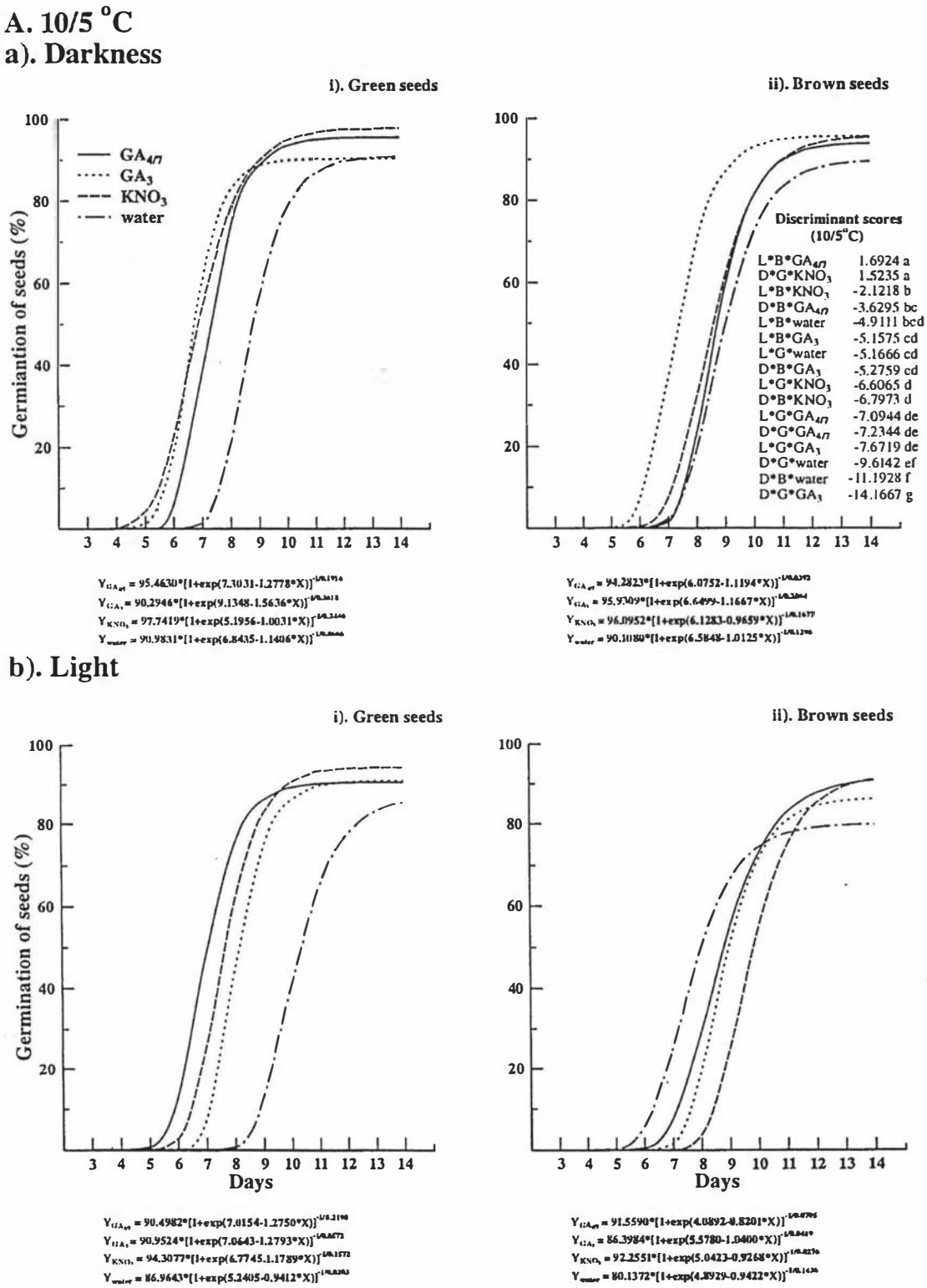


Figure 4.15a The mean Richards functions of seed germination for interaction between temperature, light, imbibant and seed colour.

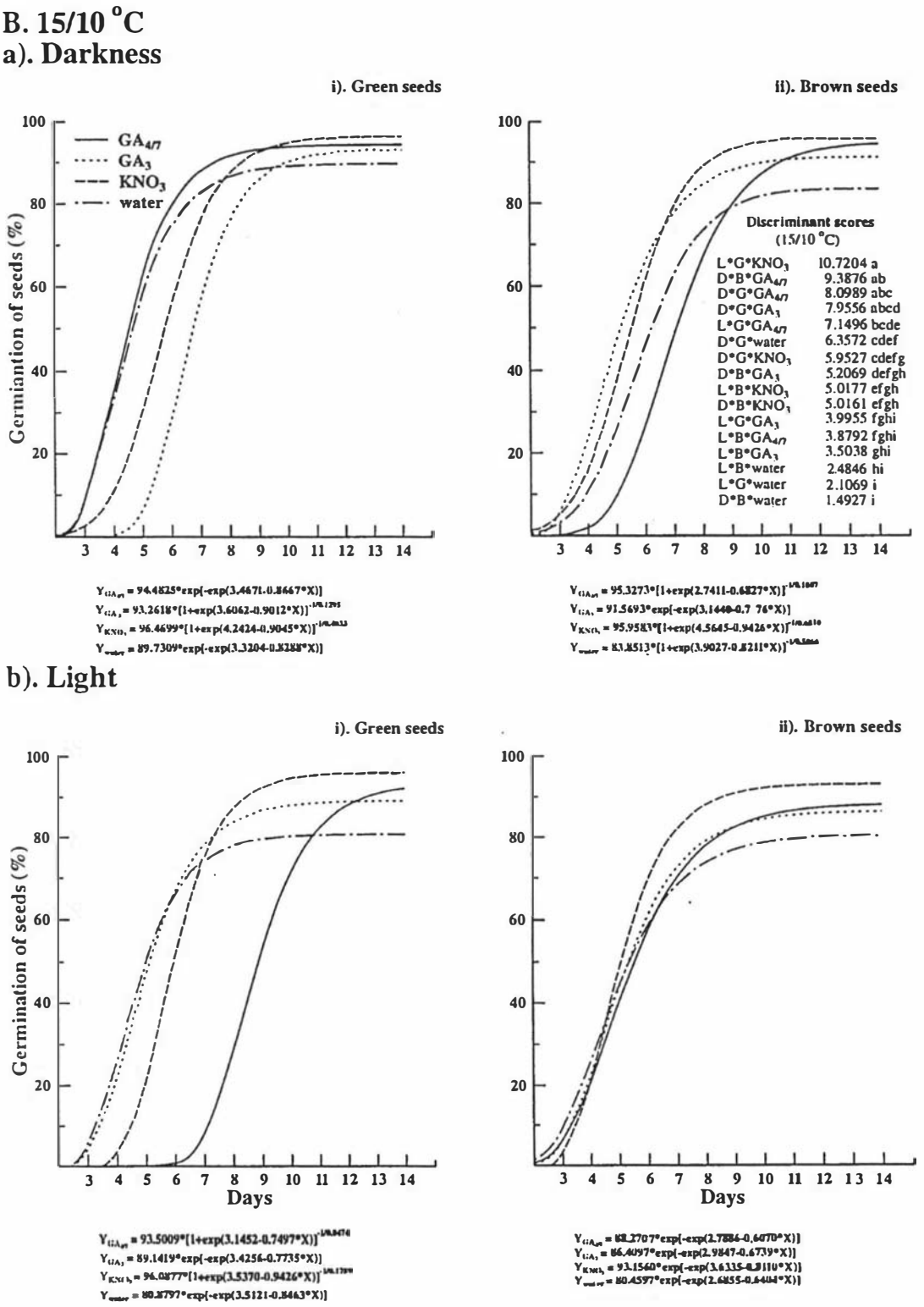


Figure 4.15b The mean Richards functions of seed germination for interaction between temperature, light, imbibant and seed colour.

4.4 Discussion

4.4.1 Optimum dormancy-breaking germination

In this study, GA_{4+7} and KNO_3 in darkness at 10/5°C resulted in high final germination (21 days after seed imbibition), regardless of pre-chilling. Water gave the lowest final germination (Table 4.5; Table 4.8). The final germination in GA_3 was intermediate. Mmolawa (1987) has suggested that KNO_3 was effective in alleviating dormancy in meadowfoam seeds. Hilhorst (1990a) has suggested that nitrate is effective because of direct binding to the phytochrome receptor protein in *Sisymbrium officinale*. Derkx *et al.*, (1994) demonstrated that the commercial GA_{4+7} mixture contained 54% GA_4 and 46% GA_7-isolactone. They found that GA_4 and GA_7-isolactone had the highest activity in *A. thaliana* seeds, and the activity of GA_4 was 10 times more than that of GA_3. The results in this study did not show such a large ratio between the two gibberellin solutions, but they did reveal the superiority of the GA_{4+7} for meadowfoam. Differences in affinity of GA receptors for various GAs are illustrated by parallel positions of GA dose response curves along the X-axis (Firn, 1986; Wyers *et al.*, 1987).

Darkness stimulated high germination, whereas light inhibited germination (Table 4.7; Table 4.8). The mean absolute germination rate (R) was considerably higher in darkness (Table 4.9). The results agreed with those of Toy and Willingham (1966) and Cole (1974) that meadowfoam seeds do not require light for germination and germination may even be inhibited by exposure to light.

The "warm" regime (15/10°C) increased heterogeneity of individual germination times, as reflected by a decreased mean absolute germination rate (R) and an increased duration of germination (Table 4.9), while temperature effects on final germination level were not observed in this study (Table 4.6). Other accessions of *Limnanthes* showed optimum germination at temperatures of about 5-10°C, but dropped sharply at temperatures above 15°C (Toy and Willingham, 1966; Cole, 1974). Germination was near 0 when the temperature approached 20°C. Similarly, seed germination for cultivar Mermaid was maximum at 10°C and decreased sharply at temperatures greater than 15°C (Mmolawa,

1987; Nyunt, 1987). Temperature in this study avoided these problems of high temperature.

The choice of the alternating temperature regime in the present study was based on which regime more closely resembles field conditions. Soil temperature measurements indicate that temperature fluctuations are great on or close to the surface of bare soil and in breaks in the vegetation canopy (Vàzquez-Yanes and Orozco-Segovia, 1982; Van Assche and Vanlerberghe, 1989). The air temperature during June (sowing season) in Palmerston North, New Zealand, is around 5-15 °C (Anonymous, 1983), and thus seed germination of meadowfoam may be under optimum temperature.

Early work showed that pre-chilling resulted in 60% germination of Mermaid at constant 20°C, whereas germination was reduced to near zero without pre-chilling at that temperature (Mmolawa, 1987). This indicated that pre-chilling may be effective in promoting germination at higher temperatures. This work has shown no advantage from pre-chilling at the (lower) temperatures used (Table 4.6). However, green seed after pre-chilling in KNO_3 were less sensitive to light irrespective of temperatures (Table 4.8). A similar result was found in GA_{4+7} but only at 15/10°C. GA-deficient seeds only germinated after application of the commercially available GA_{4+7} mixture, both in darkness and in light (Derkx and Karssen, 1993c; Karssen *et al.*, 1989). The percentage of germination in Florence fennel seeds in GA_{4+7} was high in the light at temperature from 20 to 30°C, although it was better in the dark than in the light, the high temperature cut-off points being between 27.2 and 29.4°C (Thomas, 1994). A short chilling treatment sometimes enhances the light effects on both GA sensitivity and GA biosynthesis, but it can also directly increase GA sensitivity, without the interference of light (Derkx and Karssen, 1993a; Karssen *et al.*, 1989;). Although elevated levels of the active GA_4 were found in darkness after chilling, light did not further increase this level, whereas light was an absolute requirement for germination (Derkx and Karssen, 1994). Thus, the role of light in stimulating germination of chilled seeds is still rather obscure.

From this study, it is recommended that optimum dormancy-breaking germination for meadowfoam seed can be achieved by using KNO_3 and GA_{4+7} imbibant in darkness at

10/5°C, without pre-chilling. A "standard" germination test could follow the usual pattern, using water as imbibant, and with constant temperature of 10°C, in the dark with no pre-chilling. The differences between the special germination (for dormancy-breaking) and the water imbibant in this work (Table 4.8) indicated that some primary dormancy remained in these seeds. Jolliff *et al.*, 1994, also reported primary dormancy in meadowfoam.

4.4.2 The effect of seed-colour on germination

The results showed that germination was higher in green seeds than in brown seeds (Table 4.7; Table 4.8). Brown seeds slowed down more in the cooler germination than the warmer (Table 4.8). This may indicate that late coat-imposed dormancy was incipient. Bewley and Black (1994) have suggested that seed coat properties depend on the rate and degree of drying, and on the oxidizing of phenolic substances to dark-coloured compounds. These changes may affect germinability. Moreover, these results showed that seeds could be harvested before they are "brown" ripe, with the possibility of reducing shattering losses. Loss of harvested yield through excessive seed shatter has been noted (Jolliff *et al.*, 1981; Higgins *et al.*, 1971). Seed losses due to shattering ranged from 20 to 37% at maturity or 1 week after maturity harvest (Johnson *et al.*, 1978). However, meadowfoam is valuable as a potential new crop because its seed oil is a source of renewable industrial raw materials and energy. The yield of seed oil, therefore, is anther important factor. The relation between green seeds and seed oil yield should be explored in the future research, although meadowfoam seed could be harvested a week before full maturity and shattering stage without any loss of oil quality (Jain and Abuelgasim, 1981).

4.4.3 Biological applications of the quantitative analysis of seed germination

The germination profiles of meadowfoam seeds clearly was sigmoidal, which was not unexpected (Bewley and Black, 1994). In this study, nonlinear functions (Richards or Gompertz) provided convenient and credible descriptions of the progress of seed germination. (If the Gompertz is regard as a special case of Richards function, the term "Richards function" can be used as a generalization.) The Richards function has also

proved superior to other curve-fitting approaches considered for seed germination data of cress (Lehle and Putnam, 1982). The results of this experiment (Figure 4.1; Table 4.2) also showed that the Richards function represented the primary data reliably. Thus, the Richards function is a suitable and convenient empirical function to summarize seed germination and to describe the influence of environmental factors on the seed germination. The original data, disturbed by irregular errors, are replaced by a smoother continuous function (Richards, 1969).

Moreover, the multivariate analysis of variance (MANOVA), which provided a valid simultaneous analysis of all four (correlated) statistics which define any Richards function, made it possible to test significant differences amongst the functions (germination profiles) themselves (Table 4.3). Therefore, this study brought together the biological and the mathematical (mainly statistical) aspects of seed germination analysis.

Subsequently, the functions allowed estimation of other aspects, such as germination percentages at key times, germination rates and various timings. Results may be presented as "mean functions" following MANOVA, as has been done here. A similar result has been reported by Schimpf *et al.* (1977), who, however, used only the logistic function to fit the germination curve. (Only two of our Richards functions approached logistic.) Most of the germination profiles were Gompertz functions (141/192 functions); and a further 39 functions were between Gompertz and logistic ($0 < \nu < 1$) ("unspecified Richards functions"). This result agreed with Nichols and Heydecker (1968), who noted that most germination curves were positively skewed, a fact which the (symmetrical) logistic cannot accommodate. Compared with the univariate final germination level (ANOVA) (Appendix 4.3), multiple analysis of variance (MANOVA) was much more sensitive in discriminating amongst germination profiles. Whether this degree of sensitivity will always be useful biologically was a point of caution: it may sometimes be over-sensitive with respect to *a priori* knowledge from physiology. However, in this case, it has revealed much information even in the face of unknown effects of after-ripening.

The Richards function fitting yields estimates of A, ß, k and ν, of which only A and ν appear to provide clear biologically information. Parameter A gives the asymptotic

maximum germination of seeds, and v described the type (or shape) of the germination curve. The point of inflexion is a function of v (in fact $L_I/A= (v+1)^{-1/v}$, Causton and Venus, 1981). The parameter v has a profound effect on the Richards function, as it changes the X-axis placement and slope as well as inflexion. Slope is complex in any case, being influenced not only by k (obviously), and v, but also by A. (In fact, slope $(\rho)=(kL)/(vA^v)(A^v-L^v)$, Causton and Venus, 1981). Furthermore, as all four statistics are correlated, only MANOVA can supply valid F-tests for profile testing. However, as this work has convincingly shown, these complexities are all surmountable, and highly satisfactory results can emerge.

Further useful comparisons of curves were made from two biologically meaningful variables, namely, the mean absolute germination rate (R) and the germination duration (D)(Table 4.9). The mean absolute rate (R) should be considered together with the asymptote (A) if a useful picture of profile shape is to be obtained (Causton and Venus, 1981). The duration of germination loosely measured the time required for the major portion of germination. However, it does not correspond to the time of 50% germination, nor to the time of inflexion, nor to the time between (say) 0.05A (onset of germination) and 0.95A (end of germination) (Table 4.9; Table 4.10): so its meaning is not clear.

In this study, the functions of KNO_3 and GA_{4+7} in darkness at 10/15°Cfor green seeds were the "optimal" patterns of germination, and they had high mean absolute rate (R) and relatively short duration (D) (Table 4.9), as well as high upper asymptote (Figure 4.15a and 4.15b); the similar results were found for brown seeds, but the onset of germination delayed (Table 4.10). GA_3 for green seeds at 10/5°C in darkness yielded higher mean absolute rate (29.89), but had shorter duration of germination (3.0 days), which resulted in a low upper asymptote compared with GA_{4+7} and KNO_3. These secondary statistics led to a better understanding of seed germination profiles, since rate and duration were affected differently by different factors. The derived parameters (R and D) have also been employed usefully by Dennett *et al.*, (1978) and Dennett *et al.*, (1979) in the description on leaf growth in *Vicia faba* (broad bean).

The asymptote points must be chosen carefully since growth rates here are generally small and a small error in fixing the area may cause large errors in defining the growing time (Dennett *et al.*, 1978). In this study, thus, the onset and the end of germination were defined the days to reach 5% and 95% upper asymptote germination.

In prediction results, KNO_3 consistently showed high germination. However, GA_{4+7} (non-chilling) gave relatively lower germination in prediction (Table 4.2), which was influenced by prediction window (from 3 to 14 days after seed imbibition). Because two long tails of x-axis attenuation near the y-axis asymptote might affect on the shape of curve in the fitting of the function, the decision of a prediction window was made. However, the results for observation and prediction were mostly corresponding, because only less than 5% primary data were affected by predicting window (Table 4.2). Moreover, Jolliff *et al.*, (1994) reported that germination by day 21 for all seeds lots was the same as measured on day 14 in meadowfoam sees. From an agricultural point, a stand establishment is expected in the field. In addition, the viability of seeds may be influenced by long duration of germination. Therefore, the fitted functions may be expected to regain much of the clarity with which reality was perceived by the whole set of observations.

Response of seed germination to environment could have ecological consequences for field emergence. Reliable information on seed germination and dormancy of this new oilseed crop is needed for choosing the optimum field seeding dates and rates in New Zealand. Predictable, rapid and complete germination of meadowfoam seed could facilitate commercial production through optimum establishment timing and plant populations. Hence, KNO_3 and GA_{4+7} were the best imbibants for seed germination of meadowfoam, since it gave high germination within short duration.

4.4.4 Mechanisms on seed dormancy of meadowfoam

Germination of meadowfoam seed was better in darkness at the cooler temperature than in light at the warmer temperature (Figure 4.7; Table 4.9 and 4.10). Interestingly, the warmer temperature and light inhibition were overcome by KNO_3 (Figure 4.9 and 4.10; Table 4.9

and 4.10). A number of studies have shown that the effect of nitrate is dependent on P_{fr} (Hilton, 1983; Probert *et al.*, 1987; Hilhorst *et al.*, 1986). Nitrate direct binding to the phytochrome receptor protein has been suggested (Hilhorst, 1990a). The combined action of red light and nitrate could be replaced by addition of GA_{4+7}, which inhibited by the growth retardant tetcyclacis, an inhibitor to GA biosynthesis. It is indicated that the combination of red light and nitrate stimulated GA biosynthesis (Hilhorst and Karssen, 1988). With the Hilhorst and Karssen work, it appeared that the active form of phytocrome (P_{fr}) triggered germination of light-requiring seeds, and possibly also of light-independent seeds. The levels of preexisted P_{fr} in these seeds may be higher than the threshold for germination (Cone and Kendrick, 1985). If these ideas apply to meadowfoam, the effect of KNO_3 may be explained in that it stimulated GA biosynthesis during seed germination.

Phytochrome receptors and GA receptors have been proposed to be membrane-bound (Taylorson, 1988; Hooley *et al.*, 1991). Hilhorst, (1990a and 1990b) suggested that the effects of temperature, light and nitrate on germination and dormancy breaking interact on a common reaction site, the plasma membrane. Temperature may affect the rate of receptor synthesis, or the availability of receptors, by altering physical properties of membranes (Di Nola and Mayer, 1986; Hilhorst, 1990b). Also the affinity of the receptors may be influenced. In a response chain which initiated by phytochrome-receptor interaction, the biosynthesis of GAs is stimulated and sensitivity to GAs is enhanced (Hilhorst and Karssen, 1988; Derkx and Karssen, 1993a). GA sensitivity can also be directly influenced by temperature, without the interference of light (Derkx and Karssen, 1994). Germination of meadowfoam seeds has been shown to be sensitive to high temperature (Toy and Willingham, 1966; Cole, 1974; Jolliff *et al.*, 1994), with indirect confirmation from this work. The results in this study showed that 15/10°C decreased mean absolute germination rate (R) and an increased duration of germination (Table 4.9). Moreover, light inhibition had a greater slowing effect in the cooler temperature regime (Figure 4.7; Table 4.9 and 4.10). Therefore, biosynthesis of GAs and/or sensitivity to GAs might be involved in seed germination of meadowfoam. Further specific work is needed to explore this point.

4.4.5 Germination testing and dormancy

The most efficacious germination procedure herein involved the use of either KNO_3 or GA_{4+7}, both of which have long been associated with dormancy breaking in many species. The test results that these imbibants supply, therefore, measure the basic germinative ability of the natural whole-seed system: that is "germinative maturity". Using these results, we could define the dormancy-breaking test as: imbibant 0.2% KNO_3 solution (for economic reasons) in darkness, at temperature 10/5°C (12/12 hrs) without prechilling, and assessment at 14 days from imbibition (test M). Poor germination in this test would represent "immaturity". A "standard" germination test usually has water as the imbibant (ISTA, 1985), and a steady temperature. From this study, darkness, a cool temperature (10°C), and no pre-chilling would be recommended, with assessment at 14 days (test S). The difference between the two tests would represent dormancy (D) plus inviability (I), the latter being separated following a tetrazolium test (Perry, 1987).

Thus: maturity (%) = M/V × 100, where V (viable seeds) = Total - I;
dormancy (%) = (M-S)/V × 100, (on a live-seed basis); or
dormancy (%) = (M-S)/M × 100, (on a maturity basis).

From the present results, the asymptote of KNO_3 at 10/5°C of this seed-lot was 95.1%±0.34 (Figure 4.9). We do not have a simple "standard" test in these treatments: the nearest was water at 10/5°C. The asymptote of water at 10/5°C was 87.0%±0.32. Using that estimate, this seed-lot has 8.1% dormancy. The standard error of the KNO_3 asymptote (from pooled errors of the NLIN fits) provides a standard error for germinative maturity, and was, in this case, 0.34% (as noted already). A standard error for dormancy also is obtainable, as that of the difference between the two asymptotes (A_{KNO3} - A_{H2O}). As these are independent estimates, that standard error is:

$$\hat{\sigma}_{Dorm} = (\hat{\sigma}^2_{A_{KNO_3}} + \hat{\sigma}^2_{A_{H_2O}})^{1/2}.$$

For this case, the standard error for dormancy was 0.47%.

Chapter 5. Seed development and afterripening

5.1 Introduction

The development program proceeds in plants from zygote formation *via* seed development and germination to seedling growth. In nearly all species, it is interrupted by a period of developmental arrest, characterized by dehydration and cessation of growth and resulting in either quiescent or dormant seeds. Quiescent seeds can germinate under suitable conditions, whereas dormant seeds require a specific stimulus in order to break dormancy, before germination will occur under suitable conditions.

Both arrest of growth and primary dormancy have long been associated with the presence of ABA during seed development. The roles of endogenous ABA during seed development *in vivo* are mainly restricted to: (1) the concurrence of the maximum rise in ABA content and the most active phase of fresh and dry weight increase and cell enlargement (Hsu, 1979; King, 1982); and (2) initiation of primary dormancy on the parent plant (Karssen *et al.*, 1983).

Primary dormancy occurs during seed development and maturation. It is often, but not always, removed by exposure of dry seed to elevated temperatures (afterripening) or of imbibed seed to low temperatures (chilling) (Bewley and Black, 1985). Germinability, both during development and after maturation, is sometimes directly correlated with ABA content; lack of a correlation may be explained by assuming lack of sensitivity to ABA or other overriding factors, *e.g.* osmoticum (Hilhorst, 1995). Despite numerous studies in this field, very little is known about the mechanisms underlying induction and breakage of primary dormancy, although a role for ABA in the induction of primary dormancy is now firmly established (Black, 1991; Hilhorst and Karssen, 1992; Hilhorst, 1995).

Mature, dry seeds usually contain 5-15% water and under these conditions, metabolic activity is reduced. Physiological studies in seeds of *Avena fatua* (Adkins and Simpson,

1988) showed that breakage of dormancy is characterized by an increasing sensitivity to germination-stimulating factors such as azide, nitrate and GA_3. Hormone-deficient mutants of *Arabidopsis thaliana* were found to be useful for assessing the role of ABA and gibberellin A_4 (GA_4) in the transition between the dormant and non-dormant states (Hilhorst and Karssen, 1992). It was concluded that ABA played a pivotal role during the development of primary dormancy and that gibberellins are involved in the induction of germination (Karssen and Laçka, 1986).

In this chapter, seed development, afterripening and germination of meadowfoam have been investigated. Results have been used to suggest potentially useful selection criteria in breeding methods.

The objectives of the experiments were:

1) to investigate the patterns of seed development *via* moisture, dry mass, and germination during afterripening;

2) to study germinative maturity and dormancy of meadowfoam seeds, in order to estimate the duration of dormancy;

3) to investigate the effect of afterripening and the interaction between GA_{4+7} and prechilling on primary dormancy release;

4) to establish the relationship between seed dormancy and the levels of endogenous ABA in meadowfoam seeds.

5.2 Material and methods

5.2.1 Choosing eight lines

Eight lines of meadowfoam were chosen from the open-pollinated composite "Moginie" (discussed in Chapter 3) to study seed development. A preliminary principal components (PC) analysis of data for field experiment was done in order to choose representative lines. The preliminary PC analysis considered only 9 variables, *i.e.*, fresh flowers at date 2 (middle flowering), maximum seed set, seed set at date 4 (late seed set), late seed colour turning and 5 post-harvest data (seed set, flowers plant^{-1}, seeds plant^{-1}, 100 seed weight and yield plant^{-1}). Plant choice was based on first two PC components, which accumulated 63.9% of total variance (Appendix 5.1). The factor structure and standard coefficients of the principal components were used to interpret the components (Appendix 5.2). PC component 1 (42.4% of total variance) could be summarised as "Prolific", since maximum seed set, yield plant^{-1}, flowers plant^{-1} and seeds plant^{-1} after harvest were highly correlated with PC component 1, with higher standard coefficients. Seed set at date 4 and seed set after harvest were highly associated PC component 2 (21.5% of total variance) and had higher standard coefficients as well. Thus, PC component 2 could be named "Fertile". The eight lines were chosen from extreme differences in the two PC component combinations (Appendix 5.3). This approach maximized discrimination of phenotypes amongst plants. Seed from each was harvested separately, and provided the 8 half-sib lines used in this study. These were the seed packets numbered: 51, 130, 134, 137, 422, 423, 440 and 448 (Appendix 5.3).

This experiment was carried out in two stages. The first was in one of the HortResearch controlled environmental rooms (Appendix 5.4). A spring simulation of Palmerston North in New Zealand was used, with alternating temperature 15/6.5 °C and 14 hours day length. At the beginning of the experiment, the plants were raised in a glasshouse to a pre-reproductive growth stage (budding). The experiment was a randomised complete block design (RCB) with three blocks. Each experimental unit consisted of 6 plants in separate pots. Stage 2 was a shelf afterripening following the controlled environmental room. Seeds were stored at a 20°C room in darkness.

5.2.2 Seed development

Flowers were tagged (with date) at anthesis, so that subsequent seed age could be determined. Serial samples over the whole period of seed development were harvested from the tagged flowers, and attributes measured at each sampling. Regression analyses of each variable (seed dry mass, seed moisture and germination percentages) as a function of seed age (in days after anthesis) were estimated. Significant differences amongst these regressions would indicate whether different plants (phenotypes) existed.

Fifteen to twenty flowers were sampled at 5 or 7 day intervals, starting from 7 or 10 days after anthesis. The ages of the seed were expressed as the numbers of days after anthesis (DAA). The following attributes were measured.

a. Seed dry mass and seed moisture content

A sample of ten to fifteen seeds was weighed immediately after removing from the flowers, and then dried at 60°C for 48 hours. The dry seeds were reweighed, after cooling to room temperature in a desiccator for one hour. Seed dry mass was expressed in mg $seed^{-1}$. The seed moisture content was expressed as a percentage of fresh weight, following usual cereal technology practices.

b. Standard and potential germination

Two samples (10 - 15 seeds) were obtained for germination tests. For standard germination, the seeds were put into a 9 cm petri dish on No. 1 Whatman filter paper, and 5 ml of distilled water added. Germination was carried out at 10°C without light.

Potential germination was researched in Chapter 4, and a dormancy-breaking procedure was defined, as follows. Seeds were put into a 9 cm petri dish on No. 1 Whatman filter paper, the germination medium being 5 ml gibberellin solution (100 mgGA_{4+7} L^{-1}). The petri dishes were kept in a dark germination cabinet with alternating temperatures 10/5°C (12 h for each temperature).

The number of germinated seeds in both tests was counted after 2 weeks of incubation. Seeds were considered germinated when the embryo ruptured the testa. Ungerminated seeds were tested in 1% triphenyl tetrazolium chloride solution for viability. The data were adjusted for viability. Germination was thus expressed as the percentage of germinated seeds based on total viable seeds.

Dormancy percentages were estimated as the difference between the standard germination and potential germination (adjusted for viability).

$$\textbf{Dormancy (\%)} = \frac{\textbf{Potential G - Standard G}}{\textbf{Potential germination}} \text{x}100$$

c. Concentration of seed ABA

In order to assess possible links between dormancy and abscisic acid, ABA immunoassay was performed on freeze-dried samples collected at sampling time. Ten to forty seeds were put immediately into liquid N_2, and then stored in a deep freeze (-75°C). The samples were lyophilized as soon as possible and stored with desiccant (blue silica) at -75°C.

d. Subsequent characters

Following curve-fitting (plot by plot) of the primary moisture data (at the conclusion of the experiment), estimates of harvest ripeness and embryo maturity were made. Following Gordon *et al.*, (1979), an arbitrary level was used: that is, harvest ripeness on the day of first dehydration to 12.5% moisture. Seed dry mass at harvest ripeness was obtained from the fitted functions.

The day at which seeds attained 90% in the potential germination-test was used to indicate "embryo maturity". This can be regarded as the time at which most whole-seeds are capable of germination once dormancy mechanisms have been by-passed.

5.2.3 Afterripening

Since the potential germination was around 10% or less at harvest ripeness for all lines, an afterripening experiment was carried out. Seeds of the eight lines (about 12.5% moisture) were harvested from the controlled environmental room and stored in a dark room at 20°C. Germination tests were begun after 2 weeks storage, and thereafter at intervals of 2 or 3 weeks. The tests terminated when seed germination reached 90% or more. The line differences in time to attain 90% germination were regarded as a measure of the degree of primary dormancy.

Three samples, each of ten to fifteen seeds, were subjected to different germination tests: standard test, dormancy-breaking test (section 5.2.2), and another dormancy-breaking test with the addition of prechilling.

The number of germinated seeds in each test was counted after 2 weeks of incubation. Seeds were considered as germinated when the embryo ruptured the testa. Ungerminated seeds were tested in 1% triphenyl tetrazolium chloride solution for viability. Germination was thus expressed as the percentage of germinated seeds based on total viable seeds used.

The differences in germination arising from these three germination tests were expected to confirm the "special" dormancy-breaking germination test (as discussed in Chapter 4).

5.2.4 Data analysis

5.2.4.1 Curve fitting

The curves of dry mass, moisture of seeds and seed germination (afterripening) were expected to be sigmoid, such as Richards function. However, the data of this experiment did not fit Richards function because of insufficient data points around one or both asymptotes. A matrix error (Jacobian matrix singular) occurred for dry mass, moisture and germination when using NLIN (NonLINear regression) of the SAS statistical package. Where original plots suggested that curves other than sigmoidal asymptotics might be appropriate, several criteria of regression were considered concurrently to search for the "best fit".

5.2.4.2 Criteria of "best fit"

Criteria for judging the curve of "best fit" involved both accuracy of regression and appropriateness of the resultant curve (*i.e.* curve placement and biological expectations). With respect to biological expectations, whole or partial sigmoidal asymptotics were preferred generally to power, exponential, logarithmic, quadratics and exponential quadratics. A linear regression was also compared. However, if one of the "open-ended" functions fitted the data, they were accepted. Such apparent exponentials can be treated as incomplete sigmoids (Bliss, 1970).

The next criterion of best fit was to favour curves with higher coefficients of determination (R^2). The highest was not necessarily accepted, depending on other criteria. Also, the actual shape of the expected curve with respect to the observed points was considered by examining plots and residuals. When an alternative still existed, the simpler equation was favoured. Finally, when sets of curves were being compared, the commonest "best fit" was chosen for all lines.

5.2.4.3 Regression equations

a) Power function: $Y = AX^B$, with linear form:

$$\ln Y = \ln A + B(\ln X),$$

then, $\ln Y = \beta_0 + \beta_1(\ln X)$ in regression form;

b) Exponential function: $Y = AB^X$, with linear form:

$$\ln Y = \ln A + (\ln B)\, X,$$

then, $\ln Y = \beta_0 + \beta_1 X$, in regression form;

c) Logarithmic function: $B^Y = AX$, with linear form:

$$Y = (\ln A / \ln B) + (1 / \ln B) \ln X,$$

then, $Y = \beta_0 + \beta_1 \ln X$, in regression form;

d) Quadratic function: $Y = \beta_0 + \beta_1 X + \beta_2 X^2$;

e) Exponential quadratic function: $Y = e^{\beta_0+\beta_1 x+\beta_2 x^2}$, with linear form:

$\ln Y = \beta_0 + \beta_1 X + \beta_2 X^2$;

and f) linear function: $Y = \beta_0 + \beta_1 X$.

The procedures followed were straightforward applications of linear regression as appropriate, using PROC REG of the SAS (SAS Institute Inc., 1990). The interactive of PLOT statement with the OVERLAY option in SAS was used in order to look at scatter plots of data and diagnostic statistics. A plot of predicted and observed values helped to evaluate the model and detect outliers in the data.

5.2.4.4 Tests for outlying observations

When sample data were plotted, often one or more observations were far from the regression line. Such extreme values could be outliers. The outlier indicates a data point which is not at all typical of the rest of the data (Draper and Smith, 1981). Thus, outliers were examined carefully in this study, as the data in this experiment were variable.

Behnken and Draper (1972) have stated that the Studentized residuals (Z) are of interest in plots and tests for outliers in regression. The Studentized residuals (Z) are the residuals divided by the square root of their estimated variances ($Z = (Y - \mu)/\sigma_Y$). In the plot of Studentized residuals (Z), a large number of observations with absolute values greater than two indicates an inadequate model (SAS Institute Inc., 1990). Probability of Studentized residual (Z) being greater than 2 is less than 0.0228 (Steel and Torrie, 1981).

In PROC REG, the options of P and R were used in the MODEL statement, thereby providing the standard errors of the predicted value, the residual and Studentized residual for each observation. The plot of Studentized residuals were also printed. In this study,

observations with absolute values of Studentized residuals (Z) greater than 1.645 ($P<0.05$) (Steel and Torrie, 1981) indicated outliers in the data.

The power and exponential quadratic functions provided the best fit for the data of seed dry mass according to the coefficients of determination (R^2) (Appendix 5.5). Following the method to identify outliers, 14 observations were outliers ($P<0.05$) in the power function, but in the quadratic exponential function there were 16 outliers. In order to eliminate model influence and also minimize deleting observations, 10 observations, which were indicated outliers for both models, were deleted. The coefficients of determination of regression (R^2) for seed dry mass fitting power and exponential quadratic functions are shown in Appendix 5.6 with adjusted data.

The quadratic and exponential quadratic functions were the best fit for seed moisture according to the coefficients of determination (R^2) (Appendix 5.5). According to Studentized residuals, 19 observations were outliers significantly ($P<0.05$) in the quadratic function, but in the quadratic exponential function there were 17 observations. Nine observations, which were indicated outliers for both models, were removed. The coefficients of determination of regression (R^2) for seed moisture fitting quadratic and exponential quadratic functions are shown in Appendix 5.6 with adjusted data.

The quadratic and exponential quadratic functions were also the best fit for the data of seed germination according to the coefficients of determination (R^2) (Appendix 5.7). There were 61 and 52 outliers ($P<0.05$) in quadratic and exponential quadratic functions, respectively. Twenty-nine observations were significant outliers for both functions, leading to a new data file without these 29 observations. However, several R^2 after rerunning PROC REG with new data file were much lower than before (Appendix 5.8), which might be caused by lack of data points for a regression fit. Therefore, the method of adjusting outliers based on both models might not be suitable for germination data in this study.

The coefficients of determination (R^2) was higher in the quadratic function than in the quadratic exponential function (Appendix 5.7). The shape of curves was as expected with

respect to the observations. Thus, the quadratic function was check to be the best fit for this germination data. Although 61 of the observations were significant outliers ($p<0.05$) in the quadratic function, fourteen of these data in the plot of predicted and observed values were visible outliers. In order to minimize deleting data, only 14 observations were deleted as outliers for the germination data. After re-runing PROC REG with this adjusted data set, higher coefficients of determination (R^2) were obtained (Appendix 5.8).

5.2.4.5 Profile analysis

This experimental design was originally a RCB with three blocks. However, there were not enough tagged flowers because of plant decay in the later stages of seed development. External replications for each line were pooled in order to get 8 separate functions per character. Because of this lack of external replications, multivariate analysis for statistics of regression function could not be carried out (as in Chapter 4). Thus, Hotelling T^2 test was used to examine the differences between functions. Hotelling T^2 test is extended one- and two-sample tests on the means of normal variates to multinormal mean vectors (Morrison, 1990).

The Hotelling T^2 statistic was computed from the vector (p x 1) of $X_{ik} - X_{i'k}$ and the difference of the dispersion or variance-covariance matrix, the (p x p) vector ($D_{ik} + D_{i'k}$) (Morrison, 1990).

$$\Delta_{ii'} = X_{ik} - X_{i'k}$$

$$D_x = D_{ik} + D_{i'k}$$

$$T^2 = \Delta' D_x^{-1} \Delta$$

for i = 1, 2, 3, ... n (line); and k = β_0, β_1, β_2, ...β_n (parameters of function). Then,

$$F = \frac{N - p}{(N-1)p} T^2$$

the F distribution with p and N-p degrees of freedom (Morrison, 1990). Here, N was pooling N_{ik} and $N_{i'k}$, and p was number of parameters. If $F > F_{\alpha;\, p,\, N-p}$, this indicated that functions were different.

5.2.4.6 Estimate of heritability

In this study, samples were insufficient for RCB analysis because of plant loss at late seed development; and external replications were pooled for the regression work. Thus, analysis of variance (ANOVA) for RCB could not be applied. However, the variance of $\hat{Y}$ ($\sigma^2_{\hat{Y}}$) can be estimated at arbitrary points from linear and quadratic regressions. Thus, a parallel ANOVA was carried out. The variance of $\hat{Y}$-estimates across the 8 lines (from regression) was taken as genotypic variance (σ^2_G), and the pooled error-variances of $\hat{Y}$ ($\sigma^2_{\hat{Y}}$) of the 8 lines (within regression) was taken as the environmental variance (σ^2_E). Therefore, the broad heritability for seed moisture, dry mass and seed germination (afterripening) could be estimated.

$$h^2 = \frac{\sigma^2_G}{\sigma^2_P} = \frac{\sigma^2_G}{\sigma^2_E + \sigma^2_G}.$$

The sampling variances of estimates of phenotypic variance (V_P), and of the heritability estimate (V_h^2) were calculated based on the method of Osborne and Paterson (1952).

$$V_P = V_E + V_G + 2\,\mathrm{cov}(\sigma^2_E,\ \sigma^2_G);$$

$$V_{h^2} = \frac{[\sigma^2_G V_P + \sigma^2_P V_G - 2\sigma^2_G \sigma^2_P \,\mathrm{cov}(\sigma^2_G, \sigma^2_P)]}{(\sigma^2_P)^4}.$$

where V_E and V_G were variances of environmental and genotypic variance, respectively. Here, $\mathrm{cov}(\sigma^2_E, \sigma^2_G)$ was assumed equal to zero, because both environmental and genotypic variances were independent analysis. Thus,

$$\mathrm{cov}\,(\sigma^2_G, \sigma^2_P) = \mathrm{cov}(\sigma^2_E, \sigma^2_G) + \sigma^2_G\sigma^2_G = V_G.$$

The significant test for heritabilities was carried out by t-test.

The variance of $\hat{Y}$ ($\sigma^2_{\hat{Y}}$) can be calculated for quadratic function as follows (Draper and Smith, 1981):

$$\sigma^2_{\hat{Y}} = \begin{bmatrix} 1 & X & X^2 \end{bmatrix} \begin{bmatrix} \sigma^2_{\beta_0} & \text{cov}(\beta_o,\beta_1) & \text{cov}(\beta_o,\beta_2) \\ \text{cov}(\beta_o,\beta_1) & \sigma^2_{\beta_1} & \text{cov}(\beta_1,\beta_2) \\ \text{cov}(\beta_o,\beta_2) & \text{cov}(\beta_1,\beta_2) & \sigma^2_{\beta_2} \end{bmatrix} \begin{bmatrix} 1 \\ X \\ X^2 \end{bmatrix} = x'Vx .$$

In linear function, the variance of $\hat{Y}$ ($\sigma^2_{\hat{Y}}$) can be simplified from quadratic equation as follows (Draper and Smith, 1981):

$$\sigma^2_{\hat{Y}} = \sigma^2_{y,x}[\frac{1}{n} + \frac{(\hat{X} - \overline{X})^2}{SS_X}].$$

From the fitted functions for seed moisture, harvest ripeness (12.5% moisture) was around 86 days after anthesis for 8 lines. Therefore, 86 days after anthesis was selected as a key stage for seed moisture and seed mass, and the heritabilities were estimated at that point. The differences for seed moisture and seed mass amongst 8 lines were also examined at 86 days after anthesis.

From the fitted functions for seed germination, the periods to reach 90% germination (dormancy-breaking) were from 15 weeks or more. Thus, a high value (after 15 weeks storage) was selected as a key stage for line comparisons. In order to investigate the effect of afterripening, an arbitrary low value (after 5 weeks storage) was also selected as a key stage for releasing dormancy. The heritabilities after 5 and 15 weeks storage were estimated for standard germination and dormancy-breaking germination. The differences amongst treatments and lines were also tested after 5 and 15 weeks storage.

5.3 Seed ABA analysis

The method of ABA analysis is a modification of Walker-Simmons (1987) immunoassay method.

5.3.1 ABA extraction and purification

Seed samples were ground in a mortar and pestle, and then transferred to 40 ml polypropylene tubes. The samples were suspended in 80% methanol containing 10 mgL^{-1} BHT (butylated hydroxytoluene) at a ratio of 0.01 g dry tissue to 1.0 ml extracting methanol. BHT is an antioxidant and was included in order to prevent oxidation of ABA. A 100µl aliquot of [^{3}H]ABA (18,000 DPM per 100µl, specific activity) (Appendix 5.9) was added to each of the samples as an internal standard. [^{3}H]ABA added was well below the detection limits of the immunoassay. Samples were extracted overnight in a dark and cold room at 4 °C on a shaker, then filtered through Whatman No 1 filter paper under vacuum and dried in a Savant centrifugal evaporator. This procedure gives optimum extraction of ABA as measured by immunoassay (Walker-Simmons, 1987).

For ELISA (enzyme-linked immunosorbent assay), the dried samples were resuspended in 5 ml 0.01M ammonium acetate (pH>7). The extract solutions were loaded onto a Sep-Pak Cartridge (Waters Associates, Milford, U.S.A.) column. The columns were rinsed with 5 ml distilled water and eluted with 5 ml 80% methanol. The eluent was collected into a vacutainer tube and dried in the centrifugal evaporator. The dried samples were redissolved in 4ml TBS (Tris-buffered saline) (Appendix 5.9) for ELISA.

Duplicate extracts (200µl) were mixed with 1ml of scintillation cocktail until a stable emulsion was formed; and the radioactivity of extracts counted in a liquid scintillation counter (Wallac 1409/11 LSC version 1.6). Percentage recovery was estimated from the proportion of radioactivity recovered (DPM_r) and added (DPM_a) to assess each sample for variation in loss of ABA.

$$\text{Recovery (\%)} = \frac{DPM_r}{DPM_a} \times 100.$$

The recovery rate was used to compensate for loss. The average recovery rate was 88.2% (Appendix 5.10).

5.3.2 ELISA assay procedure

The method was sensitive to (+)-ABA concentration between 5 - 2500 pg per 100 µl. Available evidence indicates that the naturally occurring ABA is a single enantiomorph, specifically the dextrorotatory compound, (+)-ABA (or (*S*)-ABA) (Moore, 1989). In this study, therefore, the content of seed ABA related specifically to (+)-ABA.

The procedure described below was used for both standards and sample extract material. All samples and solutions were kept in the dark during incubations. Nunc-Immuno Plate MaxiSorp™ microtitration plates having 96 wells were used in the procedure. ELISA assay materials were presented in Appendix 5.9.

At day 1, a 200 µl aliquot of the conjugate was added to each well of the microtitration plate. Plates were incubated at 4°C overnight. The conjugate binds strongly to the surface of the well and contains sites for binding the ABA monocloned antibody (mouse derived antibody from Idetek, USA).

On the same day, a 350 µl aliquot of the ABA standards or sample extracts was pipetted into microcentrifuge tubes; and 300 µl of MAb solution was added and mixed. The solutions were incubated overnight at 4°C. This provided enough material for three replicate well assays. The purpose of this step is to allow binding of sample/standard ABA molecules to MAb molecules after which the remaining unbound MAb molecules can then bind with the ABA conjugate coated onto the wells in the ELISA plate. Three additional microcentrifuge tube were prepared, two having 350 µl TBS + 300 µl of MAb for nil ABA (Bo) and one having 350 µl TBS + 300 µl of TBS-BSA (without Mab) for non specific binding (NSB).

At day 2, plates with wells coated with conjugate were inverted and then washed three times with washing buffer, leaving the wash in for 5 minutes on the 2nd and 3rd wash and then discarding. Then, 200 µl aliquots of the samples or standards incubated with MAb was added to three replicate wells. Plates were incubated for 2.5 hours. This incubation and all the following steps were performed at room temperature. The purpose of this step is to attach the free MAb (*i.e.* that which has not already conjugated with the sample ABA) to the conjugate molecules fixed to the well surface. The binding is on a one-to-one basis between the ABA-end of conjugate and the MAb.

Wells were washed three times with washing buffer (as described above). Rabbit antimouse alkaline phosphatase conjugate (200 µl) was added to each well and plates were incubated for 2 hours. Washing removes any excess MAb molecules that are not complexed with ABA molecules. The 2nd antibody binds to the MAb end of the conjugate-MAb complex. The concentration of ABA in the samples/standards determines the amount of free Mab (*i.e.* available to bind onto the conjugate), and therefore, the amount of 2nd antibody bound. The 2nd antibody catalyses the breakdown of *p*-nitrophenyl phosphatase to a yellow compound, *p*-Nitrophenyl and phosphate group. The optical density of *p*-Nitrophenyl was measured to determine the concentration of ABA in the original sample incubated with MAb, by comparison with a standard curve as described below.

Wells were washed three times with washing buffer. *p*-Nitrophenyl phosphate substrate (200 µl) was added to each well. The sample absorbance was measured at 410 nm with a Dynatech MR5000 Enzyme Immunoassay Reader. Plates were incubated for around half hour until the Bo (control samples containing no ABA) was approximately 1.0. The absorbance of the samples is inversely proportional to the amount of ABA in the original sample incubated with MAb.

The percentage binding of each standard or sample was calculated by the following:

$$B / B_0 = \frac{\text{Standard or Sample absorbance - NSB absorbance}}{\text{Bo absorbance - NSB absorbance}} \times 100 .$$

The B/Bo was plotted against the concentration of ABA. A linear curve was drawn using a logit function (with logit fitted against ABA concentration):

$$\text{Logit } B/Bo = \text{Ln}[\frac{B/Bo\%}{100 - B/Bo\%}].$$

Replicate ABA standards were assayed for each plate. A typical standard curve is shown in Figure 5.1 ($Y=\beta_0 + \beta_1 \ln(x)$). All sample results were the average of replications within the linear range of the ABA standard curve. Thus, the amount of ABA in the seed extract samples was determined from the ABA standard curve.

5.3.3 Validation of ELISA assay for ABA of meadowfoam seed

A validation test for the ELISA procedure for seed ABA of meadowfoam was conducted, in order to test for interference by the plant extract material. The sources of potential interference peculiar to immunoassays fall within two groups: 1) Compounds antigenically (structurally) similar to the plant hormone under study, which may exhibit high affinities for the antibody and thus will cross react even when present at low levels; 2) Factors interfering with certain steps of the assay procedure (*e.g.* detergents or solvent residues impairing antibody function, desorbing antibody from the solid phase etc.) (Weiler *et al.*, 1986).

Meadowfoam seeds were extracted and purified using a Sep-Pak cartridge (Section 5.3.1) and then aliquots (diluted 1/300 and 1/600) added to known amounts of ABA standard (25 - 250 pg (+)-ABA per 100 µl. The mixtures were then assayed by ELISA. Extracts diluted 1/300 and 1/600 were equivalent to 135 and 270 pg per 100 µl of ABA, respectively. The known amounts of pure ABA standard were also assayed by ELISA, to judge the linearity of the immunoassay.

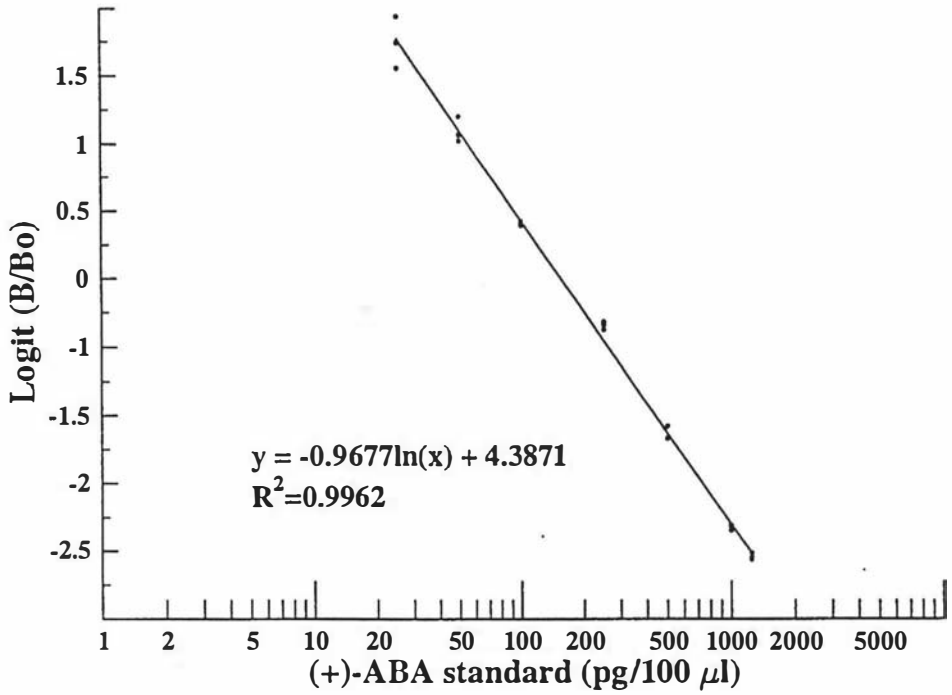


Figure 5.1 A typical curve of (+)-ABA standard by ELISA.

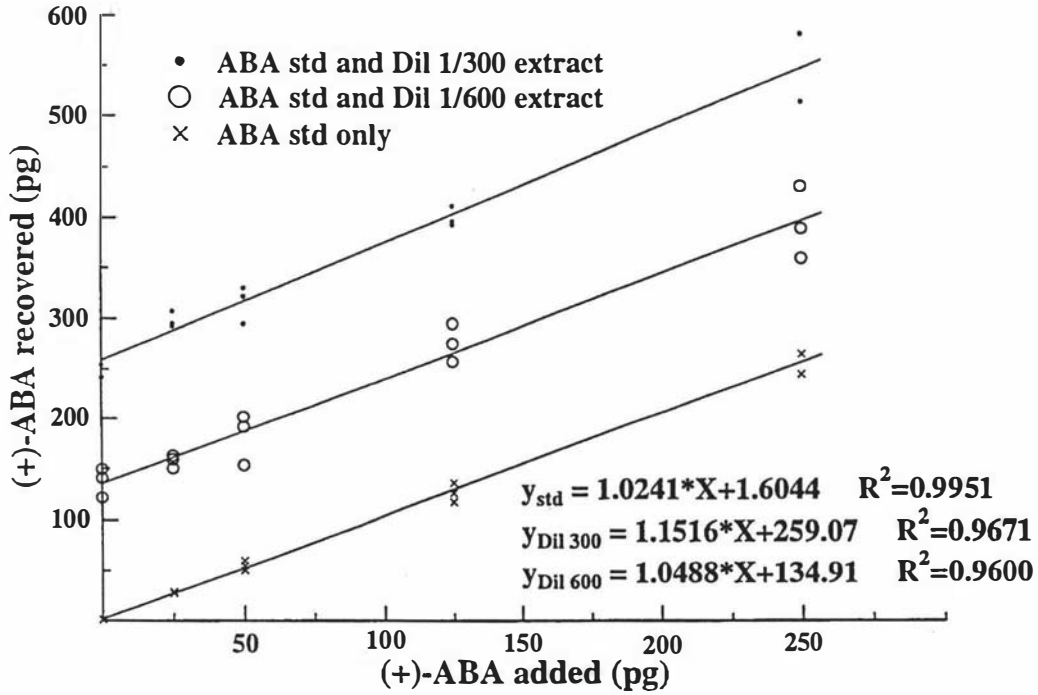


Figure 5.2 Validation of the method of ABA analysis for meadowfoam seeds.

Table 5.1 Comparison of regressions for the (+)ABA standards with or without added seed extract.

	$f_{y.x}$	Y intercept (β_o)	s.e. β_o	Slope[1] (β_1)	s.e. β_1
Standard	11	3.2088	5.9541	1.0241 a	0.0218
Dilution 1/300	11	518.14	15.1121	1.1516 a	0.0640
Dilution 1/600	13	369.82	15.1420	1.0488 a	0.0594

[1]: The same letters indicated none significantly differences between slopes by t-test.

Linear regression for the ABA recovered against ABA added was applied, using PROC REG of the SAS statistical package (SAS Institute Inc., 1990).

The differences between slopes of pure ABA standard (β_{1std}) and extracts with added ABA (β_{1ext}) were tested by t-test.

$$t = \frac{\left|\hat{\beta}_{1std} - \hat{\beta}_{1ext}\right|}{(\sigma^2_{\hat{\beta}_{1std}} + \sigma^2_{\hat{\beta}_{1ext}})^{1/2}}$$

t with N (pooling N_{ik} and $N_{i'k}$) degrees of freedom. If $t > t_{\alpha; N}$, this indicated that the slopes of lines were different.

The added ABA was accurately detected with the immunoassay. The slopes of the lines with added seed extract were not significantly different from that of ABA standards alone (Table 5.1), *i.e.* lines for extracts with added ABA being parallel to the standard line (Figure 5.2). The results indicated that the plant extract material did not interfere with the linearity of the immunoassay. Therefore, the ELISA assay was valided for ABA of meadowfoam seed.

5.3.4 Analysis of ABA Curves

The plots of ABA concentration against days after anthesis did not follow any obvious functions. Furthermore, there were no replications (because of insufficiency of tagged flowers). Thus, the previously used regression analysis and analysis of variance could not be applied for the ABA plots. The test of the curve differences among lines was

carried out by principal components analysis. PC analysis can be used for exploring polynomial relationships and for multivariate outlier detection. In this case, the pattern analysis by PC analysis was considered superior to regressions (over time) because of few time-nodes, and it avoided the need to find optimum functions (as discussed in Chapter 3).

An overview survey revealed that there were two peaks of ABA for most lines, but only one peak for Line 7 and Line 8. Three variables per peak were recorded (*i.e.* peak height, peak width and time to reach the high point of each peak) for each of the 8 lines. For the second peak of Line 7 and Line 8, time, height and width of peak were set to 0.

Any differences in pattern for these ABA variables could be ascertained by using the PC scores to ordinate the lines. The PRINCOMP procedure of SAS was used to effect the PC analysis. The analysis was done on standardized data. In order to minimize the number of principal components utilized for each attribute (parsimony), it was decided to use only those components which accumulated at least 70% of total variances in this study (as discussed in Chapter 3).

Following PC analysis, a cluster analysis was done on the PC scores in order to recognize the groups of lines. The data matrix for clustering consisted of two standardized PC scores (accounting for 80.52% of total variances) for each line. The similarity matrix was based on Euclidean distance, and the Ward method of clustering (minimum increment of within-cluster sum square) was used (details in 3.3.3). The CLUSTER procedure of SAS was used to find hierarchical clusters of PC scores.

After clustering, program CLUMEM (I.L. Gordon, unpublished) was used to process the output from CLUSTER, in order to reveal relationships among the clusters. The dendrogram truncation points were defined at the minimum F-probability value for the ratio of amongst cluster mean squares to within cluster mean squares (Teow, 1978; Cullen, 1981).

5.4 Results

5.4.1 Seed dehydration

The criteria of best fit included high coefficients of determination (R^2), goodness of fit from plots, and curve definition of prediction window. According to the criteria of best fit, the best equation was the quadratic function for seed moisture.

$$y = \beta_0 + \beta_1 X + \beta_2 X^2$$

where y: seed moisture (%);
x: age of seed expressed in days after anthesis (DAA).

The estimated statistics of the equations and R^2 are given in Table 5.2. Data points and fitted curves for seed moisture of 8 lines are given in Figure 5.3-5.10.

Table 5.2 Estimated statistics of final curves (linear form) describing changes in seed moisture during seed development in 8 lines.

Statistic	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8
β_0	80.3155	77.2226	83.5667	77.8042	82.5376	78.6376	79.8093	83.8234
s.e. β_0	6.8442	1.9476	3.7538	1.9646	3.8973	1.9774	2.3713	6.0552
β_1	0.6195	0.7249	0.0614	0.4922	0.4190	0.7552	0.5598	0.3914
s.e. β_1	0.4492	0.1193	0.2243	0.1316	0.2236	0.1207	0.1499	0.3702
β_2	-0.0141	-0.0172	-0.0101	-0.0143	-0.0137	-0.0176	-0.0161	-0.0130
s.e. β_2	0.0051	0.0013	0.0024	0.0015	0.0024	0.0013	0.0017	0.0041
$\sigma_{x.y}^2$	109.35	6.4712	16.139	9.9684	18.703	6.8424	11.038	70.336
R^2	0.7679	0.9851	0.9675	0.9713	0.9638	0.9860	0.9806	0.8278
F for regression	16.540**	362.89**	163.77**	203.35**	146.61**	421.37**	302.50**	28.85**
Equation form	$Y = \beta_0 + \beta_1 X + \beta_2 X^2$ (X: days from anthesis)							

**: Significance level at 1% level ($P<0.01$).

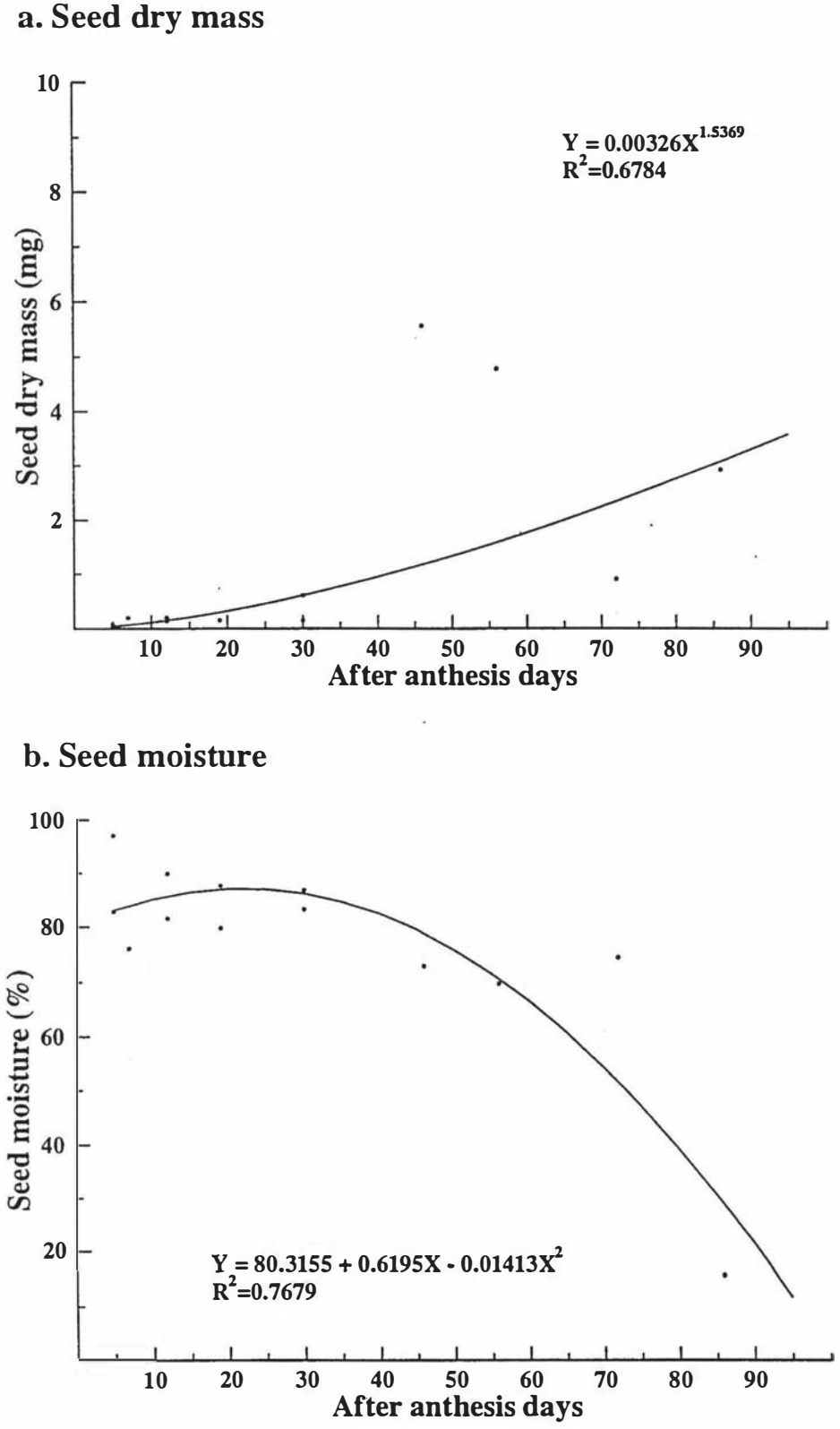


Figure 5.3 The functions of seed dry mass and seed moisture for Line 1.

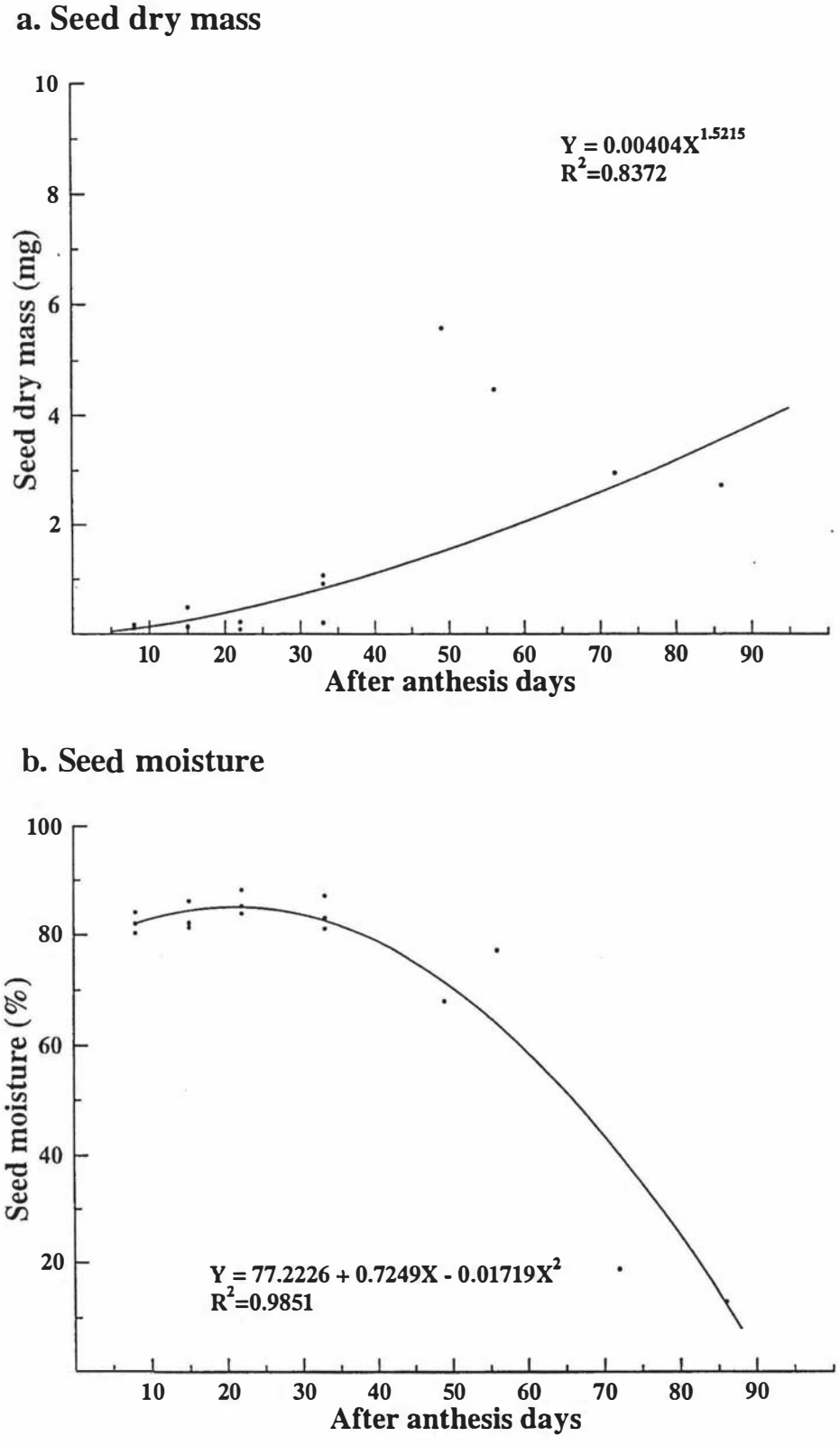
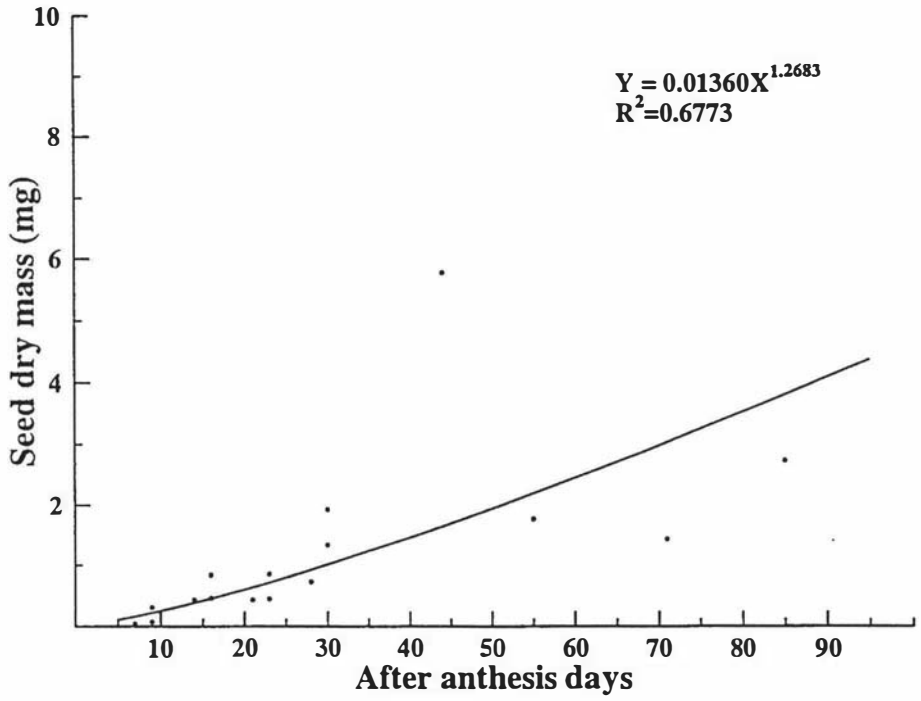


Figure 5.4 The functions of seed dry mass and seed moisture for Line 2.

a. Seed dry mass



b. Seed moisture

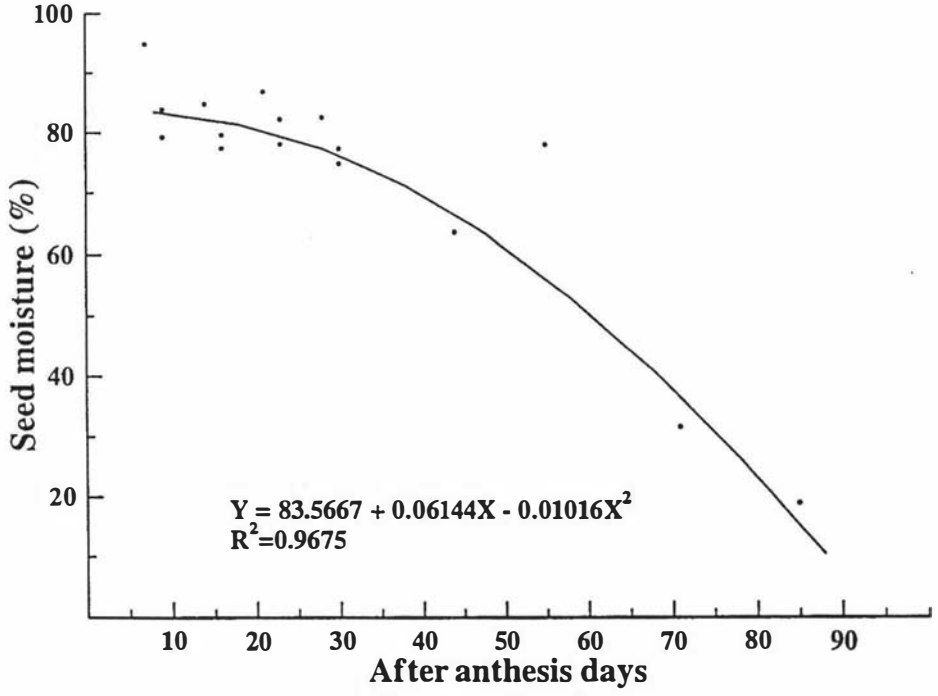
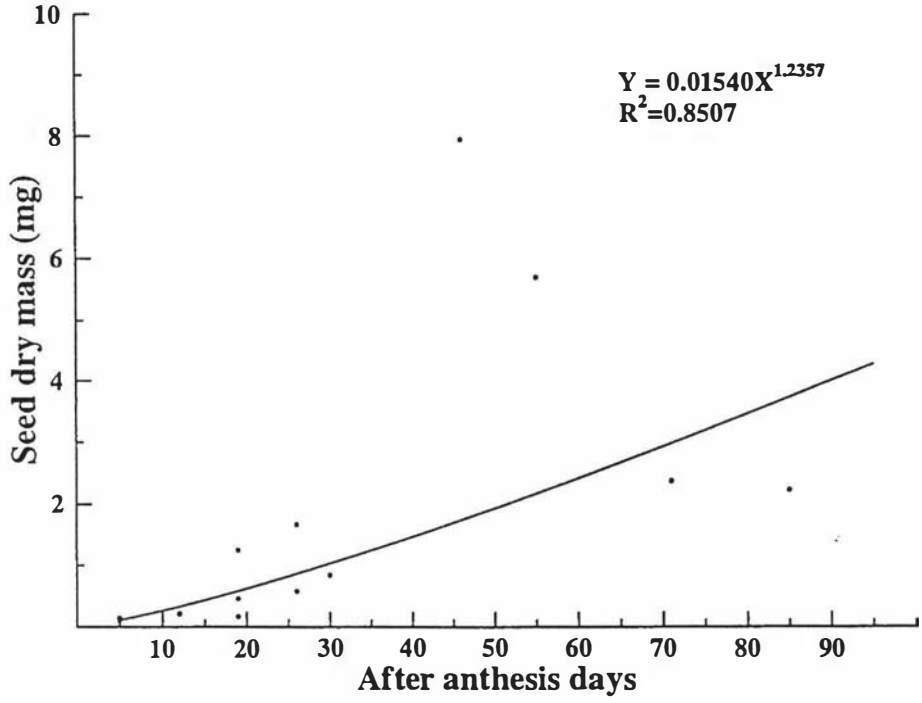


Figure 5.5 The functions of seed dry mass and seed moisture for Line 3.

a. Seed dry mass



b. Seed moisture

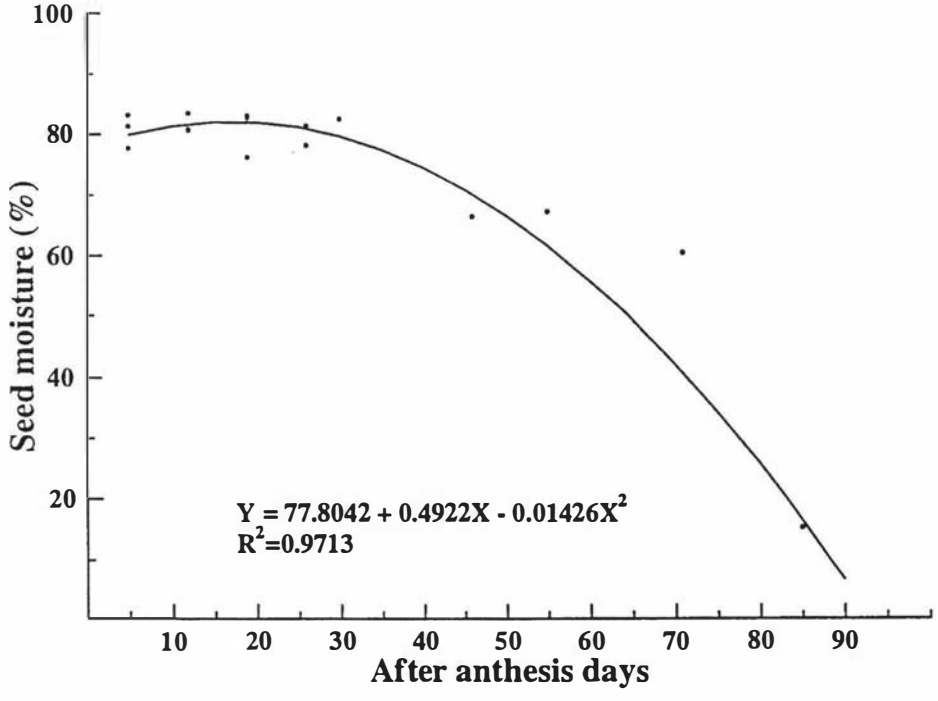
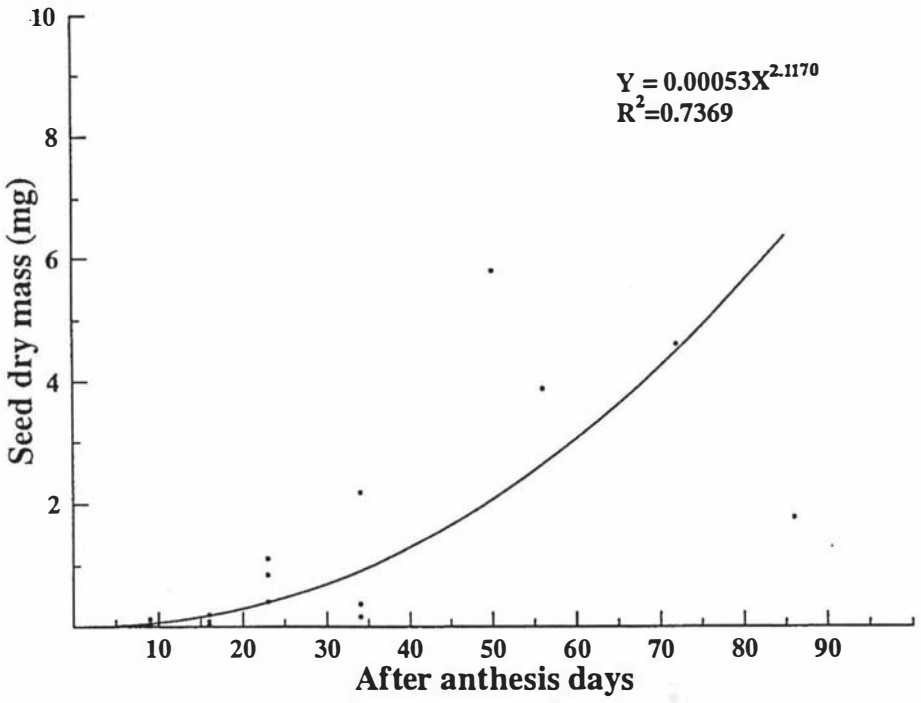


Figure 5.6 The functions of seed dry mass and seed moisture for Line 4.

a. Seed dry mass



b. Seed moisture

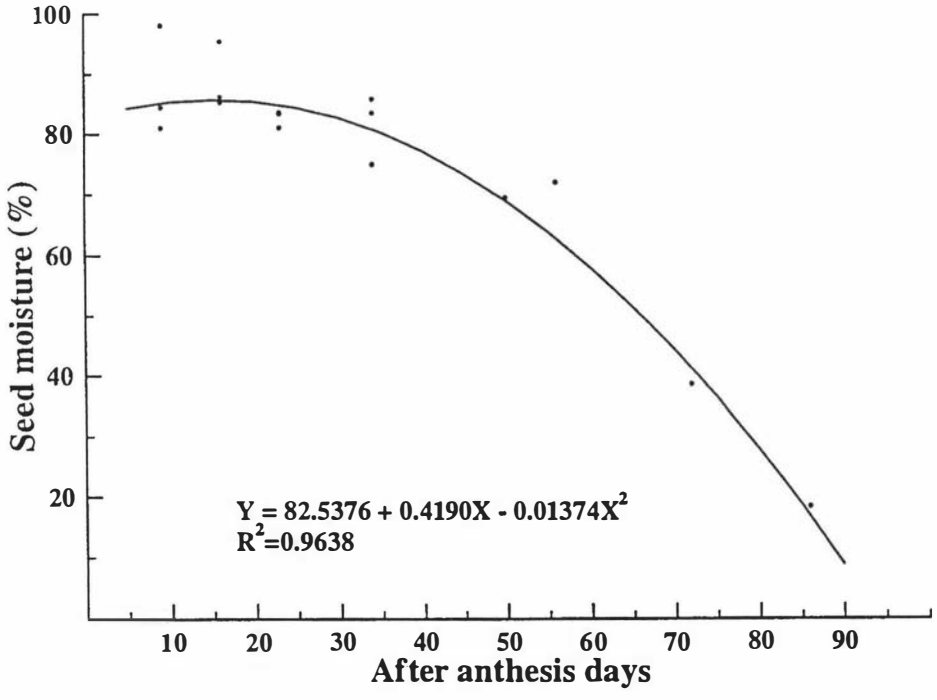


Figure 5.7 The functions of seed dry mass and seed moisture for Line 5.

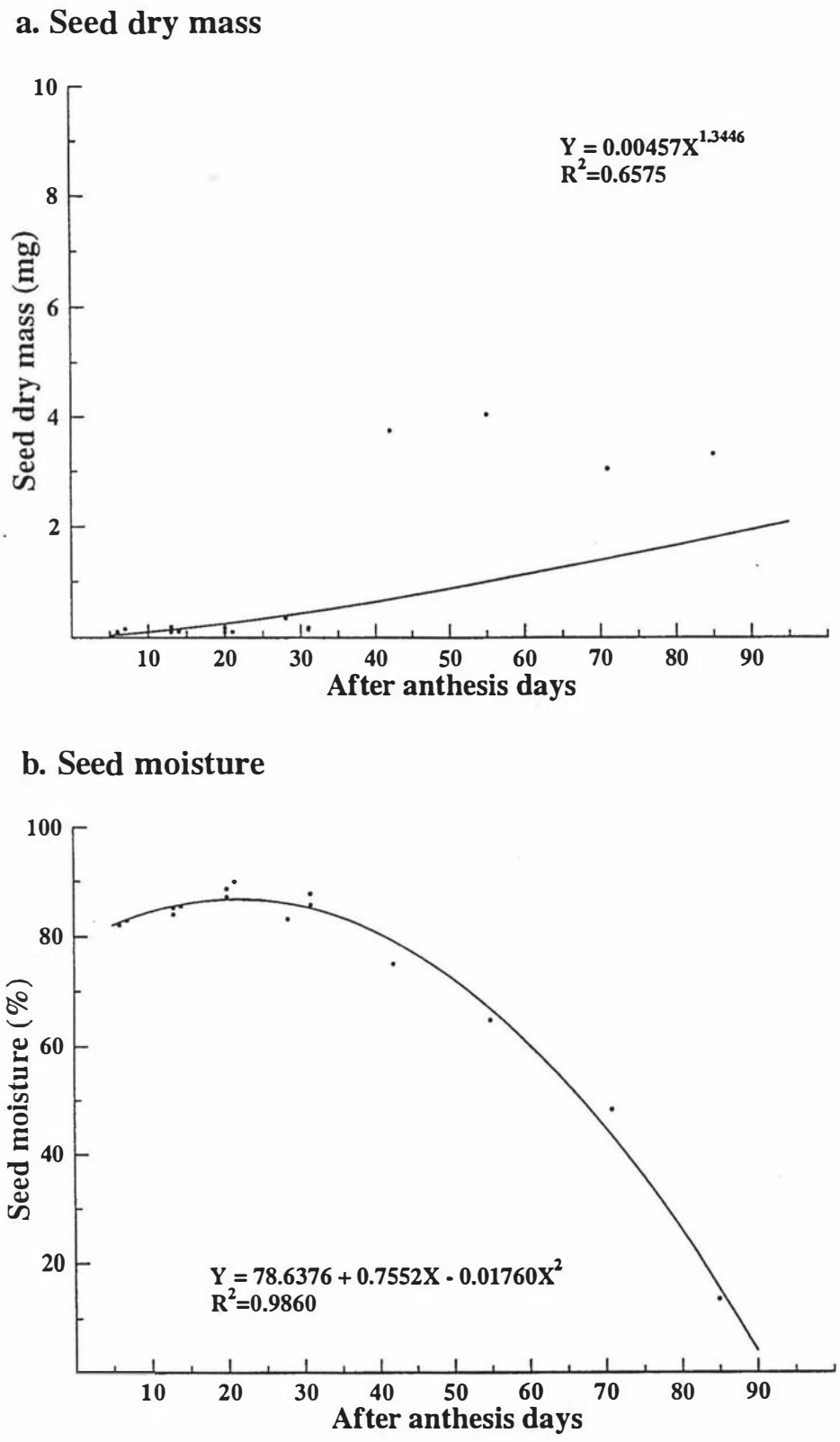
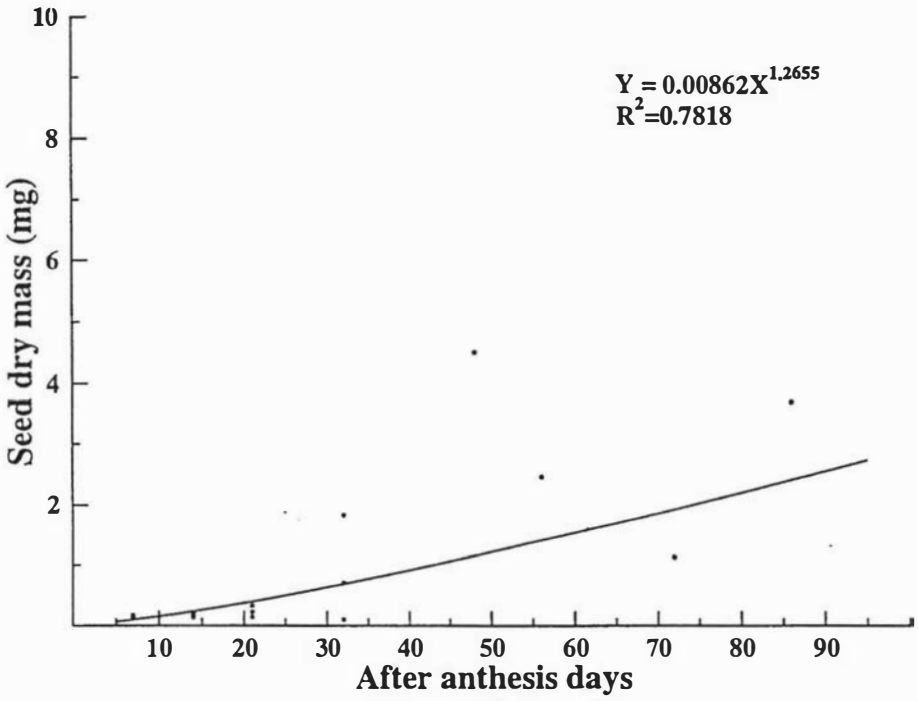


Figure 5.8 The functions of seed dry mass and seed moisture for Line 6.

a. Seed dry mass



b. Seed moisture

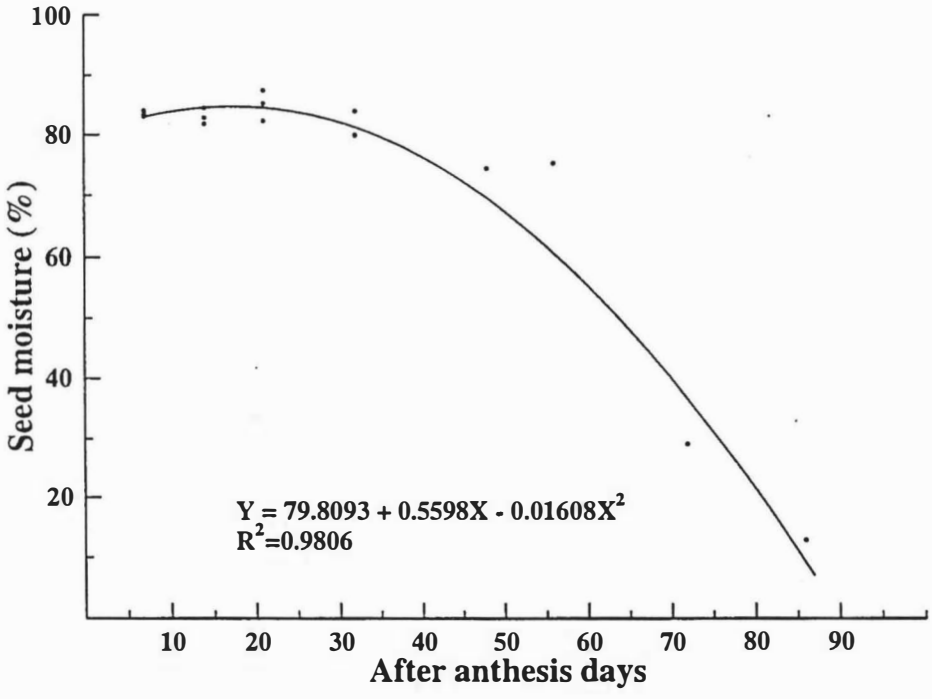
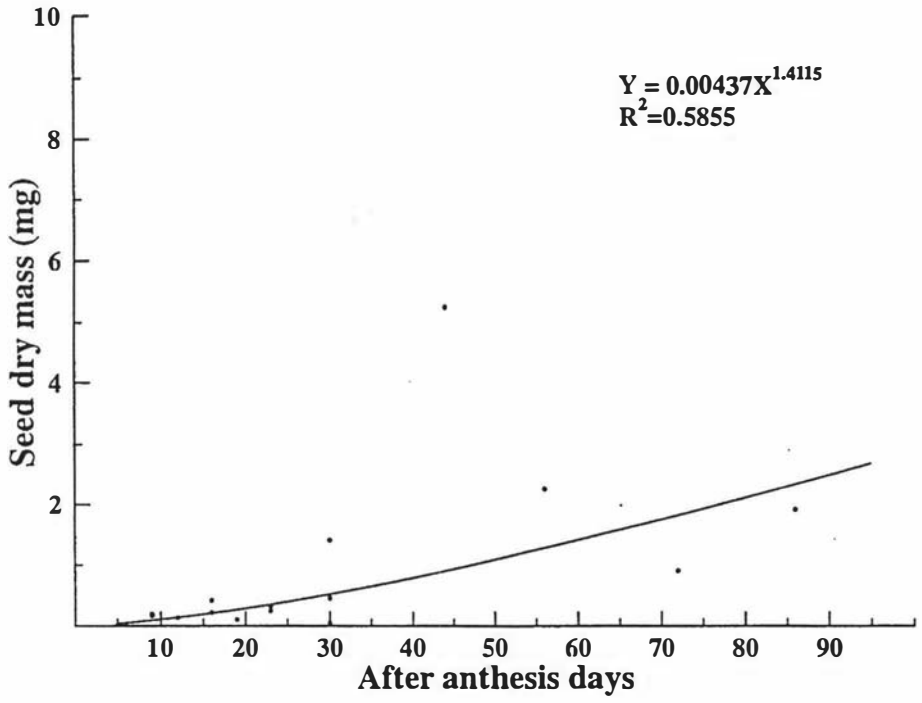


Figure 5.9 The functions of seed dry mass and seed moisture for Line 7.

a. Seed dry mass



b. Seed moisture

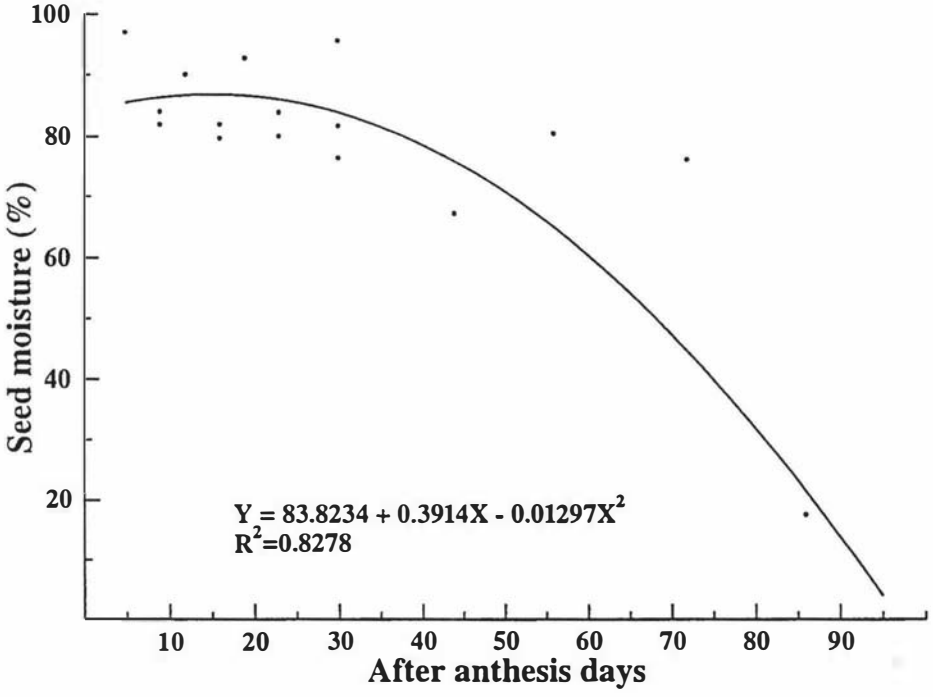


Figure 5.10 The functions of seed dry mass and seed moisture for Line 8.

The differences between functions amongst 8 lines tested by Hotelling T^2 testing are shown in Table 5.3 for seed moisture. Line 3 had a significantly different pattern of seed dehydration from Line 1 ($p<0.10$) and Line 2 ($p<0.01$). The functions between Line 4 and 6 also differed ($p<0.01$). The results indicated there was a general pattern for seed moisture in meadowfoam.

Table 5.3 The differences between functions of 8 lines for seed moisture.

	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8
Line 1								
Line 2	ns							
Line 3	(*)	**						
Line 4	ns	ns	ns					
Line 5	ns	ns	ns	ns				
Line 6	ns	ns	ns	**	ns			
Line 7	ns	ns	ns	ns	ns	ns		
Line 8	ns	ns	ns	ns	ns	ns	ns	

**: significance at the 1% probability level; (*): significance at the 10% probability level.
ns: no significance at the statistics level.

Table 5.4 The number of days from anthesis to harvest ripeness and seed moisture (%) at 86 days after anthesis for 8 lines.

Line	Days (SMC=12.5%)	Seed moisture (%)[1] (86 days)	s.e.
Line 1	94.6	29.1 a	9.0315
Line 2	86.0	12.5 a	2.5120
Line 3	86.7	13.7 a	3.6554
Line 4	87.1	14.7 a	3.1572
Line 5	88.2	16.9 a	3.7690
Line 6	86.4	13.4 a	2.3514
Line 7	84.4	9.1 a	20.829
Line 8	90.8	21.5 a	8.1144

[1]Means with the same letter are not significantly different by t-test.

Table 5.5 Variance components and heritability estimates for seed moisture at 86 days after anthesis.

Partition	Variance (σ^2)	s.e.	h^2	s.e.
Genotype	39.5905	21.1620	0.3292 **	0.0020
Environment	80.6691	11.0289		
Phenotype	120.2596	23.8635		

**: Significance level at 1% level ($P<0.01$).

Harvest ripeness was estimated at the 12.5% moisture point in days after anthesis following work on wheat (Gordon *et al.*, 1979), by using backward regression. Harvest ripeness was estimated as days after anthesis for 8 lines (Table 5.4). Line 7 was the earliest to reach harvest ripeness (84.4 days after anthesis). There were around 86.0 to 88.2 days after anthesis for Line 2, 3, 4, 5 and 6. Lines 1 and 8 took longer to reach harvest ripeness, 94.6 and 90.8 days after anthesis, respectively.

At 86 days after anthesis (arbitrary point), seed moisture for 8 lines are also shown in Table 5.4. Seed moisture for Line 7 was less than that of harvest ripeness. Line 2 just reached harvest ripeness, whereas other lines did not reach harvest ripeness. However, no significant differences for seed moisture at 86 days were found amongst 8 lines. This might be due mainly to large standard errors for these estimates (Table 5.4).

The variance components estimates and the heritability estimates for seed moisture at 86 days after anthesis are shown in Table 5.5. Harvest ripeness proved to be largely influenced ($p<0.01$) by the environment ($h^2 = 0.3292$). It is not surprising that the environment had a large influence, since the definition of harvest ripeness is based on seed moisture content which itself is largely influenced by environmental variables, *e.g.* temperature and humidity.

5.4.2 Seed dry mass

After examination of initial plots, the power function was used to fit the growth patterns of seed dry mass of all lines.

$$Y = A X^B, \text{ or}$$

$$\ln Y = \beta_0 + \beta_1(\ln X)$$

where y: seed dry mass (mg),
x: age of seed expressed in days after anthesis (DAA).

The estimated statistics of the equations in their linear form and R^2 values are given in Table 5.6. Data points and the equations in power form are given in Figure 5.3-5.10.

Table 5.6 Estimated statistics of final curves (linear form) describing changes in seed dry mass during seed development in 8 lines.

Statistic	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8
Y intercept (β_0)	-5.7259	-5.5106	-4.2978	-4.1734	-7.5495	-5.3886	-4.7542	-5.4337
s.e. β_0	1.0175	0.6466	0.8015	0.4562	1.1513	0.8403	0.6069	1.0207
β_1	1.5369	1.5215	1.2683	1.2357	2.1170	1.3446	1.2655	1.4115
s.e. β_1	0.3346	0.2023	0.2428	0.1495	0.3508	0.2692	0.1930	0.3174
$\sigma_{x.y}^2$	1.0205	0.3263	0.3814	0.2566	0.9491	0.7039	0.3329	0.9345
R^2	0.6784	0.8372	0.6773	0.8507	0.7369	0.6575	0.7818	0.5855
F for regression	21.10**	56.59**	27.29**	68.35**	36.41**	24.95**	42.99**	19.78**
Equation form	$\ln Y = \beta_0 + \beta_1 (\ln X)$ (X: days from anthesis)							

**: Significance level at 1% level (P<0.01).

Table 5.7 The differences between functions of 8 lines for seed dry mass.

	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8
Line 1								
Line 2	ns							
Line 3	ns	ns						
Line 4	ns	(*)	ns					
Line 5	ns	ns	*	*				
Line 6	ns	ns	*	**	ns			
Line 7	ns	ns	ns	(*)	ns	ns		
Line 8	ns	ns	(*)	(*)	ns	ns	ns	

**: significance at the 1% probability level; (*): significance at the 10% probability level.
ns: no significance at the statistics level.

Table 5.8 Seed dry mass (mg) at harvest ripeness (HRDW) and at 86 days after anthesis for 8 lines.

Line	HRDW (mg) (SMC=12.5%)	Dry mass (mg) (86 days)	s.e.
Line 1	3.55	3.07 (1.120) ab	(0.5925)
Line 2	3.37	3.55 (1.267) ab	(0.3165)
Line 3	3.91	3.86 (1.352) ab	(0.3362)
Line 4	3.84	3.78 (1.331) ab	(0.2670)
Line 5	6.92	6.56 (1.880) a	(0.5061)
Line 6	1.83	1.83 (0.600) b	(0.4435)
Line 7	2.36	2.42 (0.883) ab	(0.3133)
Line 8	2.53	2.35 (0.854) ab	(0.4864)

Means with the different letter are significantly different at 10 % probability level.
Values in parentheses are the values of the linear form (lnY).

Table 5.9 Variance components and heritability estimates for seed dry mass at 86 days after anthesis.

Partition	Variance (σ^2)	s.e.	h^2	s.e.
Genotype	2.1437	1.1459	0.9238 **	0.0002
Environment	0.1769	0.0234		
Phenotype	2.3206	1.1461		

**: significance at the 1% probability level.

The differences between functions for 8 lines by Hotelling T^2 testing are shown in Table 5.7 for seed dry mass. The function of Line 3 was significantly different to Line 5 and 6 at 5 % probability level and different to Line 8 at 10% probability level. The function of Line 4 also differed significantly to Line 2, 7 and 8 at 10% probability level, to Line 5 at 5% probability level and to Line 6 at 1% probability level. The results might imply that there was also a general pattern for seed dry mass.

Seed mass at harvest ripeness is one of the important components of yield. Table 5.8 shows the seed dry mass (mg) at harvest ripeness and 86 days after anthesis. Seed dry mass at harvest ripeness for Line 1, 2, 3, and 4 ranged 3.37 to 3.91 mg, but for Line 6, 7, and 8 were 1.83 - 2.53 mg. Line 5 had relatively big seeds (6.92 mg). For seed dry mass at 86 days, a weak difference ($p<0.10$) was only found between Line 5 and Line 7.

Table 5.9 shows the variance components and the heritability estimates and their standard errors for seed dry mass. The results showed that seed dry mass at harvest ripeness is predominantly ($p<0.01$) controlled by genetics ($h^2 = 0.9238$).

5.4.3 Seed dormancy

During seed development, potential germination (GA_{4+7}) was almost nil for all 8 lines at various sampling, except 10% germination in line 3 and line 5 at 85 days after anthesis and 6.7% and 13.3% germination at 71 days after anthesis in line 4 and line 8, respectively. Standard germination was absolute nil for 8 lines. In this study, seeds that attained 90% potential germination were referred to as "embryo maturity". The results, therefore, indicated that embryo was immature.

Dormancy percentages were estimated as the ratio of difference between the standard germination and potential germination to potential germination. Thus, dormancy percentages were 100% for 8 lines. The results showed that primary dormancy for meadowfoam exists.

5.4.4 Seed germination (afterripening)

Considering the criteria of best fit, the quadratic function was chosen to describe the development of seed germination for 8 lines.

$$y = \beta_0 + \beta_1 X + \beta_2 X^2$$

where y: seed germination (%);
x: time of seed storage (weeks).

The estimated statistics of the equations and R^2 are given in Table 5.10. Data points and fitted curves for seed germination of 8 lines various treatments are given in Figure 5.11-5.18.

Table 5.10 Estimated statistics of final curves describing changes in seed germination during seed development various treatments and lines.

Treatment	Line	Y intercept (β_0)	s.e. β_0	β_1	s.e. β_1	β_2	s.e. β_2	$\sigma_{x,y}^2$	R^2	F for regression
GA and prechilling	1	-1.4300	7.3710	1.6771	2.2890	0.2522	0.1393	56.491	0.9126	52.226**
	2	3.3251	11.037	10.583	2.6020	-0.3065	0.1208	231.43	0.7395	29.803**
	3	5.5944	7.4804	6.4862	1.4692	-0.1192	0.0555	159.59	0.8113	51.592**
	4	-2.2927	6.3979	8.8690	1.2054	-0.2169	0.0423	123.79	0.8482	69.836**
	5	32.269	7.1552	7.2574	1.6669	-0.2133	0.0766	91.399	0.7637	30.695**
	6	46.152	15.418	-8.2476	6.8677	1.4266	0.6933	99.154	0.6674	10.034**
	7	6.5262	10.788	1.0154	3.1408	0.3136	0.1898	129.00	0.7902	22.605**
	8	20.550	13.508	-6.9306	6.0258	1.1305	0.5945	70.061	0.6567	7.651*
GA and non-chilling	1	2.1743	11.574	5.3474	3.5940	0.0305	0.2187	139.27	0.8116	21.543**
	2	21.027	7.6193	9.4902	1.7962	-0.3121	0.0834	110.28	0.7690	34.963**
	3	16.887	8.0660	6.1919	1.6090	-0.1237	0.0617	182.14	0.7427	30.302**
	4	40.978	7.2948	2.3932	1.3514	-0.0190	0.0483	135.40	0.6188	19.482**
	5	60.633	7.4805	0.9050	1.8268	0.0174	0.0847	99.823	0.3791	5.800*
	6	46.914	20.188	-3.4178	7.2260	0.7430	0.5770	284.68	0.4790	5.057*
	7	-4.6899	14.131	7.4054	4.1659	-0.0359	0.2528	218.97	0.7434	18.836**
	8	7.9830	13.623	2.1920	4.2302	0.2585	0.2574	192.95	0.7889	18.683**
Water and non-chilling	1	4.4189	10.553	1.0394	3.2769	0.3214	0.1994	115.78	0.8578	30.163**
	2	16.179	9.6329	5.8715	2.2584	-0.1042	0.1049	174.33	0.7359	27.867**
	3	-5.1450	6.9746	7.5267	1.3585	-0.1495	0.0510	133.38	0.8583	66.615**
	4	-12.202	7.1451	8.7157	1.3465	-0.1956	0.0472	154.49	0.8491	73.123**
	5	7.1615	6.9430	9.8672	1.6415	-0.3057	0.0764	91.542	0.8446	54.366**
	6	27.920	14.905	-6.7831	5.3351	1.0995	0.4260	155.19	0.7170	13.935**
	7	0.5139	7.7054	1.1034	2.3149	0.2861	0.1390	61.068	0.8885	47.807**
	8	-5.1199	6.4386	4.4136	2.0638	0.0921	0.1241	39.018	0.9445	76.621**
Equation form		$Y = \beta_0 + \beta_1 X + \beta_2 X^2$ (X: days from anthesis)								

**: Significance level at 1% level ($p<0.01$); *: Significance level at 5% level ($p<0.05$).

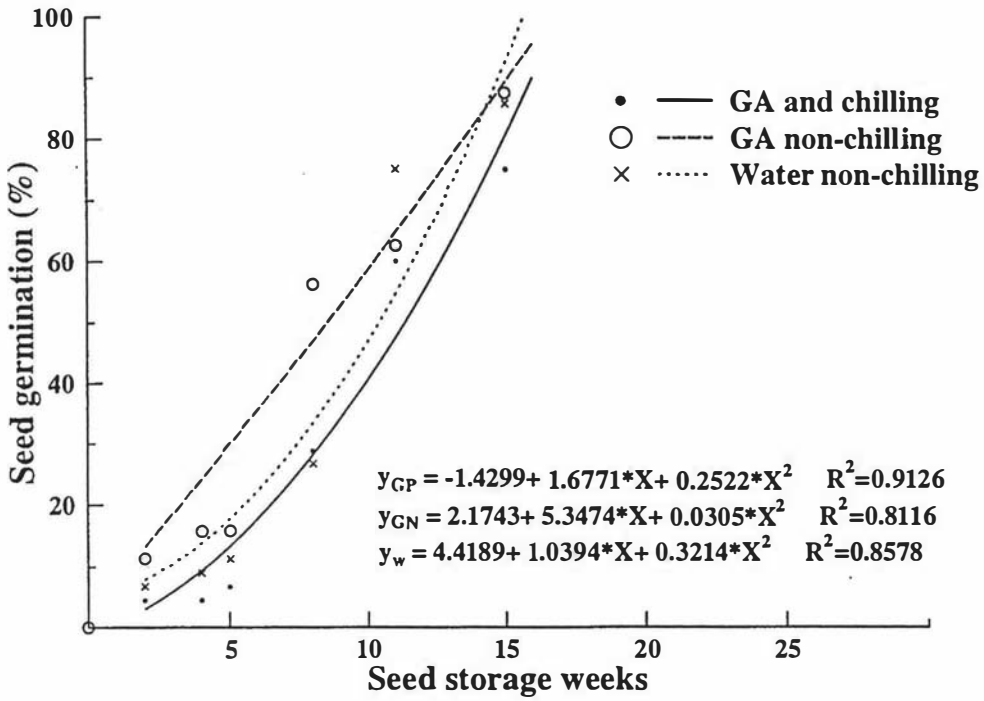


Figure 5.11 The functions of seed germination various treatments for Line 1.

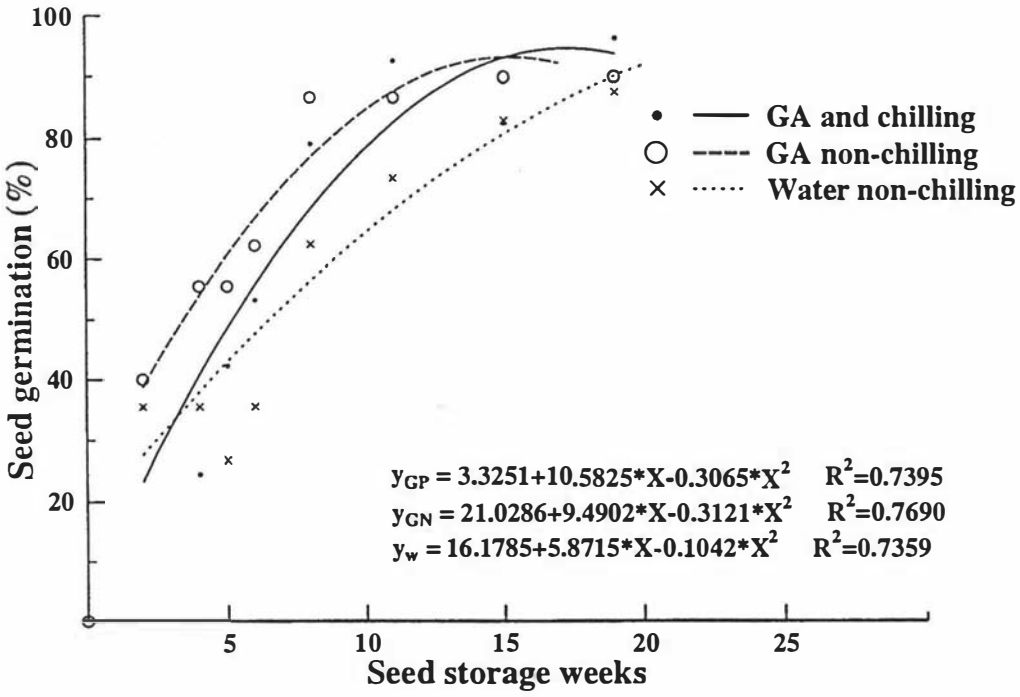


Figure 5.12 The functions of seed germination various treatments for Line 2.

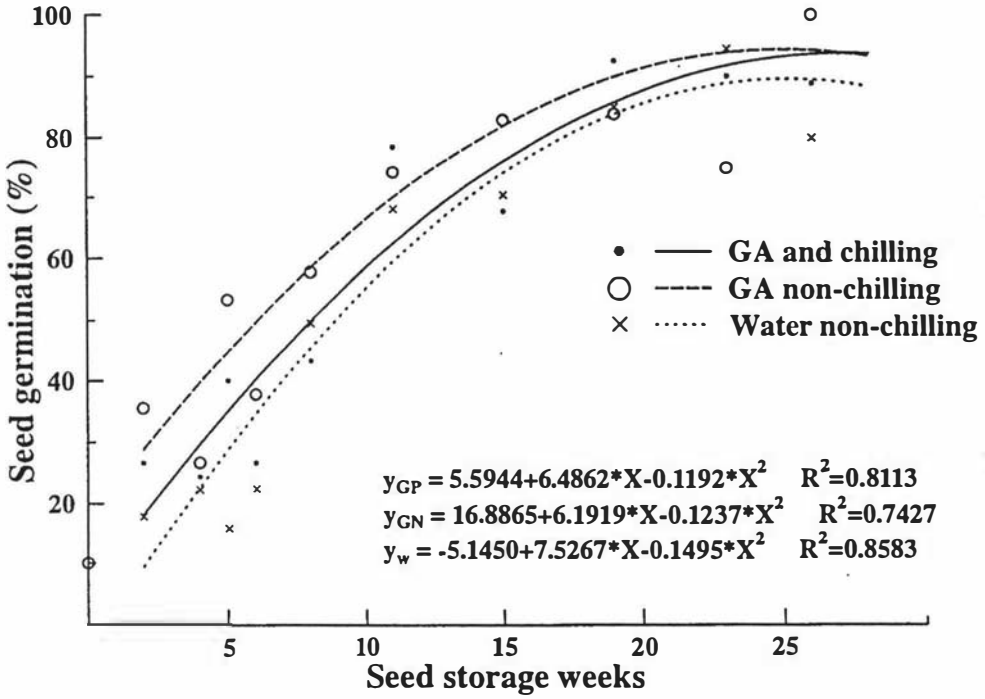


Figure 5.13 The functions of seed germination various treatments for Line 3.

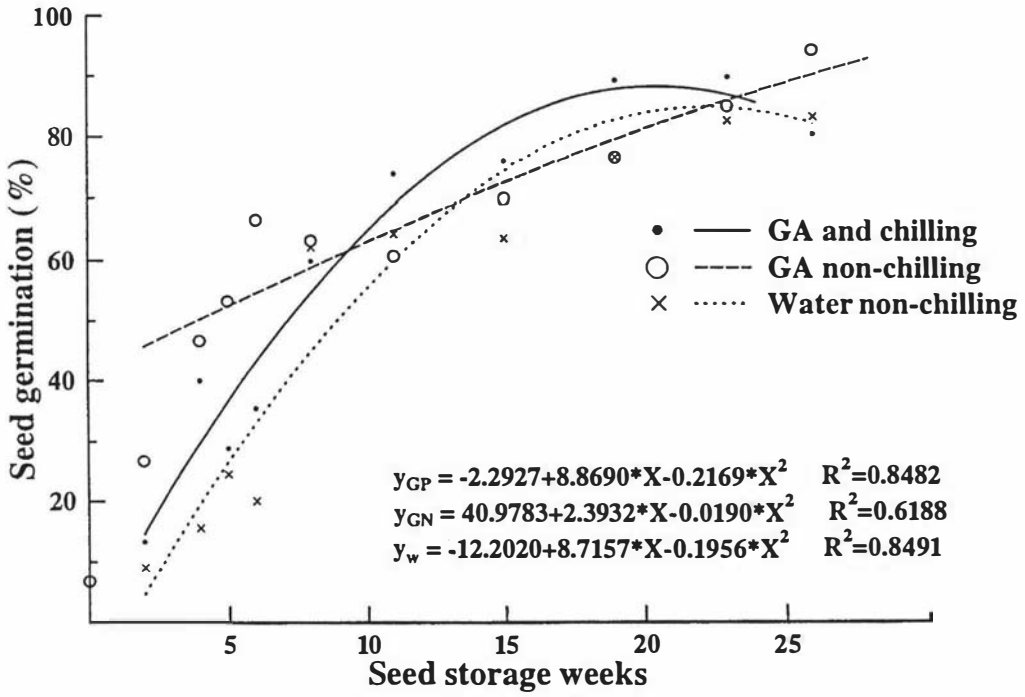


Figure 5.14 The functions of seed germination various treatments for Line 4.

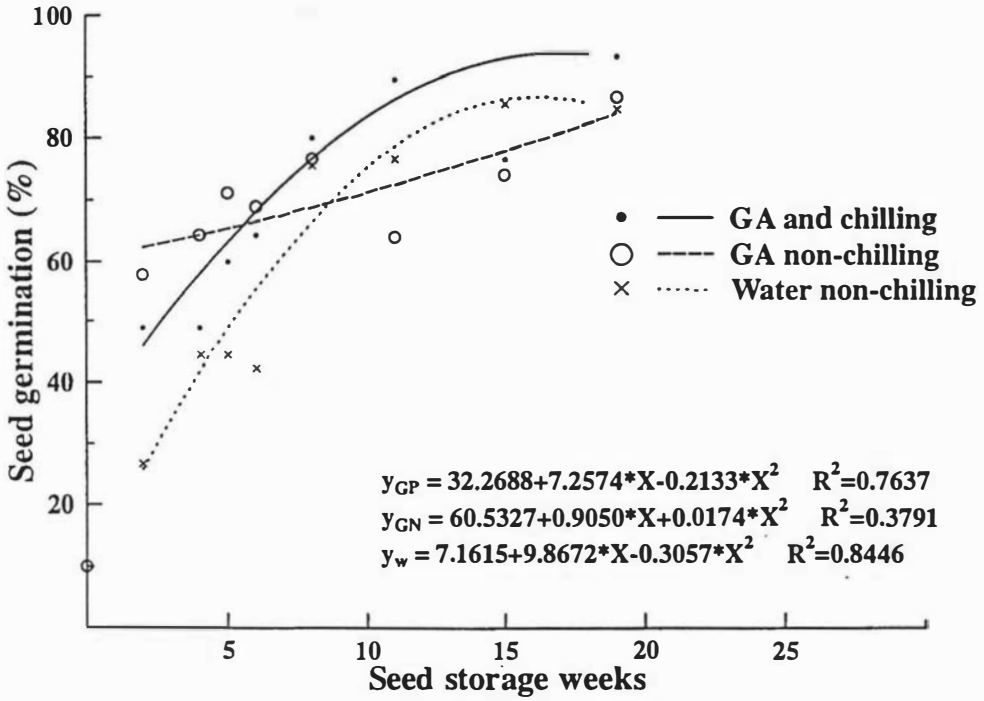


Figure 5.15 The functions of seed germination various treatments for Line 5.

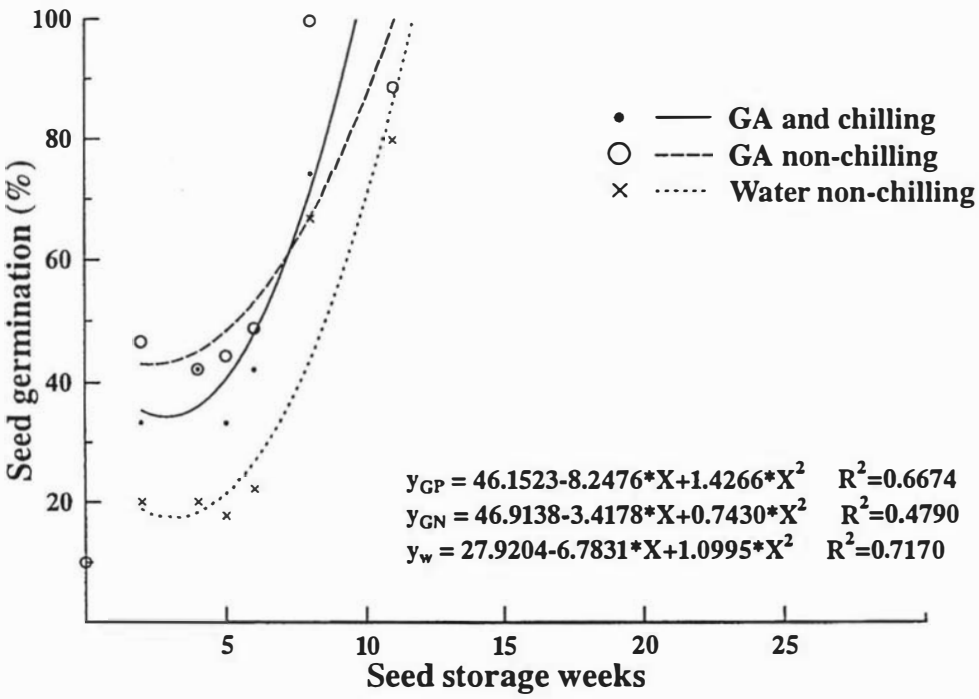


Figure 5.16 The functions of seed germination various treatments for Line 6.

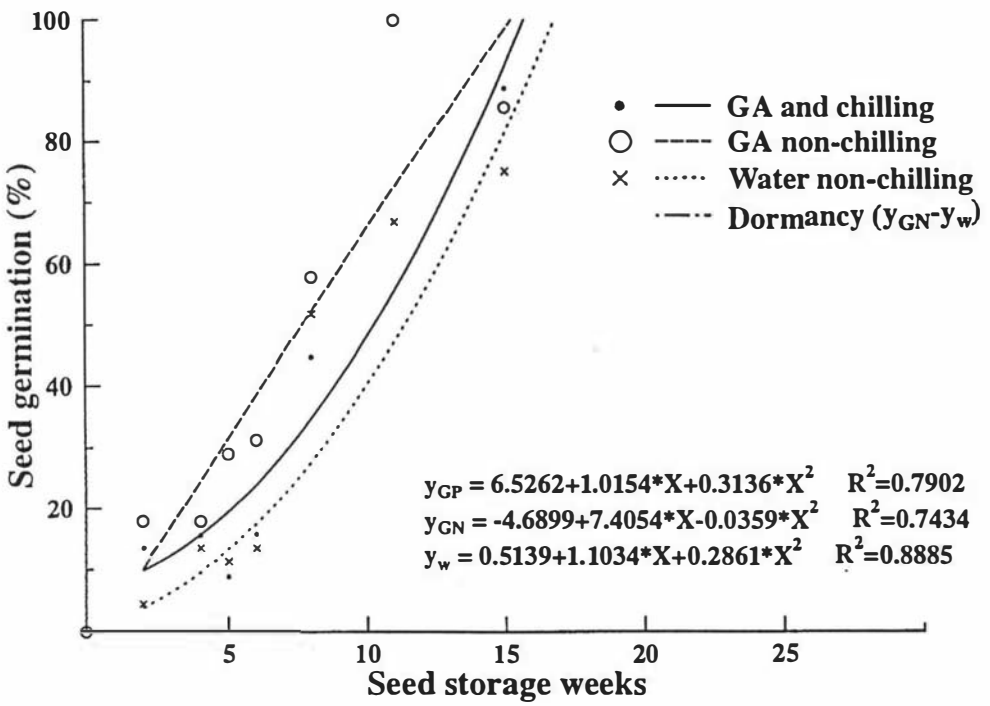


Figure 5.17 The functions of seed germination various treatments for Line 7.

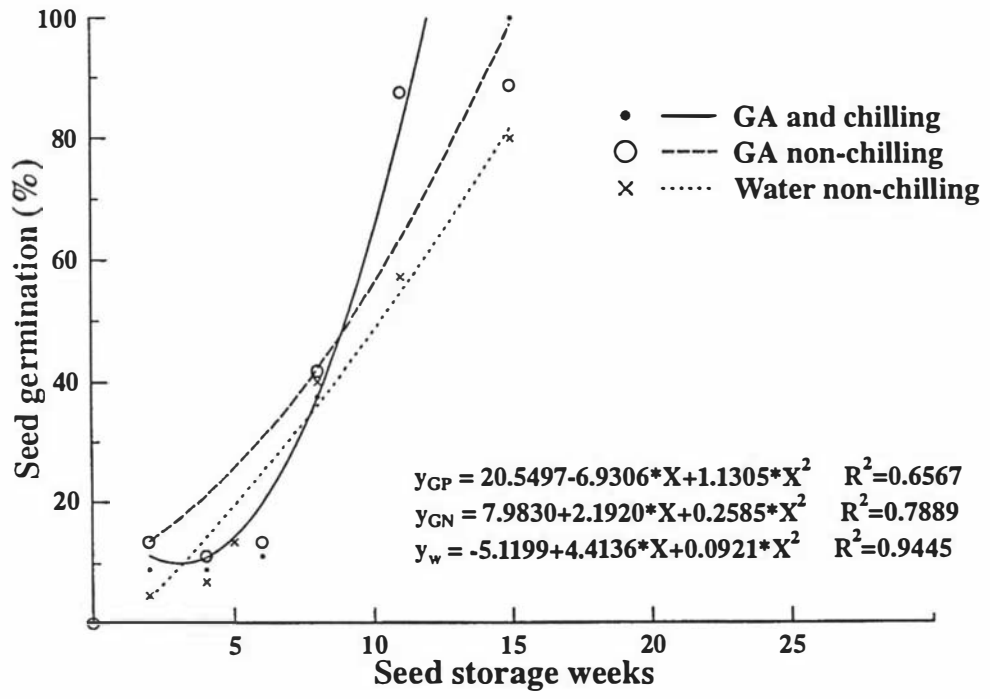


Figure 5.18 The functions of seed germination various treatments for Line 8.

5.4.4.1 Functions of seed germination comparison

The differences between functions of the 8 lines for three treatments were examined by Hotelling T^2 testing (Table 5.11). Most pairs of germination functions were significantly different at various probability levels. However, the functions of Line 1, Line 7 and Line 8 were similar, regardless of treatments. Similar results were found for Line 3 and Line 4. Line 6 was similar to all others in the GA_{4+7} with chilling test; but with GA_{4+7} without chilling, Line 6 was different from Lines 1, 5, 7 and 8. In water, Line 2 and Line 5 were similar as was Line 4 compared to Line 8.

Table 5.11 The differences between germination functions of 8 lines for three treatments.

	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8
	a. GA_{4+7} and prechilling							
Line 1								
Line 2	**							
Line 3	**	**						
Line 4	**	**	ns					
Line 5	**	**	**	**				
Line 6	ns	ns	ns	ns	ns			
Line 7	ns	**	**	**	**	ns		
Line 8	ns	**	**	**	**	ns	ns	
	b. GA_{4+7} and non-prechilling							
Line 1								
Line 2	**							
Line 3	(*)	**						
Line 4	**	**	ns					
Line 5	**	**	**	*				
Line 6	**	ns	ns	ns	*			
Line 7	ns	**	(*)	**	**	*		
Line 8	ns	**	*	**	**	**	ns	
	c. Water and non-prechilling							
Line 1								
Line 2	**							
Line 3	*	*						
Line 4	(*)	**	ns					
Line 5	**	ns	**	**				
Line 6	(*)	**	(*)	*	**			
Line 7	ns	**	**	**	**	**		
Line 8	ns	**	*	ns	**	*	ns	

**: significance at the 1% probability level; *: significance at the 5% probability level; (*): significance at the 10% probability level.
ns: no significance at the statistics level.

Table 5.12 The differences between functions of the three treatments for 8 lines.

		Water	GA_{4+7} and nonchilling
Line 1	GA_{4+7} and prechilling	ns	*
	GA_{4+7} and nonchilling	ns	---
Line 2	GA_{4+7} and prechilling	**	**
	GA_{4+7} and nonchilling	**	---
Line 3	GA_{4+7} and prechilling	ns	ns
	GA_{4+7} and nonchilling	**	---
Line 4	GA_{4+7} and prechilling	(*)	**
	GA_{4+7} and nonchilling	**	---
Line 5	GA_{4+7} and prechilling	**	*
	GA_{4+7} and nonchilling	**	---
Line 6	GA_{4+7} and prechilling	ns	ns
	GA_{4+7} and nonchilling	**	---
Line 7	GA_{4+7} and prechilling	ns	ns
	GA_{4+7} and nonchilling	**	---
Line 8	GA_{4+7} and prechilling	ns	ns
	GA_{4+7} and nonchilling	ns	---

**: significance at the 1% probability level; *: significance at the 5% probability level; (*): significance at the 10% probability level. ns: no significance at the statistics level.

The differences between germination functions of the three treatments for the 8 lines were also examined by Hotelling T^2 test (Table 5.12). During dry storage, GA_{4+7} without chilling led to an improvement ($p<0.01$) in the germination percentage, except in Line 1 and 8. GA_{4+7} with prechilling, compared with water treatment, increased germination percentage at 1% probability level in Line 2 and 5, and in Line 4 at 10% probability level. Prechilling did not advance dormancy-breaking for other lines.

Germination was better in GA_{4+7} without chilling than in GA_{4+7} with chilling for Line 1, 2, 4 and Line 5 (Table 5.12).

5.4.4.2 Dormancy release by afterripening

Two key stages, *e.g.*, after 5 and 15 weeks storage, were selected to investigate the effect of afterripening (releasing dormancy). After 5 and 15 weeks storage, germination and the standard errors for various treatments are given in Table 5.13. In GA_{4+7} with and without prechilling, there were three groups after 5 weeks storage. Line 2 and 5 had the highest germination ($p<0.01$). Line 1, 7 and 8 gave the lowest germination. Medium

Table 5.13 Germination after 5 and 15 weeks storage for various treatments and lines.

Line	GA_{4+7} and Nonchilling		GA_{4+7} and Prechilling		Water	
	Germination (%)	s.e.	Germination (%)	s.e.	Germination (%)	s.e.
a. Seed storage at 5 weeks						
Line 1	29.7 c	3.9478	13.3 c	2.5143	17.7 c	3.5995
Line 2	60.7 a	2.6083	55.5 a	3.7783	42.9 a	3.3854
Line 3	44.8 b	3.3573	35.0 b	3.0758	28.7 b	4.8584
Line 4	52.5 b	3.0159	36.6 b	2.7229	26.5 b	3.0353
Line 5	65.5 a	2.5730	63.2 a	2.4759	48.9 a	2.4448
Line 6	48.4 b	5.5381	40.6 b	0.0100	21.6 bc	4.0889
Line 7	31.4 c	4.1903	19.4 c	3.2606	13.2 c	2.3254
Line 8	25.4 c	4.6467	14.2 c	3.6637	19.3 c	2.2767
b. Seed storage at 15 weeks						
Line 1	89.2 ab	11.1563	80.5 bc	7.1052	92.3 ab	10.1719
Line 2	93.2 a	3.4274	100.0 a	4.9649	80.8 ab	4.3118
Line 3	81.9 ab	4.6165	76.1 c	3.7653	74.1 ab	20.5675
Line 4	72.6 b	3.7797	81.9 c	3.7066	74.5 b	4.0895
Line 5	78.0 b	3.4629	93.1 ab	3.9956	86.4 ab	3.1349
Line 6	100.0 ab	46.8183	100.0 a	0.0100	100.0 ab	34.5670
Line 7	98.3 ab	13.9172	92.3 abc	11.2211	81.4 ab	7.3518
Line 8	99.0 ab	13.1312	100.0 abc	58.9448	81.8 ab	5.9050

Means with the different letter are significantly different at 5% probability level.

germination was found for Line 3, 4 and 6. In water treatment after 5 weeks storage, there were similar groups as for the two GA treatments, except for Line 6 overlapping the medium and the lowest germination groups.

After 15 weeks storage, standard errors for estimates in some lines were huge so that the significance tests between lines were insensitive. This was not a surprise, because the variance of $\hat{Y}$ ($\sigma_{\hat{Y}}^2$) is usually smaller around the middle X values of regression. There was a trend that Line 3 and 4 had lower germination, regardless of treatments. In Line 6, dormancy was completely released after 15 weeks storage.

Germination after 5 weeks storage was significantly ($p<0.05$) higher in GA_{4+7} without chilling than in water, except for Line 8 (Table 5.14). GA_{4+7} with chilling after 5 weeks storage also resulted in higher germination ($p<0.05$) in Line 2, 4, 5, and 6, compared with water treatment. After 15 weeks storage, Line 2 with GA, regardless of chilling,

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

		Storage at 5 weeks		Storage at 15 weeks	
		Water	GA_{4+7} and nonchilling	Water	GA_{4+7} and nonchilling
Line 1	GA_{4+7} and prechilling	ns	**	ns	ns
	GA_{4+7} and nonchilling	*	---	ns	---
Line 2	GA_{4+7} and prechilling	*	ns	**	ns
	GA_{4+7} and nonchilling	**	---	*	---
Line 3	GA_{4+7} and prechilling	ns	*	ns	ns
	GA_{4+7} and nonchilling	**	---	ns	---
Line 4	GA_{4+7} and prechilling	*	**	ns	(*)
	GA_{4+7} and nonchilling	**	---	ns	-
Line 5	GA_{4+7} and prechilling	**	ns	ns	**
	GA_{4+7} and nonchilling	**	---	(*)	---
Line 6	GA_{4+7} and prechilling	**	ns	ns	ns
	GA_{4+7} and nonchilling	**	---	ns	---
Line 7	GA_{4+7} and prechilling	ns	*	ns	ns
	GA_{4+7} and nonchilling	**	---	ns	---
Line 8	GA_{4+7} and prechilling	ns	(*)	ns	ns
	GA_{4+7} and nonchilling	ns	---	ns	---

**: significance at the 1% probability level; *: significance at the 5% probability level; (*): significance at the 10% probability level. ns: no significance at the statistics level.

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

Partition	Storage at 5 weeks				Storage at 15 weeks			
	Variance (σ^2)	s.e.	h^2	s.e.	Variance (σ^2)	s.e.	h^2	s.e.
a. GA_{4+7} and Nonchilling								
Genotype	219.370	117.258	0.9437 **	0.0001	109.274	58.4094	0.3036 **	0.0016
Environment	13.0996	1.5385			250.669	29.4395		
Phenotype	232.469	117.268			359.943	65.4091		
b. GA_{4+7} and Prechilling								
Genotype	342.521	183.085	0.9752 **	0.0001	94.7656	50.6544	0.2618 **	0.0013
Environment	8.7046	1.0223			267.277	31.3901		
Phenotype	351.226	183.088			362.043	59.5920		
c. Water								
Genotype	157.393	84.1303	0.9306 **	0.0001	77.1574	41.2423	0.2787	0.0014
Environment	11.7342	1.3737			199.735	23.3772		
Phenotype	169.131	84.1415			276.892	47.4070		

**: Significance level at 1% level ($P<0.01$).

gave higher germination. A weak difference ($p<0.10$) between GA_{4+7} without chilling and water was also found in Line 5. No differences were detected for other lines amongst treatments. The results indicated that requirements of external GA for germination became less as storage time increased.

For GA treatments, germination was markedly higher ($p<0.05$) in nonchilling than in prechilling after 5 weeks storage for Line 1, 3, 4 and 7 (Table 5.14). Similar results were found in Line 8 ($p<0.10$). In contrast to that, prechilling gave higher germination ($p<0.01$) in Line 5 after 15 weeks storage. The same result was also found in Line 4 ($p<0.10$).

Complete relief of dormancy needed dry storage for between 10 to 25 weeks for the various lines (Figure 5.11-5.18). Dry storage for Line 6 required only 10 weeks, but around 25 weeks for Line 3 and 4. Line 1, 7 and 8 required 15 weeks afterripening, whereas Line 2 and 5 required 20 weeks. It may therefore be concluded that variation in depth of dormancy exists amongst lines. Following afterripening, dormant seeds were capable of maximum germination, even in water at constant temperatures.

The variance components and heritability for seed germination after 5 and 15 weeks storage are shown in Table 5.15. The heritability estimates were high after 5 weeks storage, regardless of treatments, whereas they were low after 15 weeks storage. The results indicated that seed germination was influenced by genetic differences at early seed storage. As storage time increased, seed germination was influenced more by environmental factors.

5.4.5 Measurement and comparison of seed ABA

ABA was measured in seeds during seed development (Figure 5.19-5.26). There were two peaks for most lines, but only one peak for Line 7 and Line 8. The curves of ABA concentration with time of seed developing did not follow any functions and did not have replications either. Thus, regression analysis and analysis of variance could not be applied for the ABA curves (as in Chapter 4). However, the detection of the peak differences amongst lines was effected by PC analysis, using peak height, width and peak time for two peaks.

Table 5.16 presents the eigenvalues and cumulative percentage of variation for each component. According to solving rule (the basis of 70% of the total variances explained), the first two PC components were used to summarise data. Table 5.17 presents the factor structure matrix and the factor scores for the first two PC components.

Table 5.16 PC analysis for ABA peaks of 8 lines.

Component	Eigenvalue	Proportion of variation	Cumulative percentage
1	3.2258	0.5376	53.76
2	1.6055	0.2676	80.52
3	0.7832	0.1305	93.57
4	0.2544	0.0424	97.81
5	0.1255	0.0209	99.91
6	0.0057	0.0009	100.00

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

	Factor structure matrix		Factor scores	
	Component 1	Component 2	Component 1	Component 2
Height 1	-0.1549	0.9282	-0.0480	0.5781
Height 2	0.8188	-0.2902	0.2538	-0.1808
Time 1	-0.1815	-0.7100	-0.0563	-0.4422
Time 2	0.9294	-0.1377	0.2881	-0.0858
Width 1	-0.9704	-0.0830	-0.3008	-0.0517
Width 2	0.8326	0.3603	0.2581	0.2244

The total variation was accounted for by component 1 and 2, 53.76% and 26.76%, respectively. Thus, component 1 was the most important. Peak 2 (height, width and time reaching the peak) was highly correlated with PC component 1 and had higher standard coefficients (Table 5.17). The width of Peak 1 was negatively correlated with PC component 1 and had a higher negative standard coefficient as well. Therefore, PC component 1 was associated with all properties of ABA Peak 2, and contrasted with width of ABA Peak 1. It could be named the "Peak 2 predominance" factor. The height of Peak 1 was highly associated with PC component 2 and had the highest standard coefficient. Time reaching Peak 1 was negatively correlated with PC component 2 and had a higher negative standard coefficient. Thus, PC component 2 highlighted early height of ABA Peak 1, and so could be named the "Early Peak 1" factor.

The scatter plot of the lines for first two scores are shown in Figure 5.27. The results of cluster analysis showed that there were 4 groups for changes in ABA content during seed development (Figure 5.28). Group A had a high PC score 1 and a low PC score 2, which included Line 1, 3, 5 and 6. These lines had an open Peak 2, but various in Peak 1. In Line 1 and 3, there were a high Peak 2, 24.53 $\mu g\ g^{-1}$ dry weight on 72 days after anthesis and 14.64 $\mu g\ g^{-1}$ dry weight on 85 days, respectively. The Peak 1 was high in Line 3 (18.51 $\mu g\ g^{-1}$ dry weight on day 33), but small in Line 1 (6.44 $\mu g\ g^{-1}$ dry weight on day 30). In Line 5 and 6, Peak 1 was around 11.5 $\mu g\ g^{-1}$ dry weight on day 34 and 28, respectively; and Peak 2 was low, 7.57 $\mu g\ g^{-1}$ dry weight on 86 days and 10.76 $\mu g\ g^{-1}$ dry weight on 71 days.

Group B (Line 2 and 4) had high scores for both PC score 1 and score 2. These lines exhibited a narrow high Peak 1 and small Peak 2. In Line 2, Peak 1 (26.43 $\mu g\ g^{-1}$ dry weight) was found on 33 days after anthesis; Peak 2 (8.08 $\mu g\ g^{-1}$ dry weight) was on 72 days. In Line 4, Peak 1 (21.35 $\mu g\ g^{-1}$ dry weight) occurred on 26 days; Peak 2 (11.14 $\mu g\ g^{-1}$ dry weight) was on 55 days.

Group C and Group D was only a single line, Line 7 and 8, respectively, which had a low PC score 1. Line 7 had a high PC score 2, but Line 8 did not. Neither line had a second peak. In Line 7, there was a high Peak 1 (26.15 $\mu g\ g^{-1}$ dry weight) on 21 days, whereas Line 8 had only a small Peak 1 (11.48 $\mu g\ g^{-1}$ dry weight) on 44 days.

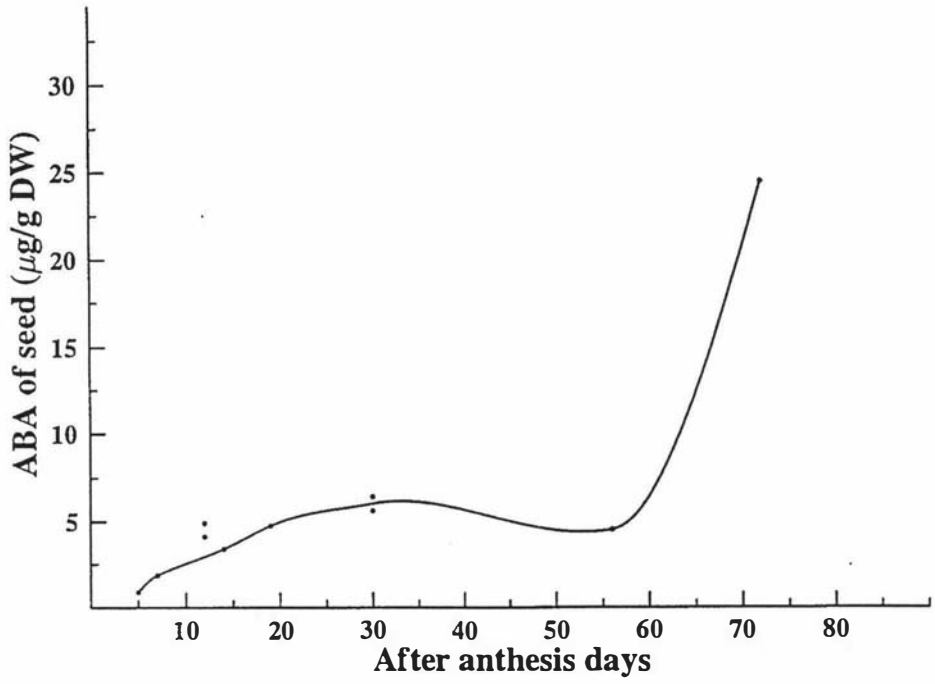


Figure 5.19 Time-dependent changes in ABA content of developing seeds for Line 1.

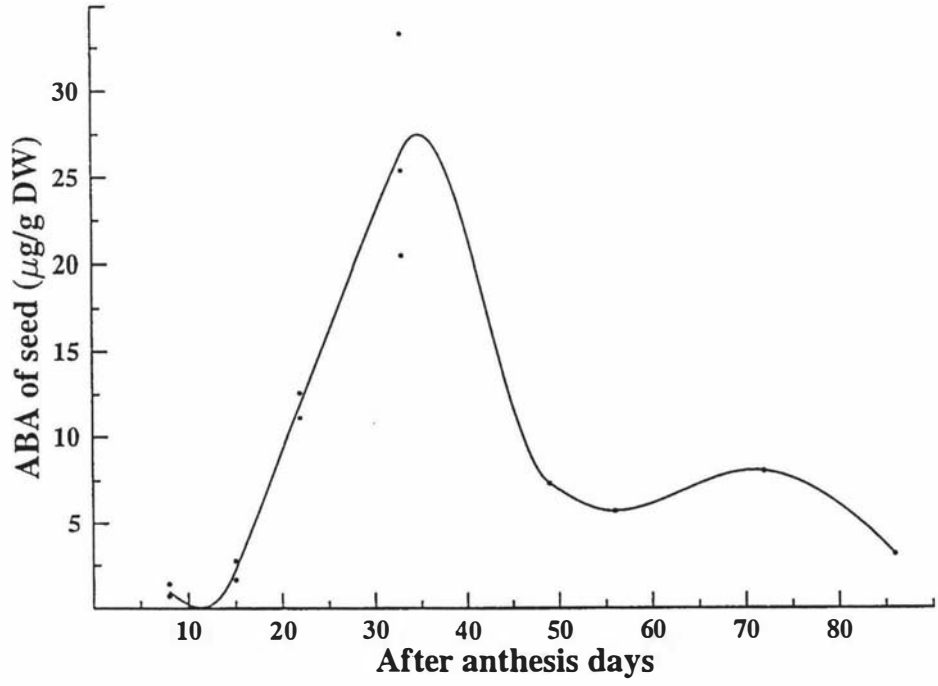


Figure 5.20 Time-dependent changes in ABA content of developing seeds for Line 2.

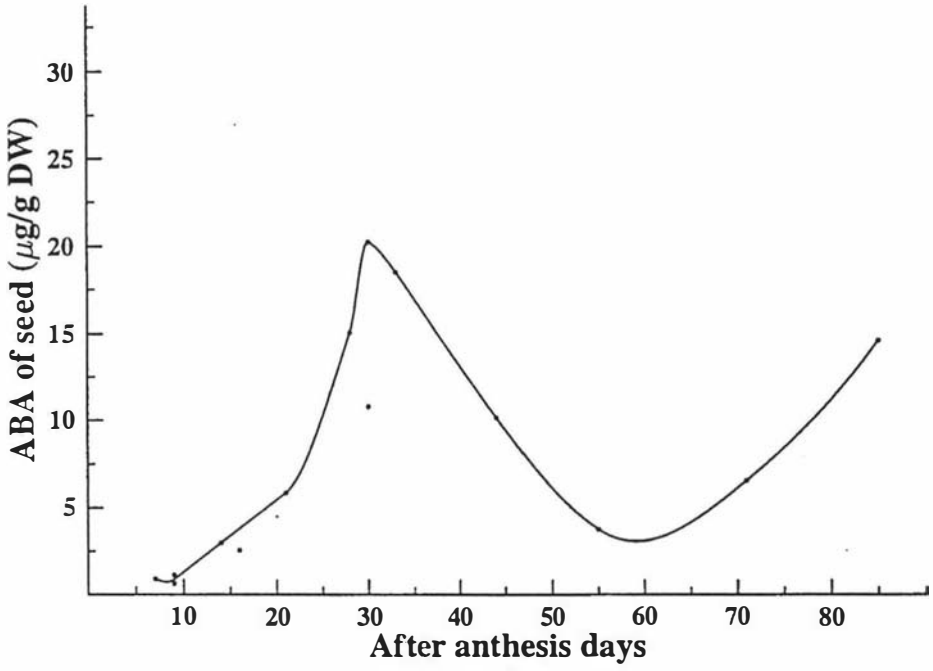


Figure 5.21 Time-dependent changes in ABA content of developing seeds for Line 3.

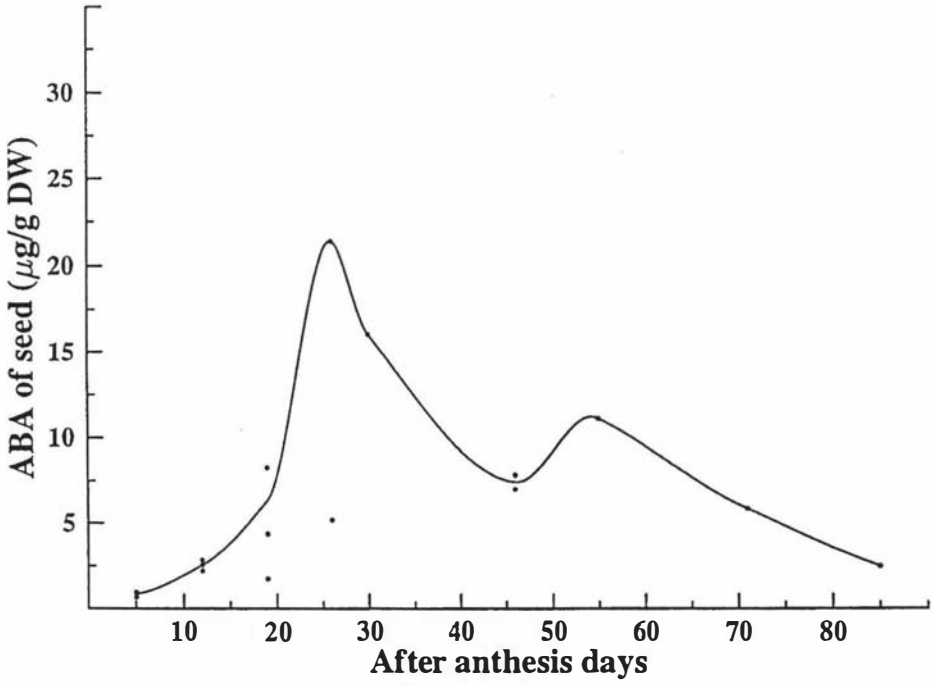


Figure 5.22 Time-dependent changes in ABA content of developing seeds for Line 4.

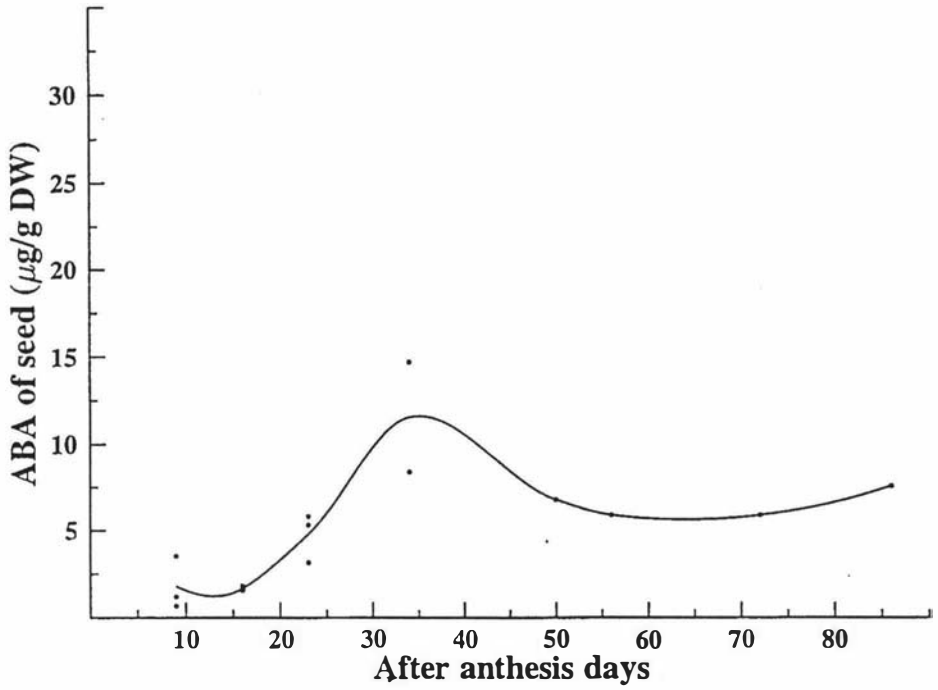


Figure 5.23 Time-dependent changes in ABA content of developing seeds for Line 5.

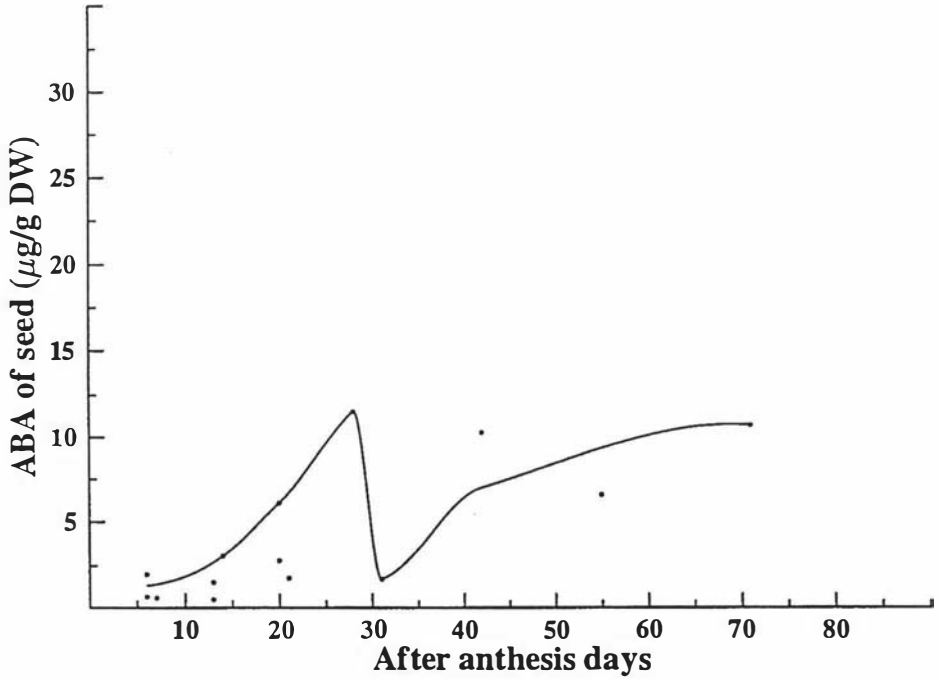


Figure 5.24 Time-dependent changes in ABA content of developing seeds for Line 6.

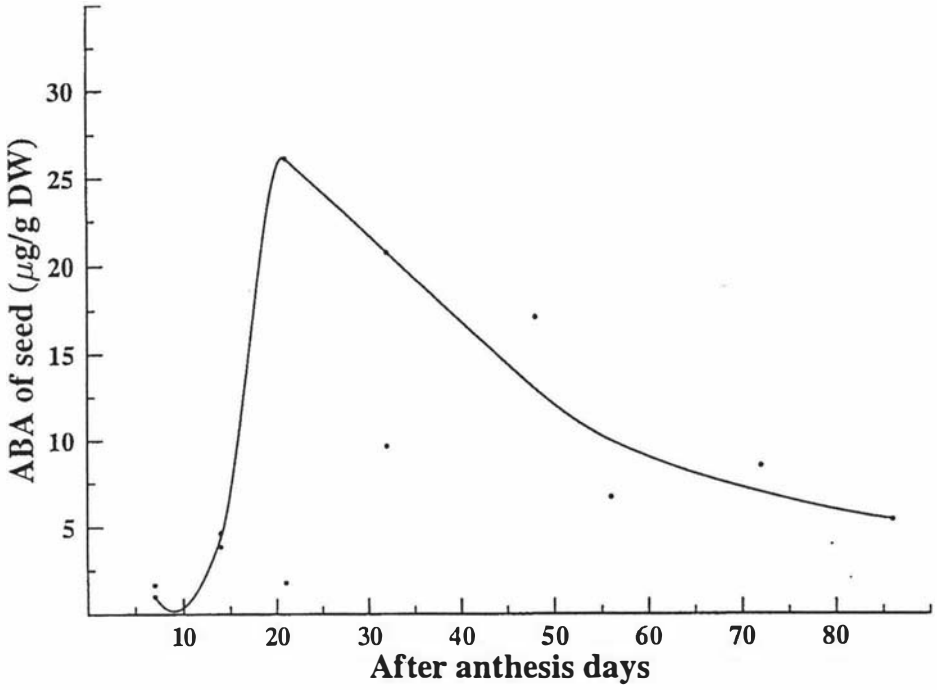


Figure 5.25 Time-dependent changes in ABA content of developing seeds for Line 7.

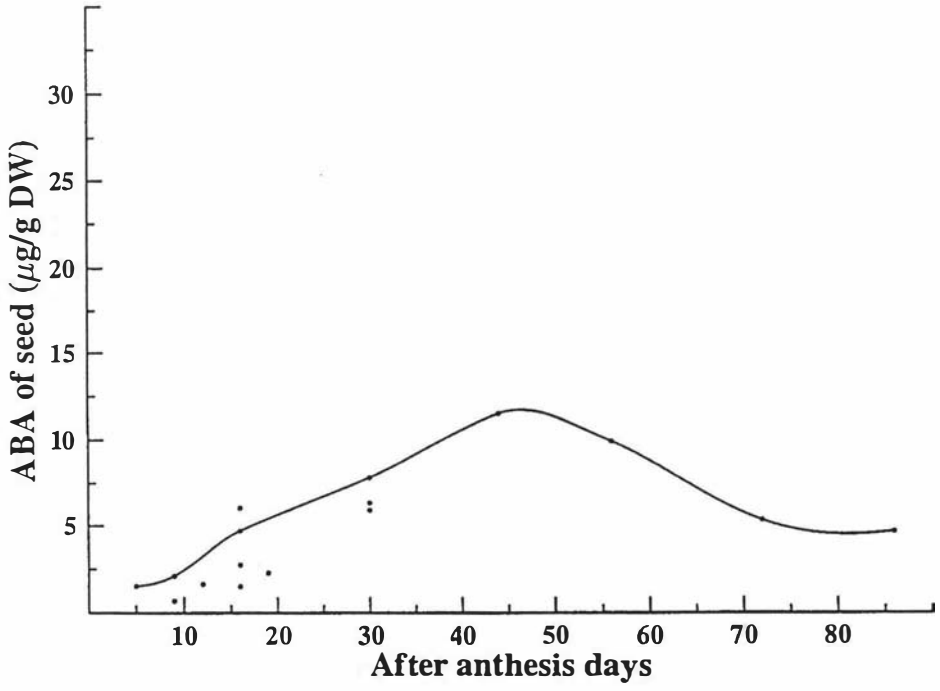


Figure 5.26 Time-dependent changes in ABA content of developing seeds for Line 8.

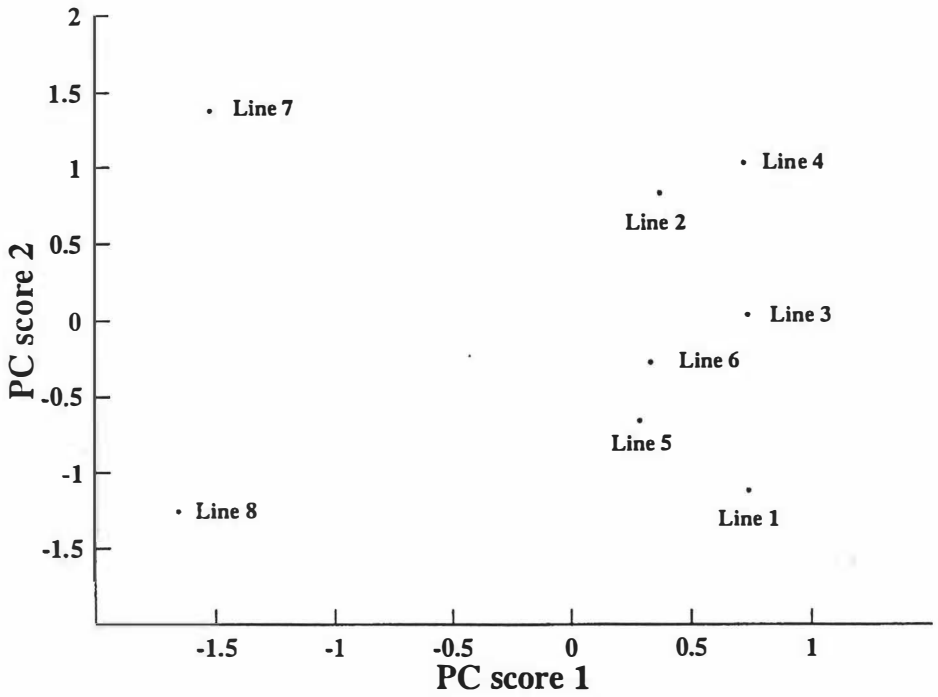


Figure 5.27 Distribution of the lines according to their response patterns on the basis of the first two PC scores.

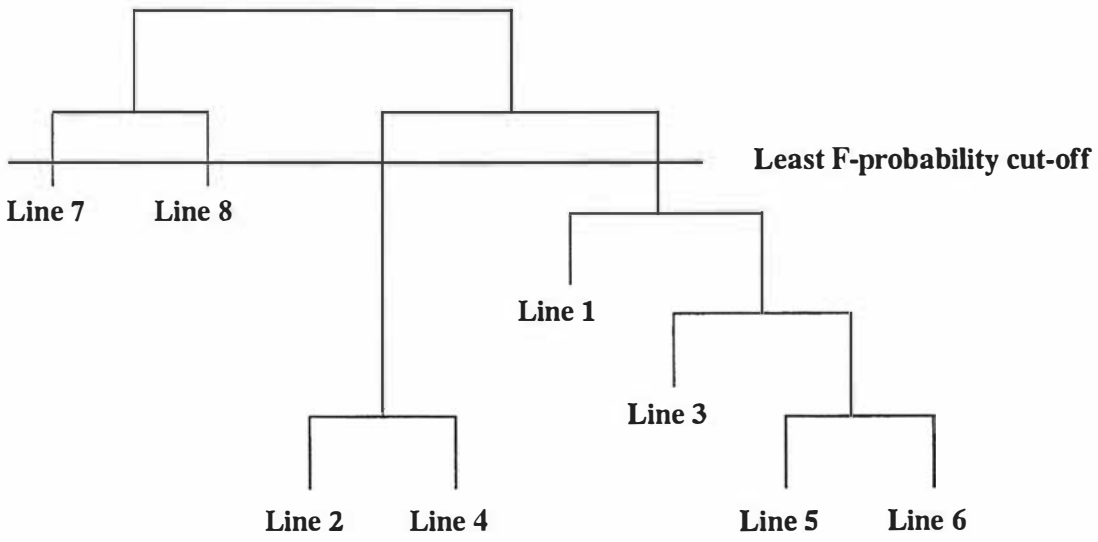


Figure 5.28 Dendrogram of cluster analysis by Ward's method for ABA content patterns during seed development.

5.5 Discussion

5.5.1 Seed development

In this study, several pairs of functions for seed dry mass and seed moisture were significantly different (Table 5.3 and 5.7), which indicated that there was a general pattern for seed moisture and seed dry mass. A general pattern for seed dry mass of 8 lines is presented in Figure 5.3-5.10. Seed dry mass increased slowly until about 30 days after anthesis. After this early increase, there was a relatively rapid increase in seed mass. Seed dry mass at harvest ripeness was 1.8 to 3.9 mg, except in Line 5 (6.9 mg) (Table 5.8).

A general pattern for seed dehydration in meadowfoam showed that moisture concentration declined slowly during early development stage, but the decline became more rapid later, reaching a maximum rate at around 30 days after anthesis (Figure 5.3-5.10). The seed moisture content values at the start of experiment (5 or 7 days after anthesis) ranged from 77.2% to 83.8%.

Harvest ripeness has been defined at 12.5% grain moisture in wheat (Gordon *et al.*, 1979). These authors found that the grain weights reached about 92 - 99% of the upper asymptotes at 12.5% moisture for the four wheat cultivars. In this study, harvest ripeness for meadowfoam seed was also defined as the time when the seed reaches 12.5% moisture content. Line 7 was the earliest to reach harvest ripeness (84.4 days after anthesis), whereas Line 1 and Line 8 took longer, 94.6 and 90.8 days after anthesis, respectively (Table 5.4). The others were around 86.0 to 88.2 days after anthesis.

The control of dehydration in developing seeds is probably biphasic. In young developing seeds, the maternal plant plays an important role by transferring nutrients, water and minerals, *via* the transfer cells to the young seeds. The efficiency of the maternal plant in these processes is reflected by the rate of growth and development of the young seed. After the connection with the maternal plant is cut severed, the environment exerts a greater influence on the rate and amount of water loss from the seed. Initial decline in moisture concentration arose from a large increase in seed dry

matter; and later decline arose from loss of water (Bewley and Black, 1994). These results also indicated that weight of water actually increased in the early stages of seed development (Figure 5.3-5.10), but eventually, the increase in dry mass exceeded this.

5.5.2 Seed ABA content

The present study has clearly demonstrated a biphasic distribution or single peak in the ABA content of the meadowfoam seeds assayed by ELISA (Figure 5.19-5.26). The free ABA content in seeds is high part way through development and is relatively low or even absent at maturity. The biphasic pattern found here is typical of that described by Black (1991). ABA content is extremely low early in development but rises as development proceeds, to reach a maximum at about one-third to one-half of the time from seed initiation to maturity, and then falls to a low value (Black, 1991). Dual peaks of ABA accumulation were also found in *Phaseolus vulgaris* (Hsu, 1979) and *Pisum sativum* (Wang *et al.*, 1987).

Principal components and cluster analysis successfully demonstrated that the eight lines could be divided into four groups with regard to the seed ABA peak height, width and time of peak (Figure 5.27 and 5.28). Group A included Line 1, 3, 5 and 6, which had an open Peak 2, but variation in Peak 1. Group B included Line 2 and 4, which had a narrow high Peak 1 and a small Peak 2. Group C was only Line 7, which had a high single peak. Group D was only Line 8, which had a low single peak.

ABA content during seed development depends on the species, and is under genetic control. During development of wheat, maximum ABA levels of embryo were approximately 1.0 and 0.8 $\mu g\ g^{-1}$ dry weight for Brevor (sprouting-susceptible) and Greer (sprouting-resistant), respectively (Walker-Simmons, 1987). In *Sorghum bicolor*, ABA content in intact developing seeds ranged approximately from 0.16 to 0.23 $\mu g\ g^{-1}$ dry weight (Benech Arnold *et al.*, 1995). In alfalfa seeds, the highest concentration of ABA was found in the endosperm (about 500 pmol mg^{-1} dry weight, or 132 $\mu g\ g^{-1}$ dry weight), peaking at about five times the value in the embryo and testa (140 pmol mg^{-1}

dry weight, or 37 μg g^{-1} dry weight), though the peak in the embryo was earlier (Xu *et al.*, 1990). In the present study, the maximum ABA content for 8 lines varied from 11.48 to 26.43 μg g^{-1} dry weight during seed development (Figure 5.19 - 5.26). In general, there were relatively high ABA peaks during seed development of meadowfoam.

5.5.3 The relationship between endogenous ABA and seed dormancy

In this study, seed germination during development was almost nil for the 8 lines. The results demonstrated that primary dormancy developed in seeds when they were on the mother plant and agrees with an earlier report that primary dormancy exists in meadowfoam seeds (Jolliff *et al.*, 1994). Lack of seed germination may depend on the presence of ABA during development, because the content of ABA was high for all eight lines (Figure 5.19-5.26). It has been concluded that *in situ* ABA synthesis was required to impose and maintain embryo dormancy (Le Page-Degivry and Garello, 1992). The availability of ABA-deficient and -responsive mutants, especially of *Arabidopsis thaliana* (Koornneef *et al.*, 1982;1984; Karssen *et al.*, 1983), tomato (Groot and Karssen, 1992) and maize (McCarty and Carson, 1991) has made a major contribution to the present notion that the absence of, or insensitivity to ABA during seed development results in the formation of non-dormant seeds.

Significantly different patterns amongst lines were observed for seed germination (Table 5.11), regardless of treatments. The results showed that seed dormancy of meadowfoam was alleviated after 10 to 25 weeks of dry storage in the various lines (Figure 5.11-5.18). The differential time requirement for afterripening may be regarded as an expression of differences in degree of dormancy. Thus, Line 3 and 4 had the deepest dormancy, whereas Line 6 exhibited light dormancy (Figure 5.11-5.18), with the others intermediate. Jolliff *et al*, (1994) also reported that there was the significant variation among the newly developed lines for their seed germination and dormancy characteristics.

In this study, the differential time requirement for afterripening might imply that the residual amount of ABA may affect the onset of seed germination. There are indications that the residual amount of ABA in tomato seeds is reduced after one year of dry storage (Hilhorst and Karssen, 1992). Afterripening reduces seed ABA content to amounts comparable with those in seeds from ABA-deficient *sitiens* mutant plants (Groot and Karssen, 1992). However, afterripening wild type seeds still had a slightly longer lag-time for germination than *sitiens* mutant seeds. It has been reported that slow drying (3 days) of dormant *Helianthus* embryos caused ABA levels to drop substantially while germinability increased (Bianco *et al.*, 1994). However, subsequent dry storage for 6 weeks did not further reduce ABA content but did promote germinability. Dry storage removes dormancy but a causal relationship between ABA content of the dry seeds and germination remains to be established (Karssen, 1995). Groot and Karssen (1992) argued that the differences in dormancy between the mature seeds are not a result of actual ABA levels in the mature seeds or fruits but a result of differences in ABA levels during seed development.

During seed development of meadowfoam, ABA content reached one or two peaks in various lines (Figure 5.19-5.26). In *Arabidopsis* and maize it has been shown that the first peak is associated with a maternal biosynthetic origin, whereas the second has a zygotic origin (Karssen *et al.*, 1983; Hole *et al.*, 1989). In this study, the first ABA peak (maternal ABA) was high in Line 2, 3, 4 and 7 (Figure 5.19-5.26). There was a high second ABA peak (embryo ABA) in Line 1 and 3. Line 3, one of deep dormancy, agrees with the report by Karssen *et al.* (1983) that dormancy is initiated only when the embryo itself can produce ABA. For Line 4 (one of deep dormancy), one might argue that maternal ABA may contribute to seed dormancy and/or that sensitivity to ABA may be involved. In *Arabidopsis*, mainly embryonic ABA is required for dormancy to set in, but maternal ABA also contributes to a small extent (Groot *et al*, 1991; Groot and Karssen, 1992). In Line 2 and 7 (moderate dormancy), the maternal ABA may also contribute to an extent for dormancy setting, since they had a high ABA Peak 1. There was no relationship between embryo ABA and degree of dormancy in Line 1 (one of moderate dormancy). Line 1 might presumably be less sensitive to ABA than Line 3. Line 6 was less dormancy than Line 5, whereas the ABA content during seed developing was

similar. Thus, line 6 might also be less sensitive to ABA. Line 8 (moderate dormancy) might presumably be sensitive to ABA, since it had a relatively low ABA Peak 1.

A link between ABA content during seed development and afterripening (dormancy degree), however, was not clear in this study. Sensitivity to ABA may possibly play a role in the expression of dormancy or inhibition of germination. Sensitivity could be the result of rapid metabolism of the hormone or signal, differential uptake, the concentration or affinity of receptors, or any rate-limiting step in the stimulus-response pathway. The sprouting-susceptible wheat cultivar Greer is considerably less sensitive to applied ABA than the sprouting-resistant cultivar Brevor throughout the developmental period (Walker-Simmons, 1987). In this study, it could also reflect inappropriate sampling, *e.g.* the samples for analysis ABA was from the intact seeds. The problem with this method of expressing ABA content was that, in a complex structures (seed), tissues of physiological importance such as the embryo or seedling axis may contain more or less of this ABA. ABA has been detected in embryo, endosperm and testa. The tissue location of ABA of course depends on the species. In the endospermic seeds (*e.g.* the cereals), in absolute quantities most of ABA is in the bulk tissue, the starchy endosperm, but at times comparable concentrations are found in the embryo and the aleurone tissue (Garcia-Maya *et al.*, 1990; Napier *et al.*, 1989). ABA levels were consistently 2 to 3 times higher in the embryo of wheat than in the remaining part of the grain until late maturation (Walker-Simmons, 1987). However, the different seed tissues may all contribute, to various extents, to the degree of whole seed dormancy. It was concluded that ABA action in dormancy regulation was not restricted to the embryo but is also located in endospermic tissue (Hilhorst, 1995).

In *Helianthus,* the lack of correlation between germinability and ABA content was ascribed to an increasing sensitivity to GAs as dry storage increased (Bianco *et al.*, 1994), a phenomenon described in several other species (Hilhorst and Karssen, 1992). It has been reported that synthesis of GAs plays no roles in dormancy breaking in dry seed (Metzger, 1983; Karssen and Laçka, 1986). It has been proposed that the mechanisms for GA_3-induced and afterripening-induced germination of dormant embryos are different (Metzger, 1983; Foley *et at.*, 1993). The use of $[^3H]GA_4$ demonstrates that enhanced

sensitivity resulting from drying was not caused by changes in gibberellin uptake or by a change in its metabolism (Bianco *et al.*, 1994). Studies on dormancy breaking of *Arabidopsis thaliana* seeds showed that the sensitivity to GAs gradually increased during the period of dry storage. This increase in sensitivity occurred both in wild type and gibberellin-deficient mutant seeds, demonstrating that the release from dormancy is independent of the capacity to synthesis GAs (Karssen and Laçka, 1986). Therefore, the increased sensitivity to GA was proposed to be correlated with an activation of GA receptors (Hilhorst and Karssen, 1992). Indeed, drying might alter membrane composition and structure which could result in structural changes of a membrane-bound or associated protein.

During dry storage, GA_{4+7} without chilling led to an improvement ($p<0.01$) in the germination percentage during storage, but not in Line 1 and 8 (Table 5.12; Figure 5.19-5.26) both of which have low early levels of ABA (ABA Peak 1). GA_{4+7} with chilling did not advance in seed germination for most lines. After 5 weeks storage, GA_{4+7} without chilling gave higher germination (Table 5.13; Table 5.14). The results agree with Karssen *et al.,* (1989) that GAs are required for germination and also indicated that prechilling was not necessary for dormancy-breaking of meadowfoam seed.

Requirements of external supply GA for germination decreased during the afterripening. The differences between germination amongst treatments were considerably less after 15 weeks storage than after 5 weeks storage (Table 5.14). After prolonged storage, dormant seeds were capable of maximum germination even in water at constant temperatures. Thus, GAs necessary for germination processes should presumably be present in seeds. Hutchinson *et al.*, (1988) identified GA_4, GA_{20} and many 15 β-hydroxy GAs in almost mature seeds. They also pointed out that axis tissues should also be competent to respond to GAs since germination occurred without any additional treatment. During storage, in this study, a relaxation in dormancy occurred in all lines. At early storage of meadowfoam seeds, presumably, ABA may prevent GA receptors forming or reduce the affinity between receptor and GA. Afterripening may then increased germinability either by reducing ABA and/or increasing affinity of GA even when ABA present. The

suppression of the *in situ* ABA synthesis, induced during dry storage, appears necessary for the release of dormancy (Le Page-Degivry and Garello, 1992; Bianco *et al.*, 1994). After a long dry storage, embryos became completely insensitive to ABA and elicited a response to GA (Le Page-Degivry *et al.*, 1996). They also reported that the sensitivity to GAs depends on the degree of dormancy, which is determined by the level of ABA synthesis capacity. It thus appears that sensitivity to GA is correlated indirectly to ABA synthesis capacity.

5.5.4 Heritability measurements

Heritability estimate was high for seed mass ($h^2 = 0.9238$) (Table 5.9) at around harvest ripeness (86 days after anthesis), showing a strong genetic control on this variable. The heritability estimate (narrow-sense) for seed dry mass in wheat was 0.74 (Gordon, 1980). Seed mass at harvest ripeness should, therefore, be a useful measure to use, and be very appropriate for improving the problem of low yield in meadowfoam.

In contrast to seed mass, heritability estimate for seed moisture was low ($h^2 = 0.3292$) (Table 5.5), indicating a considerable environmental influence. This was not unexpected because the definition of harvest ripeness is based on seed moisture content which itself is largely influenced by environmental variables. Zvomuya (1996) also pointed out that harvest ripeness was mainly controlled by the environment in wheat ($h^2 = 0.252$, narrow sense). Gordon (1980) reported that the heritability estimate (narrow-sense) for harvest ripeness in wheat was 0.62.

It was confirmed here that GA_{4+7} without chilling can be viewed as an efficacious dormancy breaking method. The results, therefore, measure the basic germinative ability of the natural whole-seed system: that is "germinative maturity" (details in Chapter 4). Germination in water was considered "standard germination". Therefore, the heritability for GA_{4+7} without chilling during seed storage was defined as "heritability of germinative maturity". The heritability for water during seed storage was defined as "heritability of dormancy-breaking".

After 5 weeks storage, the heritability of germinative maturity was high (0.9437) (Table 5.15), which may be worthy of serious consideration by breeders. The high heritability of germinative maturity may emphasize that this is a significant character, and is an important assessment of true physiological maturity. The heritability of dormancy-breaking was also high (0.9306) (Table 5.15) after 5 weeks storage, which may be considered as a dormancy assessment. However, opposite results were found after 15 weeks storage for both the heritability of germinative maturity and the heritability of dormancy-breaking. The results indicated that additional storage environments would probably induce lower estimates of heritability. These, maybe, other factors become more important with long storage (*e.g.*, biochemical deterioration).

In this study, the method for heritability measurements was unusual, using variance of $\hat{Y}$ (from regression) across 8 lines as genotypic variance (σ^2_G) and pooled (within regression) variances of $\hat{Y}$ for 8 lines as environmental variance (σ^2_E). However, the results appear to conform with biological expectations, and compare well with more conventional estimates in wheat. They also had low standard errors since heritability estimates were based on regression fits which had high level of error control. It seems, therefore, that the method was appropriate and reliable. Moreover, the heritability estimates have a reasonable generality, since they were based on a broad gene-pool, *i.e.* an open pollinated composite (as mentioned in Chapter 3).

As noted earlier, plants decayed unexpectedly in the latter part of seed development, and the seed dry mass data revealed possible unusual development. Samples collected from this latter time, therefore, may be biased. However, the results have been discussed here simply as they appeared. The possibility that this is an unusual development result should be borne in mind. Further study on these development traits would clear up this matter.

The definition of harvest ripeness (at 12.5% seed moisture) may not be appropriate for oil seeds, which tend to dry to lower levels on seed storage. The present definition makes the results directly comparable to these of wheat. A more appropriate definition requires further research.

Chapter 6 General discussion

The aim of this study was to investigate the genetic and physiological aspects of flowering, seeding, seed shattering and dormancy of meadowfoam. These are problems underlying the domestication of meadowfoam as a potential new crop in Oregon, USA and in New Zealand.

6.1 Potential for meadowfoam domestication

Meadowfoam is still a wild plant. Most wild type plant seeds have an appreciable dormancy, which has fitness value in their habitats. Variation in the degree of dormancy among individual seeds provides both a regular proportion of (surviving) seeds which germinate each year, and the potential for considerable persistence in extreme individuals. However, this "character" is not consistent with commercial agricultural production needs. Instead, stand establishment, specific timing and regular densities are essential. In this study, the results demonstrated that primary dormancy developed in seeds before they were harvest ripe in meadowfoam. Variation in the degree of dormancy among the single-plant lines was found. After 5 weeks storage, the heritability of germinative maturity (dormancy-breaking test) was very high (0.9437) (Table 5.15), which indicates high potential for manipulation by breeders. The heritability of dormancy-breaking (standard germination test) was also high (0.9306) (Table 5.15) after 5 weeks storage, which may be considered as a dormancy assessment. Selection for improvement in meadowfoam seed germination and reduced dormancy potential appears to be readily feasible using existing breeding materials. Improvement in meadowfoam seed germination characters could accelerate commercialisation of this new crop.

In this study, results also showed that the primary dormancy may be broken down between harvest and seedling time. The deepest dormancy in Line 3 and 4 released around 25 weeks after storage at 20°C room in dark (Figure 5.13 and 5.14). This finding suggests that dormancy may be a useful character against sprouting damage after

meadowfoam domestication. However, seed germination and dormancy may vary depending on pre- and post-harvest environments. Further research is need to explore the effects of environments on seed germination and dormancy.

Seed shattering at maturity is an important trait for wild races. An abscission layer is formed at a joint of articulation and collapses at maturity permitting the seed unit to fall. In domesticated races, the abscission layer is suppressed or collapse is delayed until harvest. In this study, the results of multiple regression showed that harvest shattering (Hsh) was negatively correlated (>0.310) to the seed yield (Table 3.2). The heritability of harvest shattering was 0.835 (Table 3.12), which suggested that harvest shattering is controlled strongly by genetics. In this germplasm, there were 47.6% plants around mean value of harvest shattering (μ = 0.31 seeds flower^{-1}), 27.0% and 2.8% plants with lower and lowest value, respectively (Table 3.7). The results indicated that shattering can be reduced by plant breeding. Historically, non-shattering has often featured in domesticated cereal and have been harvested in commercial quantities (Harlan, 1992).

Uniformity of maturation is another challenge to domestication of any wild plant. The results in this study showed that variability of floral initiation, general flowering time or seed set was randomly distributed (Figure 3.1-3.3). These characters had high heritabilities, ranging from 0.734 to 0.892 (Table 3.12), except for "early seed set" (seed set PC 2) which was only 0.446. The results clearly showed that flowering maturation depends to a large extent on genes, and, therefore, they may be altered under selection. In domesticated cereals, such as wheat and barley, trends toward even ripening are based mostly on shortening of the vegetative period, they bud off quickly and the flowers tend to ripen concurrently. Other work confirms that budding time and plant form (clump density) have high heritabilities for individual plant in meadowfoam; and they change noticeably from S_0 generation to the next (Chozin. 1990). These findings imply that progress for approaching uniformity of maturation can be expected.

Improvement of quantitative characters in plant breeding programs proceeds by selecting among genotypes based on their phenotypic performance. The phenotype is a result of genetic and non-genetic influences (Comstock and Moll, 1963) and selection exploits only those components of phenotypic variability that have a genetic basis. When a phenotypic

effect for a gene under selection pressure is consistently observed, achieving a response to selection is relatively straightforward. A high level of significant genotypic effect of thirteen characters associated with flowering and seeding proved the presence of considerable genetic variation for those attributes in this study (Table 3.12). Most characters had high heritability ($h^2 > 0.800$). The results suggested that meadowfoam has the genetic capability to respond to selection, but some characters may not improve independently. The rate of response can be seen from the equation $\Delta G = ih^2\sigma_p$ (Falconer, 1989). The equation provides a means of predicting the response to selection from knowledge of the heritability obtained from previous generations. The phenotypic standard deviation, σ_p, merely specifies the units of measurement. The intensity of selection, *i*, depends only on the proportion included in the selected group and, provided the distribution of phenotypic values is normal. Therefore, the general levels of genetic influences (variances and heritabilities) indicate that there is much potential for changing (domesticating) meadowfoam in these characters.

One measure of efficiency of individual selection is *h* (the square-root of the heritability), because *h* is the correlation between breeding values and phenotypic values ($r_{AP} = b_{AP}\frac{\sigma_P}{\sigma_A} = h$, where $b_{AP} = h^2$, the regression of breeding value on phenotypic value) (Falconer, 1989). Thus, individual selection may be an effective and efficient method to improve meadowfoam genotypes. The selection combined the highest performing plants from the top performing lines seems to be the most reasonable choice.

Donald (1968) proposed that increased crop yields might be gained by the development of cultivars based on crop ideotypes, plant models designed for particular purposes in specified environments. The ideotype breeding concept was proposed as an analytical and physiological basis to replace empirical breeding approaches, *i.e.*, "defect elimination" and "selection for yield". From the current studies, an ideotype of domesticated meadowfoam, may be defined as the combinations of low seed dormancy, non-shattering, mature uniformity, high seed set, erect plant and high seed oil yield. Sedgley (1991) concluded that ideotype breeding approach offers a basis for increasing the efficiency in attaining plant breeding objectives.

6.2 The role of statistical analysis in plant breeding

Increasing gains in plant breeding depends on increasing the efficiency of identifying superior germplasm. Sophisticated statistical methods can improve this efficiency. In this study, statistical analysis of the data received a lot of attention, providing maximum information from limited material for this new crop. Following the solution of the first partial derivatives with respect to each parameter of Richards function in this study, the routine use of these nonlinear functions (Richards or Gompertz) provided convenient and credible descriptions of the progress of seed germination. Moreover, the multivariate analysis of variance (MANOVA), which provided valid simultaneous analysis of all four (correlated) statistics which define any Richards function, made if possible to test significant differences amongst the functions (germination profiles) themselves. These germination functions provided more rigorous discrimination amongst treatments than the univariate final germination level (Table 4.3).

Dormancy-breaking for meadowfoam seeds can be achieved using KNO_3 or GA_{4+7} imbibant in darkness at 10/5°C, without prechilling. This special test assesses whole-seed germinative maturity; and the difference between this and standard germination provides an estimate of dormancy. From the present results, the asymptote of KNO_3 at 10/5°C of this seed-lot was 95.1%±0.34 (Figure 4.9). The water at 10/5°C was used as a simple "standard" test in these treatments (the nearest). The asymptote of water at 10/5°C was 87.0%±0.32. Using that estimate, this seed-lot has 8.1% dormancy, and the standard error for dormancy was 0.47% ($\hat{\sigma}_{Dorm} = (\hat{\sigma}^2_{A_{KNO_3}} + \hat{\sigma}^2_{A_{H_2O}})^{1/2}$).

Also, both principal components and clustering proved to be efficient analyses which maximized the discrimination of differences among plants in this segregating population. These procedures recognized 190 phenotypes within this germplasm (Table 3.10). A high level of significant genotypic variance in all characters was found (Table 3.12). The analyses explored that meadowfoam has the genetic capability to respond to selection.

A special feature of this germplasm analysis was the use of cluster analysis, to define "natural" groups. The optimum stage to truncate the dendrogram was based on the minimum F-probability of amongst-cluster mean squares to within-cluster mean squares. Gordon (pers. comm.), Teow (1978) and Cullen (1981) also used this truncation point. Furthermore, 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 (Figure 3.6 - 3.18).

Another innovation was the use of principal components analysis to identify patterns of fresh flowers, total flowers and seed set (Figure 3.1 - 3.3). The pattern analysis explored that the base population was panmictic: one large segregating gamodeme. This pattern analysis was considered superior to regressions (over time) because of few time-nodes, and it avoided the need to find optimum functions.

In seed development study, samples were insufficient for RCB analysis because plant decayed in the late days. Thus, analysis of variance (ANOVA) for RCB could not be applied. The heritability was estimated using variance of $\hat{Y}$ across 8 lines (from regression) as genotypic variance (σ^2_G) and pooled error variances of $\hat{Y}$ for 8 lines (within regression) as environmental variance (σ^2_E). However, the results appear to conform with biological expectations, and compare well with more conventional estimates in wheat. Moreover, the heritability estimates have a reasonable generality, since they were based on a broad gene-pool, *i.e.*, an open pollinated composite.

Finally, the detection of the ABA peak differences amongst lines was effected by principal components and cluster analysis, using peak height, width and time reaching peak. Because the curves of ABA concentration with time of seed development did not follow any functions and had no external replications, regression analysis and analysis of variance could not be applied to the ABA curves. Both principal components and cluster analysis successfully demonstrated that there were four ABA groups for the eight lines.

6.3 Scope for future research

In the present experiments, seed development and maturation were studied in a spring simulation of Palmerston North, New Zealand. However, variation for quantitative characters is under the control of many genes and their contribution can differ among environments. Future studies on the genetic variation and germination of meadowfoam should be directed towards a greater line numbers and their performances in various environments, to ensure that the introduction of meadowfoam is more reliable and successful.

Further specific work is also need to explore the effects of exogenous ABA, endogenous ABA in different seed tissues and endogenous GA levels on seed dormancy and germination, in order to investigate biosynthesis of ABA and GA and/or sensitivity to ABA and GA. Moreover, interaction between ABA and GA on the concentration or affinity of receptors of hormones also requires further study. Results from these studies may provide insights into possible dormancy mechanisms of meadowfoam.

In the study of seed development, the significance of several important characters have not been explored using statistics, since meadowfoam plants were decayed at later time. The plant growth, *e.g.* clump density, is also well worth investigating to facilitate a detailed plant study.

In the future, the utilization of molecular DNA markers and the linkage between molecular markers and QTL (quantitative trait loci), should also be studied to guide the selection of parental material for rapid and efficient improvement of meadowfoam.

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Appendix 4.1 χ^2 test for the homogeneity of error variances (MS_e) of the four experiments.

Experiment (k)	df	Germination test				Viability test			
		MS_e (s_i)	log(s_i)	df*log(s_i)	1/df	MS_e (s_i)	log(s_i)	df*log(s_i)	1/df
Dark-10/5	30	0.01083	-1.96538	-58.9614	0.03330	0.01079	-1.96701	-59.0102	0.0333
Light-10/5	30	0.00777	-2.10956	-63.2868	.0333	0.00909	-2.04142	-61.2426	0.0333
Dark-15/10	30	0.00648	-2.18829	-65.6488	0.0333	0.00877	-2.05701	-61.7102	0.0333
Light-15/10	30	0.00711	-2.14806	-64.4419	0.0333	0.00566	-2.24727	-67.4181	0.0333
Total				-252.339	0.1333			-249.381	0.1333
Pooling	120	0.00805	-2.09431	-251.317		0.00858	-2.06667	-248.000	
Corrected χ^2		2.2578 ns				3.0537 ns			

$$Critical\text{-}\chi^2_{\substack{df=3 \\ \alpha=.05}} = 7.81$$

$$\chi^2 = 2.3026[(\Sigma df_i).\log s_p^2 - \Sigma(df_i.\log s_i^2)]$$

$$Correction\ factor = 1 + \frac{1}{3(k-1)}[\Sigma\frac{1}{df_i} - \frac{1}{\Sigma df_i}]$$

$$Corrected\ \chi^2 = \frac{\chi^2}{correction\ factor}$$ (Steel and Torrie, 1981).

ns: none significance (χ^2 value was less than critical-χ value).

Appendix 4.2 Expected values of mean squares for a two factorials (S and Y) pooling of three factorials (A, B and C) factorial blocked.

Source of variation		Expected values of mean square (random)	Complex of F-test
S	33	e+R+ABCSY+ABCS+BCSY+BCS+ACSY+ACS+ABSY+ABS+CSY+CS+BS+BSY+ASY+AS+SY+S	(33+20+17+14+13+10+7+2)/(31+22+19+16+11+8+5+4)
Y	32	e+R+ABCSY+ABCY+BCSY+BCY+ACSY+ACY+ABSY+ABY+CSY+CY+BY+BSY+ASY+AY+SY+Y	(32+20+17+14+12+9+6+2)/(31+21+18+15+11+8+5+4)
SY	31	e+R+ABCSY+BCSY+ACSY+ABSY+CSY+BSY+ASY+SY+BC+AC+C	(31+11+8+5+1)/(30+20+17+14+2)
Blk(SY)	30	e+R	30/1
A	29	e+ABCSY+ABCY+ABCS+ACSY+ACY+ACS+ABSY+ABY+ABS+ASY+AY+AS+ABC+AC+AB+A	(29+23+20+13+12+10+9+2)/(26+25+22+21+11+8+4+3)
B	28	e+ABCSY+ABCY+ABCS+BCSY+BCY+BCS+ABSY+ABY+ABS+BY+BS+BSY+ABC+BC+AB+B	(28+23+17+13+12+7+6+2)/(26+24+19+18+11+5+4+3)
C	27	e+ABCSY+ABCY+ABCS+BCSY+BCY+BCS+ACSY+ACY+ACS+CSY+CY+CS+ABC+BC+AC+C	(27+23+14+10+9+7+6+2)/(25+24+16+15+8+5+4+3)
AB	26	e+ABCSY+ABCY+ABCS+ABSY+ABY+ABS+ABC+AB	(26+11+4+3)/(23+13+12+2)
AC	25	e+ABCSY+ABCY+ABCS+ACSY+ACY+ACS+ABC+AC	(25+8+4+3)/(23+10+9+2)
BC	24	e+ABCSY+ABCY+ABCS+BCSY+BCY+BCS+ABC+BC	(24+5+4+3)/(23+7+6+2)
ABC	23	e+ABCSY+ABCY+ABCS+ABC	(23+2)/(4+3)
AS	22	e+ABCSY+ABCS+ACSY+ACS+ABSY+ABS+ASY+AS	(22+11+8+4)/(20+13+10+2)
AY	21	e+ABCSY+ABCY+ACSY+ACY+ABSY+ABY+ASY+AY	(21+11+8+3)/(20+12+9+2)
ASY	20	e+ABCSY+ACSY+ABSY+ASY	(20+2)/(11+8)
BS	19	e+ABCSY+ABCS+BCSY+BCS+ABSY+ABS+BSY+BS	(19+11+5+4)/(17+13+7+2)
BY	18	e+ABCSY+ABCY+BCSY+BCY+ABSY+ABY+BSY+BY	(18+11+5+3)/(17+12+6+2)
BSY	17	e+ABCSY+BCSY+ABSY+BSY	(17+2)/(11+5)
CS	16	e+ABCSY+ABCS+BCSY+BCS+ACSY+ACS+CSY+CS	(16+8+5+4)/(14+10+7+2)
CY	15	e+ABCSY+ABCY+BCSY+BCY+ACSY+ACY+CSY+CY	(15+8+5+3)/(14+9+6+2)
CSY	14	e+ABCSY+BCSY+ACSY+CSY	(14+2)/(8+5)
ABS	13	e+ABCSY+ABCS+ABSY+ABS	(13+2)/(11+4)
ABY	12	e+ABCSY+ABCY+ABSY+ABY	(12+2)/(11+3)
ABSY	11	e+ABCSY+ABSY	11/2
ACS	10	e+ABCSY+ABCS+ACSY+ACS	(10+2)/(8+4)
ACY	9	e+ABCSY+ABCY+ACSY+ACY	(9+2)/(8+3)
ACSY	8	e+ABCSY+ACSY	8/2
BCS	7	e+ABCSY+ABCS+BCSY+BCS	(7+2)/(5+4)
BCY	6	e+ABCSY+ABCY+BCSY+BCY	(6+2)/(5+3)
BCSY	5	e+ABCSY+BCSY	5/2
ABCS	4	e+ABCSY+ABCS	4/2
ABCY	3	e+ABCSY+ABCY	3/2
ABCSY	2	e+ABCSY	2/1
e	1	e	

Appendix 4.3 Significance in analysis of variance (ANOVA) for parameters of Richards' function.

Source	df	F-test			
		A	ß	k	v
T	1	ns	ns	*	ns
L	1	*	ns	ns	ns
T*L	1	ns	ns	ns	ns
Blk(T*L)	8	ns	ns	ns	ns
Ch	1	ns	ns	ns	ns
I	3	*	ns	ns	ns
Col	1	(*)	ns	(*)	ns
Ch*I	3	ns	ns	ns	ns
Ch*Col	1	ns	ns	ns	ns
I*Col	3	ns	ns	ns	ns
Ch*I*Col	3	ns	ns	ns	ns
Ch*T	1	ns	ns	ns	ns
Ch*L	1	ns	**	*	ns
Ch*T*L	1	ns	ns	ns	ns
I*T	3	ns	ns	*	ns
I*L	3	ns	ns	ns	ns
I*T*L	3	ns	ns	ns	ns
Col*T	1	ns	ns	ns	ns
Col*L	1	ns	(*)	*	ns
Col*T*L	1	ns	ns	ns	(*)
Ch*I*T	3	ns	ns	ns	ns
Ch*I*L	3	ns	ns	ns	ns
Ch*I*T*L	3	ns	ns	ns	(*)
Ch*Col*T	1	ns	ns	(*)	ns
Ch*Col*L	1	ns	ns	ns	ns
Ch*Col*T*L	1	ns	(*)	ns	ns
I*Col*T	3	ns	ns	ns	ns
I*Col*L	3	ns	ns	ns	ns
I*Col*T*L	3	ns	(*)	ns	ns
Ch*I*Col*T	3	ns	(*)	(*)	ns
Ch*I*Col*L	3	ns	(*)	*	ns
Ch*I*Col*T*L	3	(*)	ns	ns	ns
Residual	120	0.0065	1.7978	0.0587	0.1653
CV (%)		6.25	27.80	25.46	300.99
Mean ($\bar{x}$)		1.2899 (92.3%)	4.8223	0.9520	0.1351
SE $\bar{x}$		0.0058	0.0968	0.0175	0.0293

T: temperature; L: light; Ch: chill; I: Imbibant; Col: seed-colour; Blk: block.
**: significance at the 1% probability level; *: significance at the 5% probability level; (*): significnace at the 10% probability level.
ns: no significance at the statistics level.

Appendix 4.4 Hotelling T^2-values (below diagonal) and approximate F-values (above diagonal) for combination of imbibant and seed-colour

	GA_3 x G	GA_3 x B	GA_{4+7} x G	GA_{4+7} x B	KNO_3 x G	KNO_3 x B	water x G	water x B
	I. F-values							
GA_3 x G	--	5.30 (*)	2.05 ns	17.04 (*)	4.62 (*)	3.75 (*)	6.44 (*)	16.40 (*)
GA_3 x B	24.37	--	1.21 ns	3.66 (*)	0.97 ns	2.06 ns	1.18 ns	7.20 (*)
GA_{4+7} x G	9.43	5.55	--	7.47 (*)	1.59 ns	0.50 ns	3.50 (*)	12.69 (*)
GA_{4+7} x B	78.37	16.83	34.37	--	2.42 (*)	6.65 (*)	6.53 (*)	11.74 (*)
KNO_3 x G	21.27	4.45	7.32	11.11	--	1.06 ns	4.25 (*)	12.69 (*)
KNO_3 x B	17.24	9.49	2.30	30.59	4.86	--	5.85 (*)	16.71 (*)
water x G	29.60	5.45	16.11	30.04	19.54	26.90	--	2.86 (*)
water x B	75.45	33.11	58.38	54.01	58.38	76.88	13.15	--
	II. T^2-values							

$$H'otellings\ T^2 = \frac{n}{2}\Delta'\, D_{(x)}^{-1}\Delta \text{ (Morrison, 1990)}$$

$$\because\ Discriminant\ score\ f_{ij} = c_{j'}\, z_j\ (standardized\ function)$$

$$\therefore\ D_{(X)} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

$$then,\ H'otellings\ T^2 = \frac{n}{2}\Delta'\Delta\ (n = 1).$$

$$Approximate\ F_{(p,N-p)} = \frac{N - p}{p(N - 1)}T^2.$$

$$here,\ F_{(4,20)} = 0.2174\ T^2\ (N = 24, p = 4);\ Critical\text{-}F_{(4,20)}^{\alpha=0.10} = 2.25.$$

(*): significance at the 10% probability level; ns: no significance at the statistics level.
N: observations for a single sample; p: attributes for a multiple population (Morrison, 1990).

Appendix 5.1 Preliminary PC analysis for data of field experiment.

Component	Percentage of variation	Cumulative percentage
1	42.40	42.40
2	21.46	63.86
3	12.22	76.08
4	9.90	85.99
5	6.83	92.81
6	3.86	96.68
7	2.19	98.87
8	0.78	99.65
9	0.35	100.0

Appendix 5.2 Factor structure matrix and factor scores of two components for preliminary PC analysis.

	Factor structure matrix		Standard coefficient	
	Component 1	Component 2	Component 1	Component 2
Fresh flowers of date 2	0.64867	-0.42499	0.16997	-0.22007
Maximum seed set	0.80560	-0.41445	0.21109	-0.21461
Seed set of date 4	0.29316	0.83845	0.07682	0.43417
Late seed colour turning	0.16997	0.09699	0.04454	0.05022
Seed set after harvest	0.27498	0.85264	0.07205	0.44152
Flowers per plant	0.87037	-0.26534	0.22806	-0.13740
Seeds per plant	0.94640	0.18840	0.24799	0.09756
100 seed weight	0.23510	-0.01130	0.06160	-0.00585
Yield per plant	0.92068	0.18259	0.24125	0.09455

Appendix 5.3 Two PC scores of preliminary PC analysis for 8 lines.

Line	PC score 1	PC score 2
1	0.14033	-1.65344
2	1.37151	-3.24029
3	-0.06995	1.86381
4	1.31683	1.99563
5	-2.04972	-1.34941
6	-0.63238	0.03097
7	0.78443	-0.02278
8	3.04692	-0.77934

Appendix 5.4 Experimental room conditions for meadowfoam seed development.

a) Lightning conditions

The lighting system used consisted of 4x1000W Sylvania 'metal-arc' high pressure discharge lamps, together with 4x1000W Phillips tungsten iodide lamps.

The photosynthetically active radiation (PAR μmol m^{-2} s^{-1}) was measured at standard trolley height using a Li-Cor 185 meter with an LI190S quantum sensor.

b) Room conditions

	Day	Night
Temperature (± 0.5°C)[a]	15.0	6.5
Relative humidity (± 5%)[a]	71.0	79.3
CO_2 concentration range	438-311	432-359
PPFD (μmol m^{-2} s^{-1})	Pre-experiment 707, Post experiment 691	
Daylength	14.0 hours (0800 - 2200)	
Nutrient	Nutrient and water were added as required by normal crop	

a: The temperature and humidity changeovers were set at 120 minutes, starting one hour before the main lights came on and then again one hour before the lights went off.

c) Nutrient: modified Hoagland's solution (half-strength)

	Items	Concentration ($g.l^{-1}$)
Stock solution A	Calcium nitrate ($Ca(NO_3).4H_2O$)	147.60
	Sequestrene (10% DTPA NaFe)	5.20
Stock solution B	Potassium dihydrogen phosphate (KH_2PO_4)	17.01
	Potassium nitrate (KNO_3)	63.20
	Magnesium sulfate ($MgSO_4.7H_2O$)	61.62
Micronutrients	Boric acid (H_3BO_3)	0.3575
	Manganese chloride ($MnCl_2.4H_2O$)	0.2262
	Zinc sulphate ($ZnSO_4.7H_2O$)	0.0275
	Cupric sulphate ($CuSO_4.5H_2O$)	0.0100
	Sodium molybdate ($Na_2MoO_4.2H_2O$)	0.00335
	Potassium chloride (KCl)	0.7875
Other	Concentrated HCl	2 ml

pH of final solution = 6.5 - 7.5.

Appendix 5.5 The coefficients of determination of regression (R^2) for data of seed dry mass and moisture fitting various functions.

Line	Seed dry mass					
	Linear	Power	Exponential	Log	Quadratic	Quadratic exponential
Line 1	0.3988	0.7218	0.6448	0.4020	0.4924	0.7487
Line 2	0.5613	0.6969	0.7119	0.5266	0.6184	0.7696
Line 3	0.3430	0.7354	0.5019	0.4096	0.4648	0.7587
Line 4	0.3633	0.7677	0.6380	0.3974	0.5483	0.8575
Line 5	0.5061	0.6859	0.5726	0.5218	0.6115	0.7249
Line 6	0.7030	0.6493	0.7700	0.5625	0.7084	0.7804
Line 7	0.5660	0.6393	0.6641	0.5017	0.5765	0.7007
Line 8	0.2850	0.5855	0.4810	0.3143	0.3935	0.5871
	Seed moisture					
Line 1	0.5930	0.2895	0.5094	0.3740	0.7679	0.7663
Line 2	0.7202	0.4099	0.6948	0.4335	0.9196	0.9376
Line 3	0.8046	0.5158	0.7715	0.5912	0.8832	0.9281
Line 4	0.7229	0.3176	0.5923	0.4164	0.9082	0.8463
Line 5	0.8220	0.4880	0.7473	0.5859	0.9315	0.9536
Line 6	0.7862	0.3767	0.6737	0.4556	0.9860	0.9385
Line 7	0.7523	0.3946	0.6891	0.4535	0.9554	0.9604
Line 8	0.5576	0.3075	0.5101	0.3831	0.6826	0.7195

Appendix 5.6 The coefficients of determination of regression (R^2) for seed dry mass and moisture fitting power, quadratic and exponential quadratic functions with adjusted data.

Line	Dry mass		Moisture	
	Power	Quadratic exponential	Quadratic	Quadratic exponential
1	0.6784	0.7212	0.7679	0.7663
2	0.8372	0.8905	0.9851	0.9937
3	0.6773	0.7159	0.9675	0.9934
4	0.8507	0.9038	0.9713	0.9658
5	0.7369	0.8016	0.9638	0.9811
6	0.6575	0.8399	0.9860	0.9385
7	0.7818	0.8316	0.9806	0.9886
8	0.5855	0.5871	0.8278	0.9041

Appendix 5.7 The coefficients of determination of regression (R^2) for data of seed germination fitting for various functions.

Treatment	Line	Linear	Power	Exponential	Logarithmic	Quadratic	Quadratic exponential
GA_{4+7} pre-chilling	1	0.8840	0.7121	0.8702	0.6745	0.9126	0.8711
	2	0.6596	0.5985	0.5559	0.6914	0.7395	0.6511
	3	0.7751	0.6590	0.6428	0.7487	0.8113	0.7074
	4	0.6885	0.7493	0.5461	0.8130	0.8482	0.7619
	5	0.5080	0.5238	0.4592	0.5570	0.5716	0.5381
	6	0.4250	0.2538	0.3504	0.3027	0.6171	0.4964
	7	0.7078	0.3260	0.4885	0.4636	0.7626	0.4989
	8	0.5015	0.2586	0.3817	0.3562	0.6567	0.5558
GA_{4+7} non-chilling	1	0.8113	0.4952	0.6059	0.6937	0.8116	0.6059
	2	0.6150	0.7314	0.5733	0.7319	0.7690	0.7653
	3	0.5931	0.5421	0.4894	0.6179	0.6556	0.5750
	4	0.5950	0.5197	0.3683	0.6682	0.6151	0.4507
	5	0.2806	0.2854	0.2686	0.2817	0.2819	0.2687
	6	0.4005	0.1719	0.2983	0.2520	0.4790	0.3809
	7	0.7430	0.5173	0.5712	0.6135	0.7434	0.5854
	8	0.7676	0.4434	0.5784	0.5749	0.7889	0.5829
Water non-chilling	1	0.8209	0.5392	0.6948	0.6234	0.8578	0.7046
	2	0.6883	0.4054	0.4541	0.6169	0.6981	0.4671
	3	0.7958	0.6617	0.6004	0.7827	0.8488	0.7051
	4	0.7287	0.7813	0.6398	0.7977	0.8120	0.8004
	5	0.7125	0.7977	0.6615	0.7882	0.8175	0.8186
	6	0.5456	0.1181	0.2878	0.3315	0.7170	0.5141
	7	0.6796	0.6614	0.7115	0.5548	0.6800	0.7257
	8	0.9411	0.9046	0.9110	0.7944	0.9445	0.9425

Appendix 5.8 The coefficients of determination of regression (R^2) for data of seed germination fitting for quadratic and exponential quadratic functions with or without adjusted data.

Treatment	Line	Quadratic (no data adjusting)	Exponential quadratic (no data adjusting)	Quadratic (deleting 29 observations)	Exponential quadratic (deleting 29 observations)	Quadratic (deleting 14 observations)
GA_{4+7} pre-chilling	1	0.9126	0.8711	0.9115	0.9078	0.9126
	2	0.7395	0.6511	0.7751	0.7767	0.7395
	3	0.8113	0.7074	0.8218	0.7208	0.8113
	4	0.8482	0.7619	0.8668	0.7770	0.8482
	5	0.5716	0.5381	0.7637	0.7538	0.7637
	6	0.6171	0.4964	0.6674	0.6528	0.6674
	7	0.7626	0.4989	0.7019	0.6447	0.7902
	8	0.6567	0.5558	0.8233	0.7489	0.6567
GA_{4+7} non-chilling	1	0.8116	0.6059	0.8116	0.6059	0.8116
	2	0.7690	0.7653	0.7889	0.8015	0.7690
	3	0.6556	0.5750	0.6540	0.6275	0.7427
	4	0.6151	0.4507	0.5806	0.4372	0.6188
	5	0.2819	0.2687	0.3592	0.3378	0.3791
	6	0.4790	0.3809	0.0399	0.0982	0.4790
	7	0.7434	0.5854	0.7963	0.6574	0.7434
	8	0.7889	0.5829	0.7889	0.5829	0.7889
Water non-chilling	1	0.8578	0.7046	0.8227	0.6681	0.8578
	2	0.6981	0.4671	0.7359	0.6175	0.7359
	3	0.8488	0.7051	0.8906	0.8161	0.8583
	4	0.8120	0.8004	0.8120	0.8004	0.8491
	5	0.8175	0.8186	0.8836	0.8701	0.8446
	6	0.7170	0.5141	0.0125	0.2500	0.7170
	7	0.6800	0.7257	0.8885	0.8127	0.8885
	8	0.9445	0.9425	0.9445	0.9425	0.9445

Appendix 5.9 ELISA assay materials

a. [^{3}H]ABA standard

[^{3}H]ABA (50 μCi per 250 μl) was diluted 1:2220 in 100% methanol (9 x 10^{-3}μCi per 100μl, 20,000 DPM per 100μl). (1μCi == 2.22 x 10^6 DPM).

b. Buffers

TBS: 6.1g Tris, 0.2g $MgCl_2$, 8.8g NaCl per liter (pH 7.8); washing buffer: TBS containing 0.5 ml Tween-20 per litre and 0.1% (w/v) BSA (bovine serum albumin).

c. ABA-4'-BSA conjugate

The conjugate was prepared according to Weiler (1980) and lyophilized. Conjugate was suspended in 0.05M $NaHCO_3$ (pH 9.6), at a concentration of 7 mg ml^{-1} and stock aliquots of 30 μl were kept frozen at -20°C. Before ELISA assay a 30 μl aliquot was thawed and diluted with 0.05M NaHCO3 (pH 9.6), to a final volume of 20 ml, which is sufficient to coat the useable assay wells of one microtitration plate.

d. Monoclonal antibody (MAb)

MAb to free *cis, trans* (+)ABA (Mertens *et al.,* 1983) was purchased from Idetek, Inc.. Two mg MAb was mixed into 60 ml TBS, containing 0.2% (w/v) BSA. Aliquots of 0.5 ml of the MAb solution were stored at -20°C. Before ELISA assay a 0.5 ml aliquot were was thawed and diluted in TBS with 0.2% (w/v) BSA to a final volume of 9.15 ml, which is sufficient for the assay wells of wells of one microtitration plate.

e. Second antibody

Rabbit antimouse alkaline phosphatase conjugate (Sigma Chemicals A3350) was diluted 1:1000 in TBS.

f. ABA standards

A synthetic ABA standard [(±)-2-cis, 4-trans-abscisic acid, M.W. 264.32, 98% pure] was dissolved in TBS in concentrations ranging from 50 to 2500 pg per 100 μl. (+)-ABA (or (*S*)-ABA) accounts for about 50% of the racemic mixtures of ABA that are made synthetically (Moore, 1989).

Appendix 5.10 Recovery rate (%) of radioactivity of internal standard ([^{3}H]ABA).

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8
Line 1	83.0	87.9	H	94.3	-	91.0	89.1	-
	-	92.5	81.7	84.1				
	87.5	92.7	-	-				
Line 2	90.4	82.6	82.3	81.7	85.4	91.5	78.1	H
	89.7	84.2	-	100.0				
	83.1	-	77.5	H				
Line 3	88.3	87.5	H	85.1	H	93.4	96.2	H
	83.1	93.8	91.9	83.1				
	99.3	78.7	86.1	H				
Line 4	88.4	H	H	84.6	83.4	98.6	H	80.6
	93.2	88.6	85.4	79.9				
	95.4	102.7	88.9	81.7				
Line 5	93.7	84.7	91.1	80.5		104.6	H	H
	86.8	93.3	90.3	84.8	88.1			
	89.1	86.2	81.2	-				
Line 6	89.0	90.1	85.8	86.2	87.5	96.2	72.1	-
	86.7	87.7	90.2	H				
	85.1	93.8	96.8	-				
Line 7	92.4	84.5	87.5	H	H	93.7	H	89.0
	-	93.0	84.3	89.9				
	88.5	H	-	-				
Line 8	84.8	85.7	H	81.3	H	99.0	95.2	92.7
	88.2	80.6	94.3	H				
	87.4	90.0	89.7	97.0				

H: Samples were contaminated by high radioactivity, recovery rate using average (88.2%).
-: Samples were missed.