

## RESEARCH REVIEW

# Structural, physiological and molecular aspects of heterogeneity in seeds: a review

Angel Matilla<sup>1\*</sup>, Mercedes Gallardo<sup>2</sup> and María Isabel Puga-Hermida<sup>2</sup>

<sup>1</sup>Departamento de Biología Vegetal, Laboratorio de Fisiología Vegetal, Facultad de Farmacia, Universidad de Santiago de Compostela, 15 782-Santiago de Compostela, A Coruña, Spain; <sup>2</sup>Departamento de Biología Vegetal y Ciencias del Suelo, Laboratorio de Fisiología Vegetal, Facultad de Ciencias, Universidad de Vigo, E-36200-Vigo, Pontevedra, Spain

### Abstract

Higher plants have several strategies to perpetuate themselves under adequate ecophysiological conditions. The production of heterogeneous seeds is one such strategy. That is, to ensure the survival of the next generation, an individual plant might produce seeds that are heterogeneous with respect to the extent of dormancy, dispersion and persistence within the seed bank. Heterogeneity can affect not only certain physiological and molecular properties related to seed germination, but also such characteristics as colour, size and shape, parameters commonly used to differentiate morphs within a heterogeneous seed population. In heterogeneous seeds, the above features determine seed behaviour and alter their mechanism of germination. In this work, emphasis is placed on the existence of seed mutants having major alterations in characteristics of the testa and hormonal response. These mutants constitute a valuable tool for elucidating the mechanism of dormancy, germination and perpetuation of seeds. Finally, ontogeny and heterogeneity are reviewed, providing the first data related to the possible hormonal control of heterogeneity in seeds. These results raise the hypothesis that one of the factors triggering differences in germination among heterogeneous seeds may be an alteration in the signalling and action mechanism of ethylene and abscisic acid (ABA).

**Keywords:** abscisic acid, ACC oxidase, dormancy, ethylene, germination, gibberellins, hardseededness,

**heterocarpy, heterogeneity, imbibition, ontogeny, positional effect, seed bank, seed coat, seed size**

### Introduction

The seed is the higher plant perpetuation unit, resulting from sexual reproduction. In the seed, usually a fully developed plant embryo is dispersed, which enables the embryo to survive the period between seed maturation and seedling establishment, thereby ensuring the start of the next generation (Hilhorst, 1995; Koornneef *et al.*, 2002). Zygotic embryogenesis, often divided into embryogenesis (*sensu stricto*), maturation and desiccation (Bewley and Black, 1994; Bewley, 1997a; Hilhorst and Toorop, 1997), includes all the morphological, structural and gene-expression changes that occur from the appearance of the zygote until the formation of a mature embryo that is ready to germinate, once the endogenous and environmental conditions are appropriate (Meinke, 1994; Torres-Ruiz, 1998; Holdsworth *et al.*, 1999). The change from embryogenesis to maturation is marked by an interruption of growth and is associated with changes in cell growth and gene expression (Holdsworth *et al.*, 1999), as well as with tolerance to desiccation, controlled mainly by abscisic acid (ABA) (Hilhorst, 1995; Leubner-Metzger, 2003). ABA is known as a positive regulator of dormancy and a negative regulator of seed germination (Bewley, 1997a; Koornneef *et al.*, 2002), and recent data suggest that it may be required to maintain seed dormancy (Grappin *et al.*, 2000). During development and germination, the action mechanisms of ABA and ethylene seem to be antagonistic. For the ethylene-dependent seeds to germinate, current evidence

\*Correspondence

Fax: +34 981 593 054

Email: bvmatilla@usc.es

suggests that ethylene synthesis during imbibition interrupts dormancy maintained by ABA, thereby triggering germination (Kende *et al.*, 1998; Beaudoin *et al.*, 2000). Also, gibberellins (GAs) control seed germination by attenuating the ABA response (Beaudoin *et al.*, 2000; Koornneef *et al.*, 2002; Leubner-Metzger, 2003). Ethylene and GA signals, which are not related to the induction of dormancy of the seed developing on the mother plant, help break dormancy during seed imbibition. However, seeds of *Arabidopsis thaliana* insensitive to ethylene (Beaudoin *et al.*, 2000) and to GAs (Steber *et al.*, 1998) are hypersensitive to exogenous ABA, suggesting that during seed imbibition, ethylene and GAs can counteract the action of ABA by inhibiting the ABA signalling cascade.

Seed development is affected by a variety of factors (e.g. genetic, physiological, source–sink relationships as well as environmental), and the changes that occur cannot be correlated simply with time after pollination. Therefore, seed development is not temporally uniform in any given population, even when plants are grown in identical environments. In short, a type of heterogeneity appears. Thus, some crucifer seeds do not mature synchronously inside the silique, but sequentially, and some pods shatter before harvesting, resulting in a substantial loss of seeds (Spence *et al.*, 1996; Roberts *et al.*, 2002; Puga-Hermida *et al.*, 2003a, b). Also, within the great biological diversity in higher plants, many species have marked seed heterogeneity (e.g. the heterocarpy in genus *Atriplex* and heterospermy in *Sinapis arvensis* and *Raphanus raphanistrum*) (Venable, 1985; Mandak, 1997).

In seed heterogeneity, described in more than 200 species of angiosperms, single individual plants produce seeds differing in their external appearance. The aim of the present work is to provide an update on this mechanism, used by some higher plants to increase their fitness throughout the phases of their life cycle. An examination is provided of the same individual that produces seeds with different morphs, physiological and molecular properties, and sometimes different dispersion strategies, to ensure the perpetuation of the individual in an appropriate ecological niche (Imbert, 2002). Heterogeneity affects seed size and seed-coat properties, with the subsequent effect on germination and/or dormancy; it also has an effect at the physiological and molecular level. All these alterations, together with the ontogeny and possible hormonal participation in the heterogeneity of *Cruciferae*, are discussed in this review.

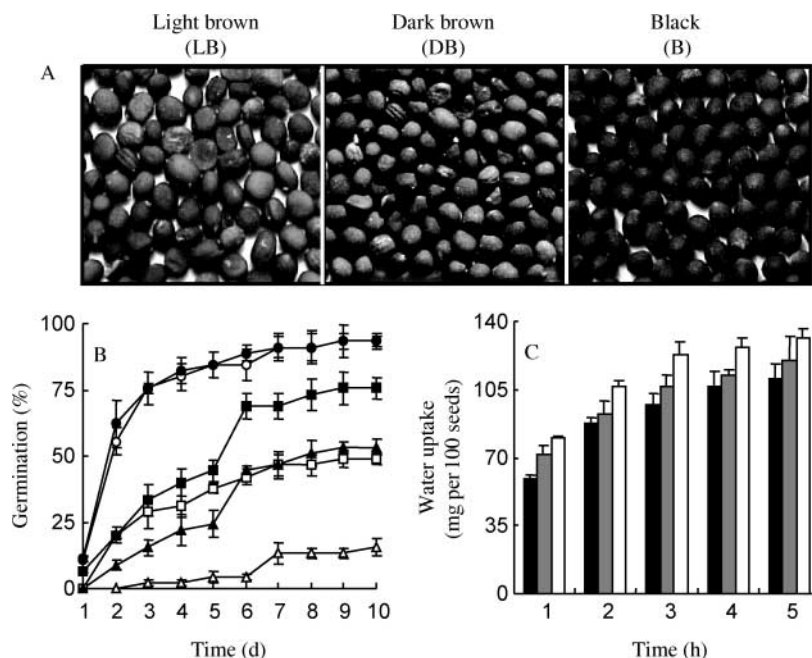
### Heterogeneity in wild seeds

Different types of seed morphs are produced by a single individual in many higher plants. In some

species, heterogeneity is of special relevance, since the morphs are ecologically distinct, helping the plants cope with spatio-temporal variability of habitats. These cases of seed diversity appear to be restricted largely to relatively short-lived, fugitive species, particularly weeds, providing a strategy to escape from the negative effects of density or competition. According to Venable and Burquez (1989), this may be a form of bet hedging in response to environments that vary spatially or temporally. These aspects of morphological heteromorphism are important because they may be associated with ecological strategies that have evolutionary significance. Ecological strategies arising from morphological heteromorphism include differential competitive performance (Weiss, 1980; Venable, 1985), dispersal (Telenius, 1992; Kigel, 1995), dormancy (Venable and Levin, 1985; Venable *et al.*, 1995), within- or among-year timing of germination (McEvoy, 1984; Tanowitz *et al.*, 1987; Zhang, 1993), vulnerability to predators (Cook *et al.*, 1971) and seedling growth (Zhang, 1993).

Seed heteromorphism may occur at the level of the inflorescence (Cavers and Steel, 1984; Venable *et al.*, 1995), individual (McGinley, 1989; Sakai and Sakai, 1996; Chmielewski, 1999), clone (Chmielewski *et al.*, 1989), population (Venable *et al.*, 1995) or species (Maun and Payne, 1989; Walck *et al.*, 1997). Morphological heteromorphism may occur in seed size (Maun and Payne, 1989), seed shape (Venable and Burquez, 1989), seed colour (McGinley, 1989; Doucet and Cavers, 1997; Puga-Hermida *et al.*, 2003a) (Fig. 1A), seed-coat texture (Williams and Harper, 1965), pattern allocation (Maun and Payne, 1989) and secondary structures (McGinley, 1989).

The heterogeneous character of seeds has been described for 18 families of angiosperms (Table 1), with the *Asteraceae* (138 species and 52 genera), *Chenopodiaceae* (18 and 10), *Brassicaceae* (12 and 8) and *Caryophyllaceae* (11 and 2) being the most represented (for a current review, see Imbert, 2002). In the *Asteraceae*, there are peripheral and central achenes of different size (e.g. *Picris amalecitana*), colour (e.g. *Crepis sancta*) and dispersion structures (pappus and trichomes, e.g. *Senecio jacobea*). Heterospermy (i.e. production, in the same fruit, of seeds differing in colour, size, shape, development or ecological functions) can be found in the genus *Xanthium*, which possesses two types of seeds, the upper seeds being heavier than the lower ones. In *Chenopodiaceae*, some species produce seeds that have different morphs during the life cycle (e.g. *Halogeton glomeratus*). However, in *Chenopodium album* the pericarp is either reticulate or smooth, and either black or brown, leading to four seed morphs; whereas in *Salsola volkensii*, heterospermic seeds (green and yellow) were found. In *Brassicaceae*, different types of heterospermy have been described. For example, *Aethionema*



**Figure 1.** Some characteristics of heterogeneous seeds of *Brassica rapa* cv. Rapa. (A) Seed lots used in the study. (B) Germination percentage of seed lots B (○,●), DB (□,■) and LB (△,▲) in the presence (closed symbols) and absence (open symbols) of 7 μM ethrel. (C) Hourly water uptake during the first 5 h of imbibition of three lots (B, black bars; DB, grey bars; LB, white bars). (Adapted from Puga-Hermida *et al.*, 2003a.)

*carneum*, *A. heterocarpum* and *A. saxatile* are heterocarpic, since individuals produce both many-seeded dehiscent siliques and one-seeded indehiscent ones. In other cases, such as *Sinapis alba*, the individuals produce partially dehiscent siliques with various

types of seeds (dispersed and not dispersed), aerial siliques (many light seeds) and underground siliques (few heavy seeds). Other cases of seed heterogeneity in crucifers (e.g. *Brassica rapa*) will be cited throughout this review. It should be noted that some authors use the term 'heterogeneity' as a synonym for polymorphism to denote seeds morphs differing in shape, colour, genetic structure or embryo size.

**Table 1.** Families displaying seed heterogeneity (data from Imbert, 2002)

Family	Number of species	Number of genera
<i>Asteraceae</i>	138	52
<i>Chenopodiaceae</i>	18	10
<i>Brassicaceae</i>	12	8
<i>Caryophyllaceae</i>	11	2
<i>Nyctaginaceae</i>	9	1
<i>Poaceae</i>	7	7
<i>Fabaceae</i>	5	5
<i>Cistaceae</i>	4	1
<i>Apiaceae</i>	3	3
<i>Papaveraceae</i>	2	2
<i>Valerianaceae</i>	2	1
<i>Commelinaceae</i>	1	1
<i>Euphorbiaceae</i>	1	1
<i>Fumariaceae</i>	1	1
<i>Plantaginaceae</i>	1	1
<i>Polygonaceae</i>	1	1
<i>Rubiaceae</i>	1	1
<i>Thymelaceae</i>	1	1

### The ontogeny of seed heterogeneity

To understand and explain the reason for seed heterogeneity in the life cycle of higher plants, we must consider a number of developmental models (e.g. heterophylly and inflorescence development). Because plants have modular architecture and plastic development, environmental factors can prompt morphological variations in the same individual (heterophylly; Wells and Pigliucci, 2000). Some studies on heterophylly have proposed possible mechanisms to explain the ontogeny of heteromorphic seeds. However, the analogy is valid only for some species, given that the prime characteristic of seed heteromorphism is its independence with respect to environmental conditions (Lloyd, 1984).

Growth and development of the inflorescence in angiosperms implies sequential maturation of the

flowers and seeds (acropetal maturation). Due to a source–sink relationship (Patrick and Offler, 2001), the proximity of seeds to vascular tissues can affect their size (Susko and Lovett-Doust, 2000). Thus, seeds from the same plant show size variation (Lokker and Cavers, 1995; Simons and Johnston, 2000) and hierarchy (Simons and Johnston, 1997; Baskin and Baskin, 1998; Susko and Lovett-Doust, 1998). This model may explain the seed heterogeneity in species with inflorescences (e.g. *Atriplex* or *Abronia*). For example, in flowers grouped in a capitulum (e.g. *Tragopodon dubius*), floral development is centripetal, and the peripheral achenes present greater mass and size than the do central ones (Maxwell *et al.*, 1994). Consequently, in most heterocarpic *Asteraceae*, the morphological differentiation between achenes is related to the position within the capitulum. However, there are examples (e.g. *Bidens pilosa*) in which central achenes weigh more than peripheral ones (Rocha, 1996). In contrast, the seeds of *Calendula* all have the same morphology regardless of position (Gardocki *et al.*, 2000), whereas in the Mediterranean composite, *Crepis sancta*, the morphological differentiation between peripheral achenes and central ones was not associated with a germination dimorphism (Imbert, 1999, 2001).

Some authors consider that some characteristics of the inflorescence in the *Asteraceae* (i.e. deficiencies of space for the achene or presence of bracts) favour heterocarpy (Venable, 1985). Sometimes, the position of the seed on the plant, or even its location within the inflorescence (positional effect), can affect the chemical composition of the seed and of the seed coat, in particular (Gutterman, 1994a; Maxwell *et al.*, 1994; Jaimand and Rezaee, 1996). In grain legumes, sequential development and spatial heterogeneity of pod position can lead to great quality differences between seeds on the mother plant (Coste *et al.*, 2001). Legumes with multi-seeded pods frequently show a positional effect that alters seed size and seed-coat permeability. Thus, there are several desert legumes in which the seeds of the pod soften at different rates – more rapidly in the proximal or distal end of the pod, depending on the species (Koller, 1972). The positional effect was studied in *Mesembryanthemum nodiflorum* (*Aizoaceae*) (Gutterman, 1980/81), revealing that seeds produced on the same mother plant differed in their germinability, even under similar day length and temperature conditions during maturation. The peripheral seeds reached a higher germination percentage than did the other seeds, which were scattered later. This differential germination was still found in seeds sown 8 years afterwards, demonstrating the possible ecological importance of the phenomenon. Similar results were also reported in the genus *Aegilops* (*Poaceae*), in which grains germinate in different years to prevent competition between plants

that germinate from the same dispersal unit (Wurzbürger and Koller, 1976).

Some plants have great variability in seed characteristics because of the positional effect. This effect can influence seed size as a result of differential seed filling (Gutterman, 1992), or because the parent plant was exposed to different environmental conditions (Michaels *et al.*, 1988; Fenner, 1993). Such species as *Triplasis purpurea* (*Poaceae*) produce heteromorphic seeds (Cheplick and Sung, 1998) in response to the maternal nutrient environment and maturation position. In some cases, the positional effect can alter dormancy, as in many-seeded fruits. In this way, either simultaneous seed germination or sister-seed competition is eliminated.

In some species, developmental deficiencies explain only seed size, but do not clarify other major morphological differences. Thus, there must be some ontogenic process that triggers morphological differentiation in the seed. Bachmann (1983) suggested a model based on the appearance of a hormonal gradient from the periphery towards the centre of the inflorescence. This model implies a morphogenetic gradient. For example, in the inflorescence of *Araceae*, sexual morphology has such a distribution: the lower part has the female flowers, the middle part hermaphroditic flowers, and the upper part male flowers. This variation is assumed to be due to similar morphogenetic gradients (Barabé and Jean, 1996). This model, which explains the ontogeny of seed differentiation, leads to the conclusion that morphological differentiation is independent of the contribution of the parental generation. But, in fact, it is the result only of maternal effects, and there are no genetic differences between seeds that are controlled by morphological differentiation. Consequently, the differences in shape are not genetic but maternal effects (Imbert *et al.*, 1999). Also, seed heteromorphism is considered a variation within an individual (Imbert, 2001).

### Implications of seed size and mass heterogeneity in the life cycle of plants

All plants possess a certain degree of seed heterogeneity, since variation in seed mass is invariably observed (Westoby *et al.*, 1992; Fenner, 1993). Currently, no genetic basis is known for the control of variation in the seed mass, although some authors conjecture that the environment controls this process (Fenner, 1993; Wolfe, 1995). Variation in seed mass within individuals is sometimes due to a positional effect on the mother plant, resulting in differences in seed filling (Gutterman, 1992), whereas differences in seed weight between plants could result from differences in environmental conditions (e.g. nutrients, light or

water) to which the mother plants were subjected during the growing season (Fenner, 1993). Seed size has been considered one of the phenotypically least flexible characters in many species (Fenner, 1993). In species with clear seed dimorphism, the characteristics of germination vary frequently with seed size or mass (Zhang, 1993, 1994; Prinzie and Chmielewski, 1994; Andersson, 1996; Khan and Ungar, 2001). It is often hypothesized that seedlings from larger seeds are more stress tolerant (Berger, 1985; Zhang, 1995). This does not seem to be the case in *Crespis sancta*, or in *Cakile maritima*, another heteromorphic-seeded species (Zhang, 1995). The composition and concentration of the nutrient reserves are similar in dimorphic seeds of *Salicornia europea* (Austenfeld, 1998), *Cakile maritima* and *Arctotheca populifolia* (Hocking, 1982). On the other hand, little information is available concerning the influence of seed size on the resulting seedlings of woody plant species. A higher percentage of large *Cecis canadiensis* seeds germinated than did small seeds (Couvillon, 2002).

Seed size appears to be related to persistence in the seed bank. However, the correlation between seed dormancy and persistence is extremely weak (Thompson *et al.*, 2003), perhaps because seed dormancy and persistence are quite different phenomena (Baskin and Baskin, 1998). Generally, small seeds tend to occupy deeper soil horizons, thereby avoiding predation (Fenner, 1993; Westoby *et al.*, 1996) and becoming more persistent in the seed bank (Bakker *et al.*, 1996). Predation is one of the selective forces that act on seed size and shape, favouring small seeds (Hulme, 1998). Some plant communities of persistent seeds have smaller and more rounded seeds than communities of transient seeds (Thompson *et al.*, 1993; Bekker *et al.*, 1998; Hodkinson *et al.*, 1998; Funes *et al.*, 1999; Moles *et al.*, 2000; Thompson *et al.*, 2001; Peco *et al.*, 2003). However, this is apparently not universal. Thus, in Australian flora, which is rich in shrubs and trees, there is no relationship at all between seed size and persistence, perhaps due to a high proportion of large-seeded species with hard seeds (Leishman and Westoby, 1998).

Although large seeds have some advantage over small ones (e.g. most suited for establishment in arid and semi-arid ecosystems) (Kigel, 1995), large seeds are much less abundant in heterocarpic species that produce multiple fruit morphs by a single individual (Venable, 1985; Gardocki *et al.*, 2000), and size appears to affect the percentage and speed of germination. Species that produce two or more seed types represent groups where divergent strategies, usually found in different taxa, are combined in one individual. Such groups, which tend to have divergent seed functions, each specialize in some aspect of environmental variation to which they are predisposed, while being

buffered by the other seed type. It is currently accepted that if a plant species produces two types of seed with differential dispersability in space and time, reproduction will be maximum when one of the seed types has high dispersability and low dormancy, and the other type the inverse. Evolution may have arrived at a trade-off with respect to seed size, since the small seeds are more suited to dispersion, whereas larger seeds favour establishment and adaptation to a particular ecosystem. However, such a generalization is awkward, since we must take into account such factors as the manner of dispersion, the properties of the ecological niche, the presence of the seed bank in this ecosystem, and the characteristics of the seed itself, among other factors.

In view of recent data, we can conclude that small and rounded seeds, compared with large and elongated or flattened ones: (1) are more easily buried (Thompson *et al.*, 1993); (2) undergo lower predation rates when buried (Hulme, 1998); (3) are less exposed to germination-promoting stimuli in seed banks (e.g. light or alternating temperatures) (Baskin and Baskin, 1998, 2004; Milberg *et al.*, 2000); and (4) are more likely to have a light requirement for germination, and thus burial depth is more likely to become a seed-survival factor (Milberg *et al.*, 2000; Baskin and Baskin, 2004). Thus, size and shape of seeds are key factors in determining seed fate and persistence in soil (Thompson *et al.*, 1993; Bakker *et al.*, 1996; McDonald *et al.*, 1996; Funes *et al.*, 1999; Cerabolini *et al.*, 2003). On the other hand, large-seeded species are less dependent on light for germination than small-seeded ones; and, therefore, light response and seed mass have coevolved (Milberg *et al.*, 2000).

### Dormancy in heterogeneous seeds

At present, few studies are available on dormancy in heterogeneous seeds. Seed dormancy, defined as the temporary failure or block of a viable seed to complete germination under physical conditions that normally favour the process (Bewley, 1997a; Baskin and Baskin, 1998; Koornneef *et al.*, 2002), can be coat-imposed and/or determined by the embryo itself (Leubner-Metzger, 2003). Seed-coat characteristics are a key in the development of physical dormancy, as they affect water uptake and gas exchange. Thus, many seeds have physical dormancy due to a hard seed coat (Bell *et al.*, 1993; Boesewinkel and Bouman, 1995; Baskin and Baskin, 1998, 2004). Chemical or physical treatments that increase the permeability of these seeds boost germination (Baskin *et al.*, 1998). However, the part of the seed coat made permeable depends on the seed type (Kelly and van Staden, 1985; Manning and van Staden, 1987; Angosto and Matilla, 1993a, b, 1994). In some seeds (e.g. *Fabaceae*, *Geraniaceae*,

*Malvaceae*), one surprising feature of the palisade cells (responsible for seed-coat impermeability), is the so-called 'light line' running in a periclinal direction through the secondary cell-wall material (Manning and van Staden, 1985; Meisert *et al.*, 2001). Some authors suggest that the osmiophilic 'light line' may be responsible for seed-coat hardness (Harris, 1987). However, this is not clear in the *Geraniaceae* (Meisert *et al.*, 2001).

In addition, some species produce heterogeneously coloured seeds with different degrees of hardness, as in the case of *Senna obtusifolia* (about 90% were green and had hard-seed-coat dormancy, whereas the rest were brown and non-dormant) (Baskin *et al.*, 1998). Some 82–93% of the brown seeds germinated, while only 15–32% of the green ones germinated. This apparent heterogeneity in *S. obtusifolia* may be an important ecophysiological strategy, since brown seeds can germinate in the spring in temperate regions, whereas green seeds cannot germinate until late spring–summer, when high temperatures cause an increase in seed-coat permeability. The argument applied to these legumes may be a way of explaining the appearance of heterogeneity with respect to the occurrence of hard-seededness within a species. In *Arabidopsis*, most testa mutants have reduced seed dormancy (Debeaujon *et al.*, 2000). Thus, the *ats* mutant forms fewer dormant seeds than does the wild type, because the testa possesses three rows of cells rather than six (Léon-Kloosterziel *et al.*, 1994). Also, seeds of the tomato *sit<sup>w</sup>* mutant (deficient in ABA) show less resistance to radicle penetration because they have a very fine testa (one layer of cells), whereas the wild type has four to five cell layers (Hilhorst and Downie, 1995). Dormancy in *Vicia faba* was studied to determine whether the genes that control dormancy are related to other genes. For example, a locus involved in dormancy (i.e. *doz*), which was characterized, proved to be linked to a locus controlling anthocyanin and pro-anthocyanidin synthesis (i.e. *sp-v*), with the genetic distance estimated to be about 25 cM (Ramsay, 1997). Some of these mutations affect primary dormancy, and therefore, the seeds can develop viviparism (Groot and Karssen, 1992; Ren and Bewley, 1998; Koornneef *et al.*, 2002). Ren and Bewley (1998), studying *Brassica rapa* ssp. *pekinensis*, reported a relationship between precocious germination and testa structure, involving the testa area through which the cotyledon penetrates. Viviparism normally causes damage, but at times serves as a mechanism for the adaptation and propagation of the species (Picciarelli *et al.*, 1994). *Amaranthus retroflexus* L. (red-root pigweed) plants produce more than 1 million homogeneously coloured seeds per m<sup>2</sup> that have different degrees of dormancy. This enables them to remain in wet soil for several years, and only a small percentage of their very large

seed bank may germinate from time to time (Cavers and Steel, 1984). On the other hand, the seeds of *Spergularia diandra*, which are heterogeneous in colour (black, brown and yellow), have different degrees of dormancy depending on the position on the mother plant, maturation conditions and post-maturation temperatures; the yellow seeds are the lightest with the highest percentage of dormancy (Gutterman, 1994a, b). In contrast, in different populations of coloured seeds from bull thistle (*Cirsium vulgare*), the proportion of dormant seeds did not appear to be associated with seed colour (Doucet and Cavers, 1997).

Plants that produce achenes have several strategies to slow down water uptake and induce dormancy, including: (1) involucre bracts covering the achene (Gutterman, 1994b); (2) differential permeability of the involucre bracts in individuals with heteromorphic seeds (Takeno and Yamaguchi, 1991); (3) differential permeability of the achene according to its location on the plant (McDonough, 1975); and (4) heterogeneity in achene size among heteromorphic individuals (Venable *et al.*, 1987; Rocha, 1996). Dormancy in achenes can be induced as a consequence of a different chemical composition of the seed coat in the morphs (e.g. concentration of water-soluble germination inhibitors or ABA content; Takeno and Yamaguchi, 1991; Beneke *et al.*, 1993), or by differences in a requirement, either physical (e.g. temperature), light (e.g. photoperiod) or chemical (e.g. salinity) to induce germination of the different seed morphs (Brown and Mitchell, 1984; Berger, 1985; de Clavijo, 1994).

The study of dormancy of seeds from inflorescences provides important information. In the spikelet of the genus *Aegilops* (*Poaceae*), two caryopsis types differ in size and dormancy level. The largest caryopses have the least dormancy and can germinate in different seasons (Maranon, 1989). In the perennial herb *Cenchrus ciliaris*, the dispersal unit contains 1–4 seeds. The units that contain one seed show much less dormancy than those with more than one (Hacker and Ratcliffe, 1989). This suggests that the interactions between the fruits developing in the same inflorescence determine the dormancy level.

#### Alterations of the seed coat of heterogeneous seeds affect dormancy and germinability

The seed coat, the main layer responsible for impermeability, often consists of prismatic, radially elongated, and compactly arranged palisade cells (Meisert *et al.*, 1999). The palisade layer of the seed coat appears to account for the variation in permeability of *Arabidopsis thaliana* seeds with different colours (Chaple *et al.*, 1994; Kuang *et al.*, 1996). Mature *A. thaliana* seeds have a dark coat due to condensed

tannins of the procyanidin type. The imbibition rate of seeds with different seed-coat colours (e.g. pea, wheat and turnip tops) is slower in dark seeds than in light ones (Huang *et al.*, 1983; Powell *et al.*, 1986a; Powell, 1989; Puga-Hermida *et al.*, 2003a) (Fig. 1C), to such a degree that water-uptake ability has been related to dormancy (Huang *et al.*, 1983), as well as to radicle emergence (Powell and Matthews, 1978, 1980). In peas and soybeans these differences in the rate of water uptake and seed vigour occur between lots within a cultivar (Oliveira *et al.*, 1984), whereas in *P. vulgaris* genotypic differences between cultivars are more important (Powell *et al.*, 1986b). In this latter case, the cultivars with an absence of anthocyanins in the seed coats imbibe rapidly (the seed coat loosens rapidly), have low vigour, suffer higher solute leakage, and show poor field emergence. The pigmented seeds imbibe slowly, the seed coat remains tightly appressed to the cotyledons, and the embryonic axis develops normally (Powell *et al.*, 1986b; Legesse and Powell, 1992). These differences in pigmentation and imbibition are determined by seed genotype (Powell, 1989). Similar results have been reported in *Panicum miliaceum* seeds (Khan *et al.*, 1996).

Seed permeability is also related to density and thickness of the seed coat, both parameters being low in light-coloured seeds. The permeability of dark seed coats can be reduced by the number of layers making up the coat, by cell density, and by certain chemical reactions (e.g. phenolic oxidation) exclusive to dark seeds. The normally dormant pigmented seeds of *Sorghum halepense* may be altered by drought during development, which produces non-pigmented, thinner-coated, more germinable seeds (Benech-Arnold *et al.*, 1992). Thick, strongly pigmented seed coats may also form a chemical barrier to pathogens (e.g. the *ortho*-dihydroxyphenols can act as a defence against herbivory; Hendry *et al.*, 1994) and perhaps promote persistent seed banks in the soil.

A striking case of heterogeneity involves seeds, belonging to the same species, that have developed at different altitudes (Angosto and Matilla, 1993a, b, 1994). The seeds of the legumes *Adenocarpus decorticans*, *Astragalus granatensis* ssp. *granatensis* and *Cytisus reverchonii* (all endemic to the Betic Cordillera, Spain) were collected from different altitudes. All seeds had the same mass and shape, and required chemical scarification to germinate, showing an optimum temperature according to altitude. The highest germination rates were correlated with the lowest amounts of ABA in all species, while putrescine was the only polyamine that varied with altitude (Angosto and Matilla, 1993a). In *Astragalus granatensis* ssp. *granatensis* the tangential-cell layer of the seed coat thinned as the altitude rose, and at lower altitudes the outside of the seed coat was rougher. However, in *Adenocarpus decorticans* the outside of the seed coat

from seeds collected at a medium altitude (1520 m) was smoother than that from seeds maturing at lower (1340 m) and higher (1800 m) altitudes. This roughness, and the consequent increase in surface area, presumably enhance the capacity of the seed to absorb water during imbibition (Angosto and Matilla, 1993b). On the other hand, seeds from two endemic populations of *Festuca indigesta* (*Gramineae*) collected at altitudes of 2250 m and 2560 m (both populations of seeds having the same mass and shape) did not require scarification, but rather strongly depended on temperature to germinate. No variation in free ABA was observed between the two types of mature wild seeds. However, whereas the seed-coat structure was similar in the two populations, the aleurone layer was composed of a single (2250 m) or a double (2560 m) layer of cells, respectively (Angosto and Matilla, 1994).

The seeds with alterations in shape of the seed coat produced by mutant plants are heterogeneous with respect to the wild type and, consequently, constitute an important tool for studying the role of seed-coat variations in the physiology of seeds. Thus, the use of *A. thaliana* mutants, such as the recessive *aberrant testa shape* (*ats*) or the *transparent testa glabra* (*ttg*), demonstrates the importance of seed-coat properties in determining seed shape, dormancy and germination (Léon-Kloosterziel *et al.*, 1994). Perhaps the absence of the mucilage layer in *ats* and *ttg* seeds allows more O<sub>2</sub> to diffuse into the seed, and this higher O<sub>2</sub> level might stimulate the breaking of dormancy (Corbineau and Côme, 1995). Several pigmentation mutants (Debeaujon *et al.*, 2000) and those with altered seed-coat structure (Léon-Kloosterziel *et al.*, 1994; Meinke, 1994) have reduced dormancy. The reduction of dormancy is more pronounced in the seed-coat-pigmentation mutants than in structural mutants (Debeaujon *et al.*, 2000). The above *Arabidopsis* mutants are characterized by a replacement of the pro-anthocyanidin polymers by anthocyanins, conferring far greater water permeability, and by a reduction of the phenolic content in the endothelium and the crushed parenchyma layers, thus thinning the seed coat.

In the *gal* mutants, which have impaired GA biosynthesis and depend on exogenous GAs for germination, the mechanical removal of the seed envelopes can substitute for the GA requirement, whereas some *transparent testa* (*tt*) mutants germinate in the absence of stratification, light or GAs (Debeaujon and Koornneef, 2000). Double mutants (*tt* × *gal*), in the absence of GAs, are able to germinate fully when they imbibe under optimal germination conditions with respect to light and temperature. These results demonstrate that the GA requirements for germination may be imposed by the seed coat, and its characteristics are responsible for the degree of coat-enhanced dormancy. Consequently, the specific

weakening of the testa is sufficient to enable germination in the absence of GAs (Debeaujon *et al.*, 2000).

In addition, the *TTG* gene (the mutant *ttg* of *A. thaliana* has an altered seed-coat surface) is a homologue of the *R* locus of maize, which is a transcription factor that activates the promoters of genes associated with the anthocyanin pathway (Lloyd *et al.*, 1992). The maize *Vp1* gene, which is expressed in seeds and is associated with ABA sensitivity (Bailey *et al.*, 1999), is also involved in flavonoid synthesis (McCarty, 1995). The main determinant of wheat caryopsis colour is the flavonoid phlobaphene (Grotewold *et al.*, 1994), which is located in the seed coat. The *taVp1* gene, an orthologue of *Vp1*, is related to the *R* locus, which controls the colour of wheat kernels (Bailey *et al.*, 1999). While the *taVp1* appears not to be related to the level of post-harvest dormancy (McKibbin *et al.*, 1999), seeds having a red colour (a dominant trait regulated by three genes) are more resistant to preharvest sprouting than white ones (Flintham, 2000; Warner *et al.*, 2000). Four facts are important in the study of the *R* locus: (1) its relationship with ABA sensitivity (Kawakami *et al.*, 1997); (2) this locus, located on chromosome 3, increases dormancy levels in wheat caryopses (Flintham, 2000; Warner *et al.*, 2000); (3) this locus can increase dormancy in *Triticum aestivum* by enhancing ABA sensitivity (Himi *et al.*, 2002); and (4) in wheat, this locus is responsible for the development of germinability, which may be controlled by one or more genes closely linked to the *R* locus (Himi *et al.*, 2002). Also, sorghum genotypes with high tannin concentrations in the seed coat avoid preharvest sprouting (Lijavetzky *et al.*, 2000), and seed-coat pigmentation regulates germinability in *Panicum miliaceum* L. seeds (Khan *et al.*, 1996).

In conclusion, *Arabidopsis* testa mutants demonstrate that the degree of seed dormancy is determined not only by characteristics of the embryo, but also by characteristics of the testa, and therefore it is possible to identify the role of the testa in determining seed shape, dormancy and germination in a number of heterogeneous seeds. However, in endospermic heterogeneous seeds, the contributions of both the testa and the endosperm layers to the degree of coat-imposed dormancy need to be considered (Hilhorst, 1995; Bewley, 1997b).

### The role of hormones in the occurrence of seed heterogeneity at the molecular level

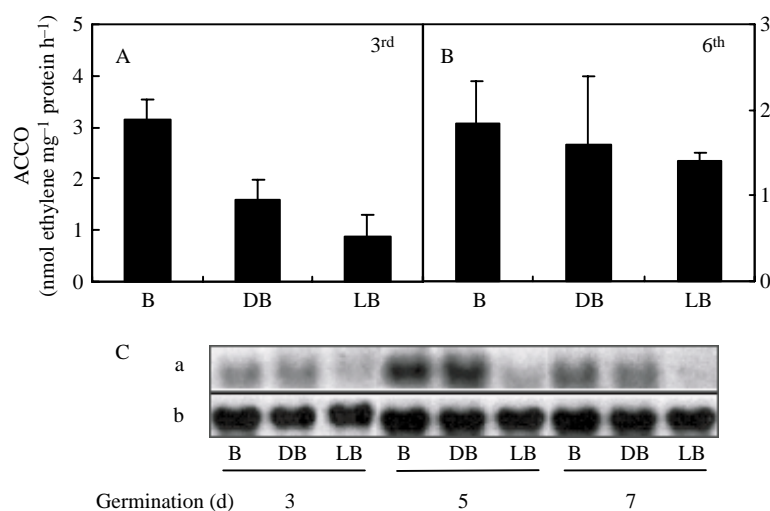
Although heterogeneity appears to be related to seed survival, at present little is known about how the behaviour of these seeds is hormonally regulated. In some *Brassicaceae*, heterogeneity refers to the fact that

seeds do not mature synchronously on the mother plant, but rather sequentially (Meinke, 1994; Spence *et al.*, 1996). Shattering of the silique causes substantial loss of viable seeds, which, in some cases, may remain in the seed bank of the soil and infest future crops. Given that the heterogeneous character of seeds can affect germination (Gutterman, 1994a; Khan *et al.*, 1996; Doucet and Cavers, 1997; Puga-Hermida *et al.*, 2003a) (Fig. 1B), and that fruiting species bear a great abundance of highly heterogeneous seeds, these seeds constitute a valuable tool to study the endogenous factors that regulate germination. We have used these characteristics of mature seeds of turnip tops (*Brassica rapa* L. cv. Rapa), which are heterogeneous in colour and size, to identify these endogenous factors. It appeared that the activity of the final step of the ethylene pathway, levels of endogenous ABA and polyamines, and capacity of imbibition are affected by heterogeneity (Puga-Hermida, 2003; Puga-Hermida *et al.*, 2003a, b; Rodríguez-Gacio *et al.*, 2004). The sensitivity to ethylene appeared not to be distributed equally among the heterogeneous seeds (Fig. 1A). Moreover, the ACC oxidase activity and expression of the transcript corresponding to the gene *BrACO1* are more intense in germinating black seeds than in dark brown or light brown seeds, respectively (Fig. 2; Puga-Hermida *et al.*, 2003b). Our results raise the hypothesis that one of the factors triggering differences in germination among heterogeneous seeds of *B. rapa* L. cv. Rapa may be an alteration in the mechanism of ABA and ethylene action. Interactions between ABA, ethylene signalling and physiology have been demonstrated recently (Beaudoin *et al.*, 2000; Grossmann and Hansen, 2001; Koornneef *et al.*, 2002).

### Concluding comments

The appearance of the seed in the life cycle of a higher plant is a special adaptation mechanism to adverse environmental conditions, making it possible for the plant species to perpetuate itself. Once zygotic embryogenesis has been completed, the seed is a completely autonomous entity (Torres-Ruiz, 1998). Seeds may be morphologically heterogeneous (e.g. colour, size, weight and shape), and some of these differences affect physiological properties (e.g. level of dormancy), which, in turn, can alter the speed and timing of dispersion (Kigel, 1995). It is not known what causes this heterogeneity, but it is an important strategy for the survival of certain plant species in adverse and variable ecosystems (Egli, 1998). Within heterogeneous seed populations, some seed lots are more persistent than others in the soil seed banks (Khan *et al.*, 1996). The reason for this persistence is not currently known, although some investigators attribute it to the seed coat (Hendry *et al.*, 1994).





**Figure 2.** ACC oxidase (ACCO) activity in seeds and seedlings of *Brassica rapa* cv. Rapa belonging to the lots of Fig. 1A and germinated in water for (A) 3 d and (B) 6 d. (C) Time course of ACCO mRNA (*BrACO1*) expression in embryonic axes of seeds belonging to B (black), DB (dark brown) and LB (light brown) lots during 3, 5 and 7 d of germination. (Adapted from Puga-Hermida *et al.*, 2003a.)

Thus, coloration and chemical composition of the seed coat might affect the capacity and rate of water uptake (Powell *et al.*, 1986a; Benech-Arnold *et al.*, 1992; Khan *et al.*, 1996; Puga-Hermida *et al.*, 2003a). In crucifers, it is well known that some type of heterogeneity results from the fact that seeds of fruits (i.e. siliques) do not mature synchronously on the mother plant, but rather sequentially (Meinke, 1994; Spence *et al.*, 1996).

Although heterogeneity appears to be related to survival, little is known about how the seeds are affected physiologically. It has been demonstrated that the ability to germinate differs within a population of heterogeneous seeds (Gutterman, 1994a; Puga-Hermida *et al.*, 2003b). In this sense, the capacity for water uptake, altered by the different structural characteristics (Benech-Arnold *et al.*, 1992; Debeaujon *et al.*, 2000) and colour (Khan *et al.*, 1996; Puga-Hermida *et al.*, 2003a) of the palisade layer (Meisert *et al.*, 1999) of the seed coat, appears to be involved. For the first time, a differential sensitivity to ethylene and capacity to synthesize it was demonstrated recently in heterogeneous seeds (Puga-Hermida *et al.*, 2003a). We suggest that during silique maturation, a degree of differential preparation for the production and perception of ethylene takes place in the seeds. In addition, the seeds of *B. rapa* cv. Rapa are heterogeneous with respect to their free and conjugated ABA content (Puga-Hermida *et al.*, 2003a, b). Our results raise the hypothesis that one of the factors triggering different germination rates in heterogeneous seeds of *B. rapa* L. cv. Rapa may be an alteration in the mechanism of ABA and ethylene action. This ABA-ethylene interrelationship would be consistent with

that put forward by Grossmann and Hansen (2001). These authors proposed a model assigning different roles for ABA (e.g. promotion of dormancy, inhibition of cell elongation and division, senescence or gravitropism) as an ethylene-triggered second hormonal messenger in the regulation of growth and development in higher plants. It appears that ABA increases tissue sensitivity to ethylene, affecting seed germination (Beaudoin *et al.*, 2000), whereas ethylene may suppress seed dormancy by inhibiting ABA action (Beaudoin *et al.*, 2000; Koornneef *et al.*, 2002).

In summary, the still meagre physiological and molecular evidence gathered suggests that an array of signals triggered by factors within and/or outside the seeds induces heterogeneity, the biological function of which is far from being fully understood. Nevertheless, the differential seed maturation, the source-sink relationship during zygotic embryogenesis, and the arrangement of the seeds within the fruit should not be neglected in future attempts to elucidate this important aspect of survival in higher plants.

### Acknowledgements

This review has been supported by grants from 'Dirección General de Investigación' BFI2000-0305 and Xunta de Galicia PGIDT00AGR20301PR. M.I.P-H is, at present, the recipient of a post-doctoral fellowship from Comunidad Autónoma de Madrid (Spain) in the CNB (Centro Nacional de Biotecnología, Spain). We are particularly grateful to two anonymous reviewers

for their constructive criticisms and helpful comments, and to colleagues sending the bibliographic material to prepare this work. The English version of the text was prepared by David Nesbitt.

## References

- Andersson, S. (1996) Seed size as a determinant of germination rate in *Crepis tectorum* (Asteraceae): evidence from a seed burial experiment. *Canadian Journal of Botany* **74**, 568–572.
- Angosto, T. and Matilla, A.J. (1993a) Germination, seed-coat structure and protein patterns of seeds from *Adenocarpus decorticans* and *Astragalus granatensis* growing at different altitudes. *Seed Science and Technology* **21**, 317–326.
- Angosto, T. and Matilla, A.J. (1993b) Variations in seeds of three endemic leguminous species at different altitudes. *Physiologia Plantarum* **87**, 329–334.
- Angosto, T. and Matilla, A.J. (1994) Modifications in seeds of *Festuca indigesta* from two different altitudinal habitats. *Seed Science and Technology* **22**, 319–328.
- Austenfeld, F.A. (1998) Seed dimorphism in *Salicornia europaea*: nutrient reserves. *Physiologia Plantarum* **73**, 502–504.
- Bachmann, K. (1983) Evolutionary genetics and the genetic control of morphogenesis in flowering plants. *Evolutionary Biology* **16**, 157–208.
- Bailey, P.C., McKibbin, R.S., Lenton, J.R., Holdsworth, M.J., Flintham, J.E. and Gale, M.D. (1999) Genetic map locations for orthologous *Vp1* genes in wheat and rice. *Theoretical and Applied Genetics* **98**, 281–284.
- Bakker, J.P., Poschlod, P., Strykstra, R.J., Bekker, R.M. and Thompson, K. (1996) Seed banks and seed dispersal: Important topics in restoration ecology. *Acta Botanica Neerlandica* **45**, 461–490.
- Barabé, D. and Jean, R.V. (1996) The constraints of global form on phyllotactic organization: the case of *Symplocarpus* (Araceae). *Journal of Theoretical Biology* **178**, 393–397.
- Baskin, C.C. and Baskin, J.M. (1998) *Seeds: Ecology, biogeography and evolution of dormancy and germination*. San Diego, Academic Press.
- Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16.
- Baskin, J.M., Nan, X. and Baskin, C.C. (1998) A comparative study of seed dormancy and germination in an annual and a perennial species of *Senna* (Fabaceae). *Seed Science Research* **8**, 501–512.
- Beaudoin, N., Serizet, C., Gosti, F. and Giraudat, J. (2000) Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* **12**, 1103–1115.
- Bekker, R.M., Bakker, J.P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P., Thompson, K. and Willems, J.H. (1998) Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology* **12**, 834–842.
- Bell, D.T., Plummer, J.A. and Taylor, S.K. (1993) Seed germination ecology in southwestern Western Australia. *Botanical Review* **59**, 24–73.
- Benech-Arnold, R.L., Fenner, M. and Edwards, P.J. (1992) Changes in dormancy level in *Sorghum halepense* seeds induced by water stress during seed development. *Functional Ecology* **6**, 596–605.
- Beneke, K., van Rooyen, M.W., Theron, G.K. and van de Venter, H.A. (1993) Fruit polymorphism in ephemeral species of Namaqualand. III. Germination differences between the polymorphic diaspores. *Journal of Arid Environments* **24**, 333–344.
- Berger, A. (1985) Seed dimorphism and germination behaviour in *Salicornia patula*. *Vegetatio* **61**, 137–143.
- Bewley, J.D. (1997a) Seed germination and dormancy. *Plant Cell* **9**, 1055–1066.
- Bewley, J.D. (1997b) Breaking down the walls – a role for endo- $\beta$ -mannanase in release from seed dormancy? *Trends in Plant Science* **2**, 464–469.
- Bewley, J.D. and Black, M. (1994) *Seeds: Physiology of development and germination* (2nd edition). New York, Plenum.
- Boesewinkel, F.D. and Bouman, F. (1995) The seed: structure and function. pp. 1–24 in Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.
- Brown, N.A.C. and Mitchell, J.J. (1984) Germination of the polymorphic fruits of *Bidens bipinnata*. *South African Journal of Botany* **3**, 55–58.
- Cavers, P.B. and Steel, M.G. (1984) Patterns of change in seed weight over time on individual plants. *American Naturalist* **124**, 324–335.
- Cerabolini, B., Ceriani, R.M., Caccianiga, M., De Andreis, R. and Raimondi, B. (2003) Seed size, shape and persistence in soil: a test on Italian flora from Alps to Mediterranean coasts. *Seed Science Research* **13**, 75–85.
- Chaple, C.C.S., Shirley, B.W., Zook, M., Hammerschmidt, R. and Sommerville, S.C. (1994) Secondary metabolism in *Arabidopsis*. pp. 989–1030 in Meyerowitz, E.M.; Somerville, C.R. (Eds) *Arabidopsis*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press.
- Cheplick, G.P. and Sung, L.Y. (1998) Effect of maternal nutrient environment and maturation position on seed heteromorphism, germination, and seedling growth in *Triplasis purpurea* (Poaceae). *International Journal of Plant Sciences* **159**, 338–350.
- Chmielewski, J.G. (1999) Consequences of achene biomass, within-achene allocation patterns, and pappus on germination in ray and disc achenes of *Aster umbellatus* var. *umbellatus* (Asteraceae). *Canadian Journal of Botany* **77**, 426–433.
- Chmielewski, J.G., Semple, J.C., Burr, L.M. and Hawthorn, W.R. (1989) Comparison of achene characteristics within and among diploid and tetraploid clones of *Solidago flexicaulis* and their significance in germination and resource allocation studies. *Canadian Journal of Botany* **67**, 1821–1832.
- Cook, A.D., Atsatt, P.R. and Simon, C.A. (1971) Doves and dove weed: multiple defenses against avian predation. *BioScience* **21**, 277–281.
- Corbineau, F. and Côme, D. (1995) Control of seed germination and dormancy by the gaseous environment. pp. 397–424 in Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

- Coste, F., Ney, B. and Crozart, Y.** (2001) Seed development and seed physiological quality of field grown beans (*Phaseolus vulgaris* L.). *Seed Science and Technology* **29**, 121–136.
- Couvillon, G.A.** (2002) *Cercis canadensis* L. seed size influences germination rate, seedling dry matter, and seedling leaf area. *HortScience* **37**, 206–207.
- de Clavijo, E.R.** (1994) Heterocarpy and seed polymorphism in *Ceratocarpus heterocarpus* (Fumariaceae). *International Journal of Plant Science* **155**, 196–202.
- Debeaujon, I. and Koornneef, M.** (2000) Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology* **122**, 415–424.
- Debeaujon, I., Leon-Kloosterziel, K.M. and Koornneef, M.** (2000) Influence of the testa on seed dormancy, germination and longevity in Arabidopsis. *Plant Physiology* **122**, 403–413.
- Doucet, C. and Cavers, P.B.** (1997) Induced dormancy and colour polymorphism in seeds of the bull thistle *Cirsium vulgare* (Savi) Ten. *Seed Science Research* **7**, 399–407.
- Egli, D.B.** (1998) *Seed biology and the yield of grain crops*. Wallingford, CAB International.
- Fenner, M.** (1993) Environmental influences of seed size and composition. *Horticultural Reviews* **13**, 183–213.
- Flintham, J.E.** (2000) Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. *Seed Science Research* **10**, 43–50.
- Funes, G., Basconcelo, S., Diaz, S. and Cabido, M.** (1999) Seed size and shape are good predictors of seed persistence in soil in temperate mountain grasslands of Argentina. *Seed Science Research* **9**, 341–345.
- Gardocki, M.E., Zablocki, H., El-Keblawy, A. and Freeman, D.C.** (2000) Heterocarpy in *Calendula micrantha* (Asteraceae): The effects of competition and availability of water on the performance of offspring from different fruit morphs. *Evolutionary Ecology Research* **2**, 701–718.
- Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. and Jullien, M.** (2000) Control of seed dormancy in *Nicotiana plumbaginifolia*: Post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210**, 279–285.
- Groot, S.P.C. and Karssen, C.M.** (1992) Dormancy and germination of abscisic acid-deficient tomato seeds: Studies with the *sitiens* mutant. *Plant Physiology* **99**, 952–958.
- Grossmann, K. and Hansen, H.** (2001) Ethylene-triggered abscisic acid: a principle in plant growth regulation? *Physiologia Plantarum* **113**, 9–14.
- Grotewold, E., Drummond, B.J., Bowen, B. and Peterson, T.** (1994) The *myb*-homologous *P* gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. *Cell* **76**, 543–553.
- Gutterman, Y.** (1980/81) Annual rhythm and position effect in the germinability of *Mesembryanthemum nodiflorum*. *Israel Journal of Botany* **29**, 93–97.
- Gutterman, Y.** (1992) Maternal effects on seeds during development. pp. 27–59 in Fenner, M. (Ed.) *Seeds. The ecology of regeneration in plant communities*. Wallingford, CAB International.
- Gutterman, Y.** (1994a) Long-term seed position influences on seed germinability of the desert annual, *Mesembryanthemum nodiflorum* L. *Israel Journal of Plant Sciences* **42**, 197–205.
- Gutterman, Y.** (1994b) Strategies of seed dispersal and germination in plants inhabiting deserts. *Botanical Review* **60**, 373–425.
- Hacker, J.B. and Ratcliffe, D.** (1989) Seed dormancy and factors controlling dormancy breakdown in buffel grass accessions from contrasting provenances. *Journal of Applied Ecology* **26**, 201–212.
- Harris, W.M.** (1987) Comparative ultrastructure of developing seed coats of 'hard-seeded' and 'soft-seeded' varieties of soybean, *Glycine max* (L.) Merr. *Botanical Gazette* **148**, 324–331.
- Hendry, G.A.F., Thompson, K., Moss, C.J., Edwards, E. and Thorpe, P.C.** (1994) Seed persistence: a correlation between seed longevity in the soil and *ortho*-dihydroxyphenol concentration. *Functional Ecology* **8**, 658–664.
- Hilhorst, H.W.M.** (1995) A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**, 61–73.
- Hilhorst, H.W.M. and Downie, B.** (1995) Primary dormancy in tomato (*Lycopersicon esculentum* cv. Moneymaker): studies with the *sitiens* mutant. *Journal of Experimental Botany* **47**, 89–97.
- Hilhorst, H.W.M. and Toorop, P.E.** (1997) Review on dormancy, germinability, and germination in crop and weed seeds. *Advances in Agronomy* **61**, 111–165.
- Himi, E., Mares, D.J., Yanagisawa, A. and Noda, K.** (2002) Effect of grain colour gene (*R*) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. *Journal of Experimental Botany* **53**, 1569–1574.
- Hocking, P.J.** (1982) Salt and mineral nutrient levels in fruits of two strand species, *Cakile maritima* and *Arctotheca populifolia*, with special reference to the effect of salt on the germination of *Cakile*. *Annals of Botany* **50**, 335–343.
- Hodkinson, D.J., Askew, A.P., Thompson, K., Hodgson, J.G., Bakker, J.P. and Bekker, R.M.** (1998) Ecological correlates of seed size in the British flora. *Functional Ecology* **12**, 762–766.
- Holdsworth, M., Kurup, S. and McKibbin, R.** (1999) Molecular and genetic mechanisms regulating the transition from embryo development to germination. *Trends in Plant Science* **4**, 275–280.
- Huang, G., McCrate, A.J., Varriano-Marston, E. and Paulsen, G.M.** (1983) Caryopsis structural and imbibitional characteristics of some hard red and white wheats. *Cereal Chemistry* **60**, 161–165.
- Hulme, P.E.** (1998) Post-dispersal seed predation and seed bank persistence. *Seed Science Research* **8**, 513–519.
- Imbert, E.** (1999) The effects of achene dimorphism on the dispersal in time and space in *Crepis sancta* (Asteraceae). *Canadian Journal of Botany* **77**, 508–513.
- Imbert, E.** (2001) Capitulum characters in the seed heteromorphic species, *Crepis sancta* (Asteraceae): variance partitioning and inference for the evolution of dispersal rate. *Heredity* **86**, 78–86.
- Imbert, E.** (2002) Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution and Systematics* **5**, 13–36.
- Imbert, E., Escarre, J. and Lepart, J.** (1999) Differentiation among populations for life history, morphology, head

- traits and achene morph proportions in the heterocarpic species *Crepis sancta* (L.) Bornm. *International Journal of Plant Sciences* **160**, 543–552.
- Jaimand, K. and Rezaee, M.B.** (1996) Variability in seed composition due to plant population and capitula zones of sunflower. *Agrochimica* **40**, 48–54.
- Kawakami, N., Miyake, Y. and Noda, K.** (1997) ABA insensitivity and low ABA levels during seed development of non-dormant wheat mutants. *Journal of Experimental Botany* **48**, 1415–1421.
- Kelly, K.M. and van Staden, J.** (1985) Effect of acid scarification on seed coat structure, germination and seedling vigor of *Aspalathus linearis*. *Journal of Plant Physiology* **121**, 37–45.
- Kende, H., Van der Knaap, E. and Cho, H.T.** (1998) Deepwater rice: A model plant to study stem elongation. *Plant Physiology* **118**, 1105–1110.
- Khan, M.A. and Ungar, I.A.** (2001) Alleviation of salinity stress and the response to temperature in two seed morphs of *Halopyrum mucronatum* (Poaceae). *Australian Journal of Botany* **49**, 777–783.
- Khan, M., Cavers, P.B., Kane, M. and Thompson, K.** (1996) Role of the pigmented seed coat of proso millet (*Panicum miliaceum* L.) in imbibition, germination and seed persistence. *Seed Science Research* **7**, 21–25.
- Kigel, J.** (1995) Seed germination in arid and semiarid regions. pp. 645–700 in Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.
- Koller, D.** (1972) Environmental control of seed germination. pp. 1–101 in Kozłowski, T.T. (Ed.) *Seed biology*, Vol. 2. London, Academic Press.
- Koornneef, M., Bentsink, L. and Hilhorst, H.** (2002) Seed dormancy and germination. *Current Opinion in Plant Biology* **5**, 33–36.
- Kuang, A., Xiao, Y. and Musgrave, M.E.** (1996) Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions. *Annals of Botany* **78**, 343–351.
- Legesse, N. and Powell, A.A.** (1992) Comparisons of water uptake and imbibition damage in eleven cowpea cultivars. *Seed Science and Technology* **20**, 173–180.
- Leishman, M.R. and Westoby, M.** (1998) Seed size and shape are not related to persistence in soil in Australia in the same way as in Britain. *Functional Ecology* **12**, 480–485.
- Léon-Kloosterziel, K.M., Keijzer, C.J. and Koornneef, M.** (1994) A seed shape mutant of *Arabidopsis* that is affected in integument development. *Plant Cell* **6**, 385–392.
- Leubner-Metzger, G.** (2003) Hormonal and molecular events during seed dormancy release and germination. pp. 101–112 in Nicolás, G.; Bradford, K.J.; Côme, D.; Pritchard, H.W. (Eds) *The biology of seeds: Recent research advances*. Wallingford, CABI Publishing.
- Lijavetzky, D., Martínez, M.C., Carrari, F. and Hopp, H.E.** (2000) QTL analysis and mapping of pre-harvest sprouting resistance in sorghum. *Euphytica* **112**, 125–135.
- Lloyd, A.M., Walbot, V. and Davis, R.W.** (1992) *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators *R* and *C1*. *Science* **258**, 1773–1775.
- Lloyd, D.G.** (1984) Variation strategies of plants in heterogeneous environments. *Biological Journal of the Linnean Society* **21**, 357–385.
- Lokker, C. and Cavers, P.B.** (1995) The effects of physical damage on seed production in flowering plants of *Saponaria officinalis*. *Canadian Journal of Botany* **73**, 235–243.
- Mandak, B.** (1997) Seed heteromorphism and the life cycle of plants: A literature review. *Preslia-Prague* **69**, 129–159.
- Manning, J.C. and van Staden, J.** (1985) The development and the ultrastructure of the testa and tracheid bar in *Erythrina lysistemon* Hutch. *Protoplasma* **129**, 157–167.
- Manning, J.C. and van Staden, J.** (1987) The role of the lens in seed imbibition and seedling vigor of *Sesbania punicea* (Cav.) Benth. (Leguminosae: Papilionoideae). *Annals of Botany* **59**, 705–713.
- Maranon, T.** (1989) Variations in seed size and germination in three *Aegilops* species. *Seed Science and Technology* **17**, 583–588.
- Maun, M.A. and Payne, A.M.** (1989) Fruit and seed polymorphism and its relation to seedling growth in the genus *Cakile*. *Canadian Journal of Botany* **67**, 2743–2750.
- Maxwell, C.D., Zobel, A. and Woodfine, D.** (1994) Somatic polymorphism in the achenes of *Tragopogon dubius*. *Canadian Journal of Botany* **72**, 1282–1288.
- McCarty, D.R.** (1995) Genetic control and integration of maturation and germination pathways in seed development. *Annual Review of Plant Physiology and Molecular Biology* **46**, 71–93.
- McDonald, A.W., Bakker, J.P. and Vegelin, K.** (1996) Seed bank classification and its importance for the restoration of species-rich flood-meadows. *Journal of Vegetation Science* **7**, 157–164.
- McDonough, W.T.** (1975) Germination polymorphism in *Grindelia squarrosa*. *Northwest Science* **49**, 190–200.
- McEvoy, P.B.** (1984) Dormancy and dispersal in dimorphic achenes of tansy ragwort, *Senecio jacobaea* L. (Compositae). *Oecologia* **61**, 160–168.
- McGinley, M.A.** (1989) Within and among plant variation in seed mass and pappus size in *Tragopogon dubius*. *Canadian Journal of Botany* **67**, 1298–1304.
- McKibbin, R.S., Bailey, P.C., Flintham, J.E., Gale, M.D., Lenton, J.R. and Holdsworth, M.J.** (1999) Molecular analysis of the wheat VIVIPAROUS 1 (*VPI*) orthologue. pp. 113–118 in Weipert, D. (Ed.) *Eighth international symposium on preharvest sprouting in cereals: 1998*. Detmold, Germany, Association of Cereal Research.
- Meinke, D.W.** (1994) Seed development in *A. thaliana*. pp. 253–295 in Meyerowitz, E.M.; Somerville, C.R. (Eds) *Arabidopsis*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press.
- Meisert, A., Schulz, D. and Lehmann, H.** (1999) Structural features underlying hardseededness in Geraniaceae. *Plant Biology* **1**, 311–314.
- Meisert, A., Schulz, D. and Lehmann, H.** (2001) The ultrastructure and development of the light line in the Geraniaceae seed coat. *Plant Biology* **3**, 351–356.
- Michaels, H.J., Wilson, M.F., Bertin, R.I., Benner, B., Hartgerink, A.P., Lee, T.D. and Rice, S.** (1988) Seed size variation: Magnitude, distribution and ecological correlates. *Evolutionary Ecology* **2**, 157–166.
- Milberg, P., Andersson, L. and Thompson, K.** (2000) Large-seeded species are less dependent on light for germina-

- tion than small-seeded ones. *Seed Science Research* **10**, 99–104.
- Moles, A.T., Hodson, D.W. and Webb, C.J.** (2000) Seed size and shape and persistence in the soil in the New Zealand flora. *Oikos* **89**, 541–545.
- Oliveira, M. De A., Matthews, S. and Powell, A.A.** (1984) The role of split seed coats in determining seed vigor in commercial seed lots of soybean, as measured by the electrical conductivity test. *Seed Science and Technology* **12**, 659–668.
- Patrick, J.W. and Offler, C.E.** (2001) Compartmentation of transport and transfer events in developing seeds. *Journal of Experimental Botany* **52**, 551–564.
- Peco, B., Traba, J., Levassor, C., Sánchez, A.M. and Azcarate, F.M.** (2003) Seed size, shape and persistence in dry Mediterranean grass and scrublands. *Seed Science Research* **13**, 87–95.
- Picciarelli, P., Piaggese, A. and Lorenzi, R.** (1994) ABA metabolite levels in developing embryos of *Secchium edule*. *Advances in Horticultural Science* **8**, 201–204.
- Powell, A.A.** (1989) The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Annals of Botany* **63**, 169–175.
- Powell, A.A. and Matthews, S.** (1978) The damaging effect of water on dry pea embryos during imbibition. *Journal of Experimental Botany* **29**, 1215–1229.
- Powell, A.A. and Matthews, S.** (1980) The significance of damage during imbibition to the field emergence of pea (*Pisum sativum* L.) seed. *Journal of Agriculture Science* **95**, 35–38.
- Powell, A.A., Oliveira, M. De A. and Matthews, S.** (1986a) The role of imbibition damage in determining the vigour of white and coloured seed lots of dwarf French beans (*Phaseolus vulgaris*). *Journal of Experimental Botany* **57**, 716–722.
- Powell, A.A., Oliveira, M. De A. and Matthews, S.** (1986b) Seed vigour in cultivars of dwarf French bean (*Phaseolus vulgaris*) in relation to the colour of the testa. *Journal of Agriculture Science* **106**, 419–425.
- Prinzie, T.P. and Chmielewski, J.G.** (1994) Significance of achene characteristics and within-achene resource allocation in the germination strategy of tetraploid *Aster pilosus* var. *pilosus* (Asteraceae). *American Journal of Botany* **81**, 259–264.
- Puga-Hermida, M.I.** (2003) The embryogenesis and germination of turnip tops seeds provokes important alterations in the ethylene production and ABA and polyamine levels. PhD Thesis, University of Vigo, Pontevedra, Spain.
- Puga-Hermida, M.I., Gallardo, M., Rodríguez-Gacio, M.C. and Matilla, A.J.** (2003a) The heterogeneity of turnip-tops (*Brassica rapa*) seeds inside the silique affects germination, the activity of the final step of the ethylene pathway, and abscisic acid and polyamine content. *Functional Plant Biology* **30**, 767–775.
- Puga-Hermida, M.I., Gallardo, M. and Matilla, A.J.** (2003b) The zygotic embryogenesis and ripening of *Brassica rapa* seeds provokes important alterations in the levels of free and conjugated abscisic acid and polyamines. *Physiologia Plantarum* **117**, 279–288.
- Ramsay, G.** (1997) Inheritance and linkage of a gene for testa-imposed seed dormancy in faba bean (*Vicia faba* L.). *Plant Breeding* **116**, 287–289.
- Ren, C.W. and Bewley, J.D.** (1998) Seed development, testa structure and precocious germination of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). *Seed Science Research* **8**, 385–397.
- Roberts, J.A., Elliot, K.A. and González-Carranza, Z.H.** (2002) Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology* **53**, 131–158.
- Rocha, O.J.** (1996) The effects of achene heteromorphism on the dispersal capacity of *Bidens pilosa* L. *International Journal of Plant Sciences* **157**, 316–322.
- Rodríguez-Gacio, M.C., Nicolás, C. and Matilla, A.J.** (2004) The final step of the ethylene biosynthesis pathway in turnip tops (*Brassica rapa* L. cv. Rapa): Molecular characterization of the 1-aminocyclopropane-1-carboxylate oxidase *BrACO1* throughout zygotic embryogenesis and germination of heterogeneous seeds. *Physiologia Plantarum* **121**, 132–140.
- Sakai, S. and Sakai, A.** (1996) Why is there variation in mean seed size among plants within single populations? Test of the fertilization efficiency hypothesis. *American Journal of Botany* **83**, 1454–1457.
- Simons, A.M. and Johnston, M.O.** (1997) Developmental instability as a bet-hedging strategy. *Oikos* **80**, 401–406.
- Simons, A.M. and Johnston, M.O.** (2000) Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *American Journal of Botany* **87**, 124–132.
- Spence, J., Vercher, Y., Gates, P. and Harris, N.** (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *Journal of Microscopy* **181**, 195–203.
- Steber, C.M., Cooney, S.E. and McCourt, P.** (1998) Isolation of the GA-response mutant *sly1* as a suppressor of *ABI-1* in *Arabidopsis thaliana*. *Genetics* **149**, 509–521.
- Susko, D.J. and Lovett-Doust, L.** (1998) Variable patterns of seed maturation and abortion in *Alliaria petiolata* (Brassicaceae). *Canadian Journal of Botany* **76**, 1677–1686.
- Susko, D.J. and Lovett-Doust, L.** (2000) Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). *American Journal of Botany* **87**, 56–66.
- Takeno, K. and Yamaguchi, H.** (1991) Diversity in seed germination behavior in relation to heterocarpy in *Salsola komarovii* Iljin. *The Botanical Magazine* **104**, 207–215.
- Tanowitz, B.D., Salopek, P.F. and Mahall, B.E.** (1987) Differential germination of ray and disk achenes in *Hemizonia increscens* (Asteraceae). *American Journal of Botany* **74**, 303–312.
- Telenius, A.** (1992) Seed heteromorphism in a population of *Spergularia media* in relation to the ambient vegetation density. *Acta Botanica Neerlandica* **41**, 305–318.
- Thompson, K., Band, S.R. and Hodgson, J.G.** (1993) Seed size and shape predict persistence in soil. *Functional Ecology* **7**, 236–241.
- Thompson, K., Jalili, A., Hodgson, J.G., Hamzehé, B., Asri, Y., Shaw, S., Shirvany, A., Yazdani, S., Khoshnevis, M., Zarrinkamar, F., Ghahramani, M. and Safavi, R.** (2001) Seed size, shape and persistence in the soil in an Iranian flora. *Seed Science Research* **11**, 345–355.

- Thompson, K., Ceriani, R.M., Bakker, J.P. and Bekker, R.M.** (2003) Are seed dormancy and persistence in soil related? *Seed Science Research* **13**, 97–100.
- Torres-Ruiz, R.A.** (1998) Embryogenesis. pp. 223–261 in Anderson, M.; Roberts, J.A. (Eds) *Arabidopsis. Annual plant reviews, Vol. 1*. Boca Raton, Florida, CRC Press.
- Venable, D.L.** (1985) The evolutionary ecology of seed heteromorphism. *American Naturalist* **126**, 577–595.
- Venable, D.L. and Burquez, A.** (1989) Quantitative genetics of size, shape, life history, and fruit characteristics of the seed-heteromorphic composite *Heterosperma pinnatum*. I. Variation within and among populations. *Evolution* **43**, 113–124.
- Venable, D.L. and Levin, D.A.** (1985) Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. *Journal of Ecology* **73**, 133–145.
- Venable, D.L., Burquez, A., Corral, G., Morales, E. and Espinosa, F.** (1987) The ecology of seed heteromorphism in *Heterosperma pinnatum* in Central Mexico. *Ecology* **68**, 65–76.
- Venable, D.L., Dyreson, E. and Morales, E.** (1995) Population dynamic consequences and evolution of seed traits of *Heterosperma pinnatum* (Asteraceae). *American Journal of Botany* **82**, 410–420.
- Walck, J.L., Baskin, J.M. and Baskin, C.C.** (1997) A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of *Solidago* (Asteraceae). 1. Germination phenology and effect of cold stratification on germination. *Seed Science Research* **7**, 47–58.
- Warner, R.L., Kudrna, D.A., Spaeth, S.C. and Jones, S.S.** (2000) Dormancy in white-grain mutants of Chinese spring wheat (*Triticum aestivum* L.). *Seed Science Research* **10**, 51–60.
- Weiss, P.W.** (1980) Germination, reproduction and interferences in the amphicarpic annual *Emex spinosa* (L.) Campd. *Oecologia* **45**, 244–251.
- Wells, C.L. and Pigliucci, M.** (2000) Adaptive phenotypic plasticity, the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3**, 1–18.
- Westoby, M., Jurado, E. and Leishman, M.R.** (1992) Comparative evolutionary ecology of seed size. *Trends in Ecology and Evolution* **7**, 368–372.
- Westoby, M., Leishman, M.R. and Lord, J.** (1996) Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society of London, Series B* **351**, 1309–1318.
- Williams, C.M. and Harper, J.L.** (1965) Seed dimorphism and germination. I. The influence of nitrates and low temperatures on the germination of *Chenopodium album*. *Weed Research* **5**, 141–150.
- Wolfe, L.M.** (1995) The genetics and ecology of seed size variation in a biennial plant, *Hydrophyllum appendiculatum* (Hydrophyllaceae). *Oecologia* **101**, 343–352.
- Wurzbarger, J. and Koller, D.** (1976) Differential effects of the parental photothermal environment on development of dormancy in caryopses of *Aegilops kotschyi*. *Journal of Experimental Botany* **27**, 43–48.
- Zhang, J.** (1993) Seed dimorphism in relation to germination and growth of *Cakile edentula*. *Canadian Journal of Botany* **71**, 1231–1235.
- Zhang, J.** (1994) Early seedling development in relation to seed mass and morph in *Cakile edentula*. *Canadian Journal of Botany* **72**, 402–406.
- Zhang, J.** (1995) Differences in phenotypic plasticity between plants from dimorphic seeds of *Cakile edentula*. *Oecologia* **102**, 353–360.

Received 4 November 2003  
 accepted after revision 4 March 2005  
 © CAB International 2005