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Seed Traits and Genes Important for Translational Biology—Highlights from Recent Discoveries

Cristina Martínez-Andújar[1], Ruth C. Martin[2] and Hiroyuki Nonogaki[3,*]

[1]Department of Plant Nutrition, CEBAS-CSIC, Campus de Espinardo, Murcia, MU, 30100, Spain
[2]USDA-ARS, National Forage Seed Production Research Center, Corvallis, OR 97331, USA
[3]Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA
*Corresponding author: E-mail, hiro.nonogaki@oregonstate.edu; Fax, +1-541-737-3479
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Seeds provide food, feed, fiber and fuel. They are also an important delivery system of genetic information, which is essential for the survival of wild species in ecosystems and the production of agricultural crops. In this review, seed traits and genes that are potentially important for agricultural applications are discussed. Over the long period of crop domestication, seed traits have been modified through intentional or unintentional selections. While most selections have led to seed traits favorable for agricultural consumption, such as larger seeds with higher nutritional value than the wild type, other manipulations in modern breeding sometimes led to negative traits, such as vivipary, precocious germination on the maternal plant or reduced seed vigor, as a side effect during the improvement of other characteristics. Greater effort is needed to overcome these problems that have emerged as a consequence of crop improvement. Seed biology researchers have characterized the function of many genes in the last decade, including those associated with seed domestication, which may be useful in addressing critical issues in modern agriculture, such as the prevention of vivipary and seed shattering or the enhancement of yields. Recent discoveries in seed biology research are highlighted in this review, with an emphasis on their potential for translational biology.

Keywords: Development • Dormancy • Germination • Seed • Shattering • Translational biology.

Abbreviations: ARF, auxin response factor; miRNA, microRNA; NIL, near-isogenic line; PHS, pre-harvest sprouting; QTL, quantitative trait locus; TPS, true potato seeds; Trx, thioredoxin.

Introduction

The vast population of the world depends on seeds for their food supply. The demand is increasing rapidly. Continuing population and consumption growth means that the global demand for food will increase for at least another 40 years (Godfray et al. 2010). Population growth, limits on arable land and fresh water, and climate change have profound implications for the ability of agriculture to meet this century's demands for food, feed, fiber and fuel (Fedoroff et al. 2010). Producing better quality seeds in a more efficient manner for food production and other purposes, such as for energy supply and pharmaceutical applications, is a challenge that we have to face in the near future. Therefore, an understanding of the biology of seeds is becoming even more critical. This review focuses on seed traits such as seed size, shattering, yields, dormancy and germination. Since most articles included in this Special Issue–Seed Biology, focus on fundamental mechanisms of seed biology, here the emphasis is on the potential for translational biology. Another focus will concentrate on key genes that have played an important role in crop domestication from wild species. The Green Revolution is probably one of the most successful cases of translational plant biology. While a Green Revolution gene, for example, *semi-dwarf1* (*sd1*) in rice, has been extensively used in modern breeding programs (Ashikari et al. 2002, Sasaki et al. 2002, Asano et al. 2007), a recent study indicates that *SD1* was already subjected to artificial selection during early stages of rice domestication in ancient times (Asano et al. 2011). Understanding the history and function of key genes selected for in seed domestication is also important, and therefore information on domestication-associated genes will be integrated into the discussions about translational biology.

Seed size

Seed size, which may have been selected since the time of hunter-gatherer societies, is probably one of the most important traits in agriculture. There is a large variation in seed size in nature. This natural variation in seed size may be a consequence of seed survival under severe, environmental selection pressure. Small-sized seeds were probably advantageous for the survival of some species in their native habitats, while seeds of other species evolved to increase their size. One of the smallest seeds

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in nature may be the orchid seed, which is smaller than the tip of a sewing needle. In contrast, seeds can grow to a considerably large size, such as 'Coco de Mer' (*Lodoicea maldivica* or double coconut) seeds that can weigh up to a few tens of kilograms.

Seed size can vary within a species, variety or genetic line, and even within an individual plant. These differences in seed size within the same genetic background are most probably controlled by environmental factors experienced by the maternal plants, or may depend on the position of seeds on the maternal plant, both of which greatly affect the physiology and development of individual seeds.

There are also many genetic factors that control seed size. Here, we will examine a few representative cases. *Sw4.1* is one of the most significant quantitative trait loci (QTLs) underlying the evolution of seed size in the genus *Solanum*, especially in species related to the cultivated tomato (*Solanum lycopersicum*). QTL analysis of tomato identified an ABC transporter that controls seed size through gene expression in the developing zygote (Orsi and Tanksley 2009). The Arabidopsis *Sw4.1* ortholog which was identified through synteny is associated with seed length variation and fatty acid deposition in seeds (Orsi and Tanksley 2009). These results of 'back translation' of tomato information to Arabidopsis suggest that the ABC transporter modulates seed size in multiple species.

HAIKU1 (*IKU1*) and *IKU2* in Arabidopsis control seed size through their effect on endosperm development and integument development which is mediated by the endosperm (Garcia et al. 2003). *IKU1* and *IKU2* encode a plant-specific VQ motif protein (Wang et al. 2010) and a leucine-rich repeat (LRR) kinase (Luo et al. 2005), respectively. While *iku* mutant plants are indistinguishable from wild-type plants, the *iku* mutants show precocious cellularization of the syncytial endosperm and a premature arrest of increase in seed size (Garcia et al. 2003). The mutations in *IKU* do not exert direct effects on maternal tissues; however, they do limit cell elongation in the integument through their function in the endosperm, suggesting a possible regulatory role of the endosperm in seed coat development (Garcia et al. 2003). Endosperm growth is also controlled by the integument (Garcia et al. 2005). Arabidopsis *MINISEEDS3* (*MINI3*), a WRKY transcription factor, acts in the same pathway as *IKU* in terms of seed size control (Luo et al. 2005). SHORT HYPOCOTYL UNDER BLUE1 (SHB1) (Kang and Ni 2006) binds the promoter region of *IKU2* and *MINI3* and enhances their expression to increase seed size (Zhou et al. 2009).

APETALA2 (*AP2*), a floral patterning regulator, is expressed during Arabidopsis seed development and decreases seed mass. In the loss-of-function *ap2* mutants, seed mass is increased. Although the *ap2* mutation affects male fertility, the increased size of *ap2* seeds may not be due to reduced male fertility or occur at the expense of reduced total seed number. Rather, *ap2* mutants increase seed size through increased cell division during morphogenesis and enhanced seed filling during the maturation phase of the embryo, both of which are subject to maternal control (Jofuku et al. 2005, Ohto et al. 2005). In *ap2* mutants, the endosperm undergoes an extended period of rapid growth, which is associated with delayed cellularization and overgrowth of the central vacuole. In addition, the integument cells of *ap2* mutants are more elongated than wild-type integument cells (Ohto et al. 2009). Thus, *AP2* affects both the embryo and the covering tissues of seeds. The mechanism of maternal control by *AP2* is not clear. It is possible that AP2 modulates the nutritional supply (e.g. carbohydrate allocation) from maternal tissues and affects endosperm and embryo development (Ohto et al. 2009). The control of seed size through endosperm development is a well known phenomenon accounting for parent-of-origin effects, which are exerted through DNA methylation (Vinkenoog et al. 2000) in interploidy crosses (Haig and Westoby 1991, Scott et al. 1998). However, *AP2* acts independently of parent-of-origin effects (Ohto et al. 2009).

As suggested in the characterization of *AP2*, carbohydrate allocation from the maternal plant to the seeds is a critical factor that determines seed size. The allocation of photosynthate is affected by both the source and sink (seeds) strength. In maize kernels, for example, sucrose is unloaded through phloem termini at the pedicel and is hydrolyzed into fructose and glucose by cell wall invertases that localize at the pedicel and the basal endosperm. Mutations in invertases, such as *miniature-1* (*mn1*), result in a reduction in seed size (Miller and Chourey 1992, Chourey et al. 2006). In rice, *GIF1* (*GRAIN INCOMPLETE FILLING 1*), a domestication-associated gene, also encodes an invertase. Ectopic expression of the cultivated *GIF1* gene with the 35S or rice *Waxy* promoter results in smaller grains, whereas overexpression of *GIF1* driven by its native promoter increases grain production (Wang et al. 2008). These results suggest that genes affecting sink strength are also good targets for modification in terms of seed size increase.

Auxin, which is a master regulator of early seed development, significantly affects the size (and shape) of a mature embryo. Auxin signaling is mediated by auxin response factors (ARFs), transcription factors that bind to auxin-responsive elements (AuxREs) in promoter regions to enhance or repress auxin-regulated genes (Abel and Theologis 1996, Guilfoyle et al. 1998, Hagen and Guilfoyle 2002). Arabidopsis mutants defective in *ARF2* (*arf2-6*, *arf2-7* and *arf2-8*) produce larger seeds compared with wild-type plants (Okushima et al. 2005). ARF2 is a suppressor protein regulating cell growth and, therefore, is thought to reduce seed size through the suppression of genes positively affecting cell expansion in Arabidopsis (Okushima et al. 2005). The Arabidopsis *megaintegumenta* (*mnt*) mutant, in which seed size and weight are dramatically increased, also has a lesion in *ARF2*, supporting the idea that *ARF2* is a key regulator of seed size. In *mnt*/*arf2* mutants, the ovule and seed cavity are enlarged compared with the wild type due to extra cell divisions in the integument (Schruff et al. 2006). This observation also suggests that maternal tissues play an important role in determining seed size.

It seems clear that *ARF2* manipulation could be used for modification of seed size. However, *arf2* plants have pleiotropic

effects in Arabidopsis, such as over-elongated petals, which prevent flower opening and reduce seed set. Even though the increase in seed size in *arf2* mutants may not be the consequence of the reduced seed number, the seed set problem in this mutant reduces seed yield, hindering the application of *arf2* mutations for translational biology. This problem has been addressed by recovering *ARF2* function specifically in the floral organs. Expression of *ARF2* using the promoter of *AP1*, a floral patterning gene, in an *arf2-9* mutant restored flower opening. The *pAP1:ARF2* (*arf2-9*) mutant retained the large seed phenotype of the original mutant while exhibiting increased fertility and greater yield than *arf2-9* (Hughes et al. 2008). These experiments demonstrated that identification of key genes for important seed traits and proper control of these genes in a seed-specific manner enable the application of knowledge obtained from basic seed biology research for enhancing agriculture production.

In rice (*Oryza sativa*), *ARF8* appears to be associated with seed development. The IAA level in developing rice seeds is approximately 40-fold higher than in other tissues (Matsuda et al. 2005, Xue et al. 2009), suggesting the involvement of auxin and its signal transduction during seed development. Unlike ARF2 which is a repressor protein, ARF8 functions as an activator of downstream target genes (Hagen and Guilfoyle 2002, Tiwari et al. 2003, Yang et al. 2006). In rice cell culture, ARF8 activates *OsGH3-2*, a gene encoding an auxin-conjugating enzyme (Yang et al. 2006) that catalyzes the conjugation of free IAA to different compounds, thereby decreasing the cellular concentration of free IAA (Staswick et al. 2002). ARF8 may function in maintaining auxin homeostasis (Yang et al. 2006). Although this function of ARF8 has not been demonstrated for developing seeds, it is possible that ARF8 regulates auxin levels in rice seeds and affects their development (Xue et al. 2009). Mutations in *ARF8* in Arabidopsis cause parthenocarpy (Goetz et al. 2006), therefore manipulation of *ARF8* provides another opportunity for translational biology, although fruit production is beyond the scope of this review. While *ARF* genes seem to affect seed size in both Arabidopsis and rice, there are crucial differences in the morphology of dicot and monocot seeds (**Fig. 1**). Therefore, transference of seed size determinants between dicots and monocots may not be straightforward and requires caution.

Interestingly, the three genes mentioned above for seed size are subject to regulation by small RNAs [*AP2* by miR172, *ARF2* by *trans*-acting small-interfering RNA (ta-siRNA) and *ARF8* by miR167] (Rhoades et al. 2002, Williams et al. 2005). Other seed size-associated genes, such as *MYB33* and *MYB65*, are also targeted by microRNA (miRNA; miR159). When *MIR159a* and *MIR159b*, which function redundantly, are mutated, *mir159a mir159b* double mutants produce small seeds (Allen et al. 2007). The regulation of seed size-associated genes by small RNAs suggests that quick turnover of these genes may be critical for seed development. Other small RNA-targeted genes are also associated with seed shattering (discussed below) and germination (Nonogaki, 2010).

Seed dispersal and shattering

Plants have evolved many strategies of seed dispersal. Some seeds developed wings and propellers to facilitate wind dispersal, while others developed seed accessories, such as hooks and spines that stick to animal furs, to take advantage of animals for dispersal. There are 'buoyant' seeds that travel thousands of kilometers in the ocean for their dispersal (Gunn 1972). In addition to these 'vehicles' or media of seed dispersal, plants have also developed efficient mechanisms for seed detachment from the maternal plants. While dispersal media are important for increasing the distance of seed dispersal, the mechanisms of seed detachment are critical for timing of seed dispersal.

Domestication of agricultural crops has probably favored traits reducing seed detachment to secure the retention of seeds in pods (e.g. peas and beans), siliques (e.g. *Brassica* oil crops) or spikes (e.g. cereal crops). In many dicot species, seed dispersal is controlled by pod or silique dehiscence. In *Brassica* species, a silique consists of two valves, each originating from a single carpel, and the replum, a partition in the middle of the silique at which the maternal plant and seeds are connected

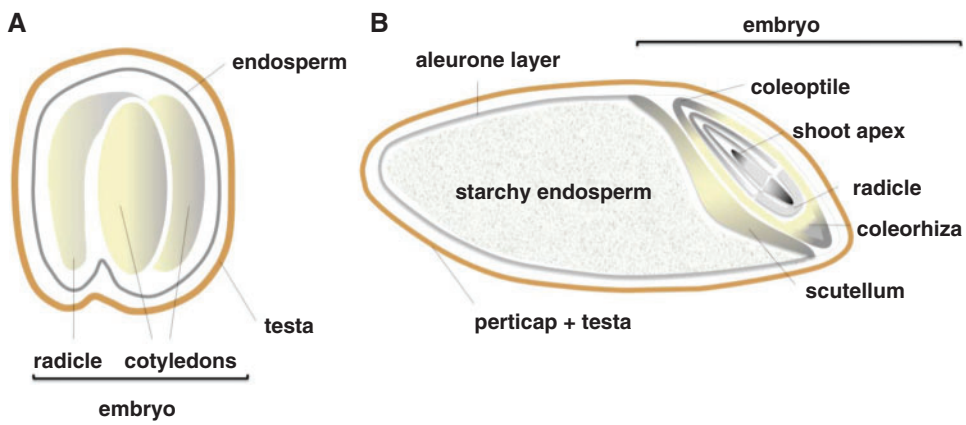

Fig. 1 Schematic representation of dicot (A) and monocot (B) seeds.

through the funiculus, an umbilical cord-like structure (**Fig. 2A**). The valve margin (valve–replum boundary) is the position where silique dehiscence occurs. The breakdown of the middle lamella between the cells at the valve margin causes dehiscence in Arabidopsis siliques (Liljegren et al. 2000). The two *MADS-box* genes, *SHATTERPROOF1* (*SHP1*) and *SHP2* [also known as *AGAMOUS-LIKE1* (*AGL1*) and *AGL5*, respectively], play a critical role in lignification of the valve margin and the adjacent cells, which is required for silique dehiscence. When these two genes are mutated, a clear dehiscence zone is not recognized and mature siliques fail to dehisce. In contrast, gain-of-function mutations in *SHP* genes cause immature, green developing siliques to split open prior to seed maturity (Liljegren et al. 2000).

FRUITFUL (*FUL*), another *MADS-box* gene in Arabidopsis is expressed throughout the valves in a complementary pattern to that of *SHP1*/*SHP2* expression. Loss- and gain-of-function mutations in *FUL* cause phenotypes strikingly similar to gain- or loss-of-function mutants in *SHP* genes, respectively, suggesting that *SHP* genes and *FUL* act antagonistically (Liljegren et al. 2000). Consistently, further analyses indicated that *FUL* is a negative regulator of *SHP* genes (Ferrandiz et al. 2000). There is another gene called *REPLUMLESS* (*RPL*) in Arabidopsis that also negatively regulates *SHP* genes. *RPL* encodes a homeodomain protein that prevents the replum cells from adopting a valve margin cell fate (Roeder et al. 2003). Genetic analyses indicate that both *RPL* and *FUL* suppress *SHP* and define the narrow stripes of *SHP* gene expression, which restricts valve margin development to the valve–replum boundary (Roeder et al. 2003) (*RPL* function in other species is discussed below).

Another factor controlling seed dispersal or shattering is the abscission of the funiculus (**Fig. 2A**). After fertilization and later in seed development, a group of small cells becomes apparent in the funiculus region adjacent to the seed body and forms the abscission zone (**Fig. 2A**), which eventually develops into a constriction at which the seed is detached (Pinyopich et al. 2003). Mutant Arabidopsis plants defective in *SEEDSTICK* (*STK*, also known as *AGL11*), a *MADS-box* gene, produce an enlarged funiculus with increased cell numbers in which a clear abscission zone is not recognized. *stk* mutant siliques fail to detach seeds, or retain them for a longer period of time (Pinyopich et al. 2003). These results indicate that proper development of the funiculus is also essential for seed shattering.

There is evidence to suggest that miR167 may be involved in seed abscission after silique dehiscence in Arabidopsis. This has been demonstrated through experiments using the 'miRNA target mimicry' technique (Franco-Zorrilla et al. 2007). This technique was developed to address difficulties in characterizing the function of plant miRNAs, many of which have gene families and exhibit redundancy. When an artificial mimic miRNA target which interrupts pairing with the corresponding miRNA family is transformed into plants, this non-cleavable mimic miRNA target competes with native targets for the miRNA family. The mutation results in sequestration of the miRNA family and deregulation of the native targets.

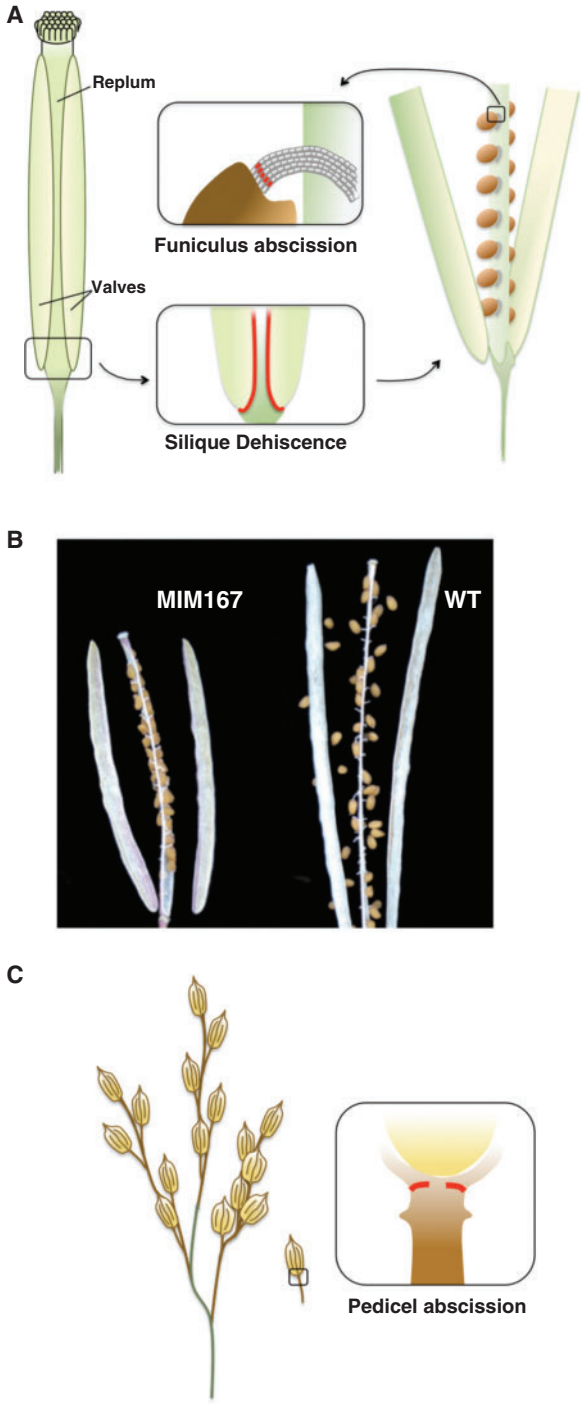

Fig. 2 Seed shattering in *Brassica* siliques and rice spikes. (A) Schematic representation of silique dehiscence and funiculus abscission in *Brassica* species. (B) Seed shattering in the wild type (WT) and seed retention in the MIM167 miRNA 'mimicry' mutant Arabidopsis. See text for details. Copyrights © Todesco et al. 2010; *PLoS Genet.* 6: e1001031. (C) Schematic representation of the abscission zone in the pedicel of rice spikes. In both (A) and (C), abscission zones are highlighted in red.

This approach has been applied to miR167 (mimic miR167: *MIM167*) (Todesco et al. 2010) which targets *ARF6* and *ARF8* (Rhoades et al. 2002). Siliques of *MIM167* exhibit a seed retention phenotype similar to *stk* phenotypes (Todesco et al. 2010) (**Fig. 2B**). As *MIM167* siliques dehisce normally, the mutation may affect funiculus abscission. These results indicate that the recently discovered mechanisms of gene regulation by small RNAs can also be applied to translational biology for crop improvement, although pleiotropic effects of miRNA mutations, such as those observed in the *MIM167* mutants (e.g. reduced seed production and germination) (Todesco et al. 2010), must be overcome with some other approaches.

Only key genes associated with seed shattering were described above. There are other transcription factors associated with seed shattering, including *INDEHISCENT* (*IND*) encoding a basic helix–loop–helix (bHLH) protein (Liljegren et al. 2004) and *ALCATRAZ* (*ALC*) encoding a myc/bHLH protein (Rajani and Sundaresan 2001), both of which function downstream of *SHP* genes, and *NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1* (*NST1*) and *NST3* (Mitsuda and Ohme-Takagi, 2008) in Arabidopsis. Enzymes such as *ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1* (*ADPG1*) and *ADPG2* are also involved in seed shattering (Ogawa et al. 2009). Since the interactions of multiple genes and the basic mechanisms of silique dehiscence are well summarized elsewhere (Mitsuda and Ohme-Takagi 2008, Moran and Halliday 2010), we will not revisit that information, but will rather focus on translational biology aspects below.

The discovery of seed shattering-associated genes opened up new possibilities for crop improvement (e.g. canola including *Brassica napus*, *B. rapa* and *B. juncea*). Unsynchronized seed shattering in oil seed crops causes serious problems for farmers. The seed shattering information obtained from Arabidopsis research is readily translatable into *Brassica* oil crops, since the genetic pathway leading to valve margin specification is highly conserved between these two groups (Østergaard et al. 2006). In fact, overexpression of the Arabidopsis *FUL* gene was sufficient to make *B. juncea* siliques resistant to seed shattering (Østergaard et al. 2006). This research demonstrated a successful case of translational biology between Arabidopsis and *B. juncea* and should be widely applicable to other *Brassica* species. These results are encouraging because they confirm that similar translation is possible between *Medicago* model species and legume crops, or *Brachypodium* model species and cereal crops.

It is probably also possible to translate information about certain traits between *Brassica* and cereal crops. Seed shattering research again provided an interesting proof for this concept. In rice, one of the most significant changes over the course of domestication is the acquisition of the 'non-shattering' phenotype. Rice seed (caryopsis) shattering is determined by its detachment at the abscission layer in the pedicel (Ji et al. 2006, Ji et al. 2010) (**Fig. 2C**). Through the analysis of a shattering-type *indica* cultivar, Kasalath, and a non-shattering-type *japonica* cultivar, Nipponbare, the QTL *seed shattering in chromosome 1* (*qSH1*) was identified (Konishi et al. 2006). A near-isogenic line (NIL) that contained a short chromosomal segment from Kasalath (shattering type) at the *qSH1* region in a Nipponbare (non-shattering type) genetic background exhibited the formation of a complete abscission layer (i.e. shattering phenotype) between the pedicel and spikelet at the base of the rice seed, suggesting that this genomic region is responsible for determining seed shattering characteristics in rice (Konishi et al. 2006). The gene responsible for the seed-shattering phenotype is most probably a BEL1-type homeobox gene. Intriguingly, this rice gene is an ortholog of Arabidopsis *RPL* (Roeder et al. 2003, Konishi et al. 2006). These results suggest that similar genes and functions are conserved for seed shattering mechanisms between the two model species Arabidopsis and rice, or between *Brassica* and cereal crops, although the positions of abscission did not perfectly match between dicot siliques and monocot spikes, in a botanical sense. While the aforementioned discoveries came from independent research in Arabidopsis and rice, these findings suggest that greater efforts in translating *Brassica* information to cereals, or vice versa, could advance crop modification, which further validates the potential of translational biology.

Seed dormancy and germination

Domestication of crops has resulted in the selection of germinable seeds. Seeds of many cereals and vegetables have little dormancy. The effects of unintentional selection of germination characteristics are well exemplified by a comparison of tomato and potato (*Solanum tuberosum*) seeds, which are genetically closely related. Potato produces small tomato-like berries (**Fig. 3A, B**), in which seeds develop. These are called 'true potato seeds (TPS)' or 'botanical seeds' (**Fig. 3C**), to distinguish them from 'seed potatoes' (tubers) that are used for vegetative propagation of potatoes for production. TPS are very similar to tomato seeds, in terms of seed shape and internal structures, although TPS are much smaller compared with tomato seeds (**Fig. 3D, E**). While tomato seeds generally have little dormancy, TPS exhibit deep dormancy, which is more exaggerated at relatively high temperatures (>25°C, called 'thermodormancy') (Alvarado et al. 2000). This is probably because potato tubers have been used predominantly for potato propagation and TPS were not selected for germinability over a long period of time, while tomatoes are produced mainly through seed propagation.

TPS dormancy can be broken by fluridone, a carotenoid biosynthesis inhibitor (Alvarado et al. 2000), as is seed dormancy in other species, such as lettuce (*Lactuca sativa*) and Arabidopsis (Yoshioka et al. 1998, Grappin et al. 2000). Fluridone inhibits phytoene desaturase, a key enzyme in the carotenoid biosynthetic pathway (Chamovitz et al. 1993), upstream of the ABA biosynthesis pathway. The discovery of the importance of the inhibition of the ABA biosynthesis pathway

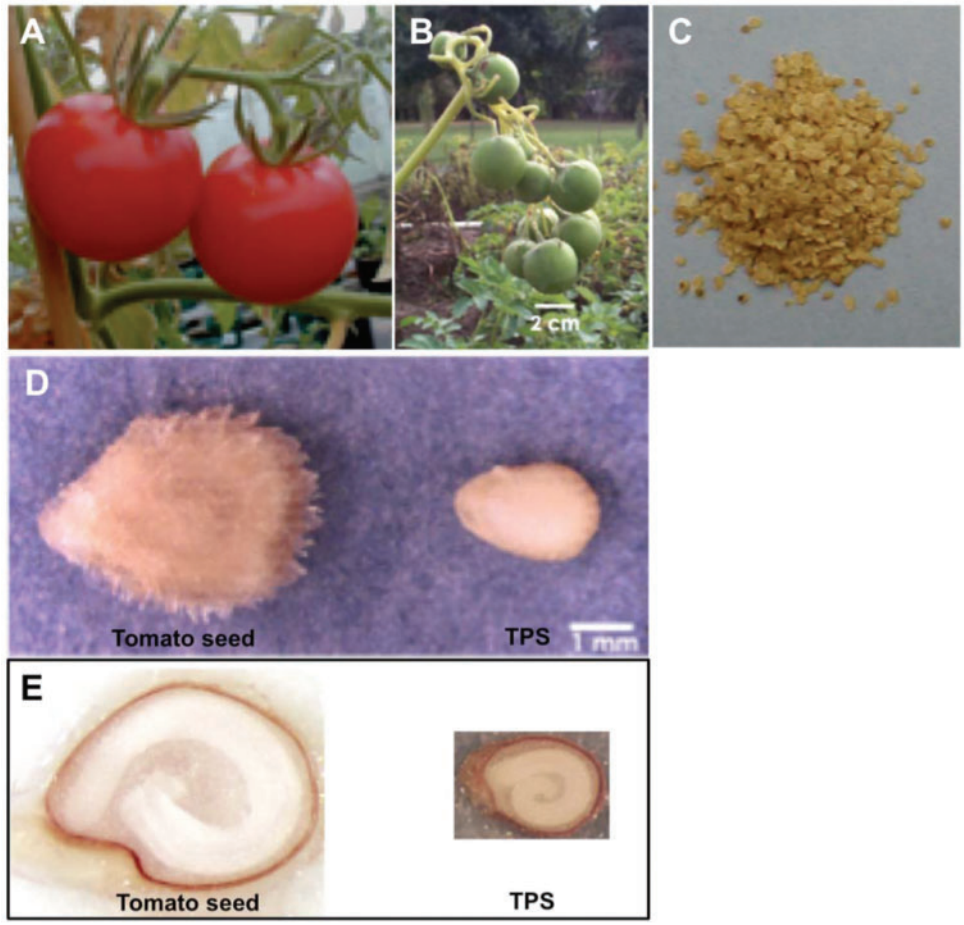

Fig. 3 Photographs of tomato fruits (A), potato berries (B) and botanical or true potato seeds 'TPS' (C). The appearance (D) and internal structures (E) of individual tomato and potato seeds are also shown. While TPS are smaller than tomato seeds, the basic structures (a curled embryo surrounded by the endosperm and testa) are similar in the two species.

greatly advanced our understanding of the mechanisms involved in the hormonal regulation of seed dormancy and germination. These discoveries accelerated the identification, isolation and characterization of genes encoding hormone metabolic enzymes, such as 9-*cis*-epoxycarotenoid dioxygenase (NCED), a rate-limiting enzyme for ABA biosynthesis (Schwartz et al. 1997, Lefebvre et al. 2006), and CYP707A2, an ABA deactivation enzyme (Kushiro et al. 2004). In contrast, gibberellin (GA) antagonizes ABA and promotes seed germination. GA 3-oxidase (GA3ox), a rate-limiting enzyme for GA biosynthesis (Yamaguchi et al. 1998), and GA 2-oxidase (GA2ox), a GA deactivation enzyme (Sakamoto et al. 2001), have also been isolated. Expression and functional analysis of these genes addressed major questions in seed biology that had been elusive for a long period of time, such as 'How does cold stratification (hydration at low temperature) break seed dormancy?' or 'What are the interactions between light signals and germination-promoting chemicals, such as GA and nitrate?'

Now, we understand that GA biosynthesis genes are up-regulated by cold stratification, e.g. in Arabidopsis seeds (Yamauchi et al. 2004). Seed responses to high temperatures can also be explained by changes in hormone metabolism. Germination is inhibited by exposure of seeds to temperatures above the optimum range, which is called thermoinhibition. In Arabidopsis seeds, the levels of ABA increase at high temperatures. Expression analysis indicated that these changes are associated with increased expression of *NCED2*, *NCED5* and *NCED9* which may be the key regulatory genes in ABA biosynthesis and germination inhibition at high temperatures (Toh et al. 2008). Seeds of *nced9* mutants show tolerance to thermoinhibition, but seeds of *nced2* and *nced5* have no apparent phenotype, suggesting that *NCED9* plays a major role in thermoinhibition. In lettuce seeds, the reduction of ABA levels normally seen in imbibed seeds is suppressed at supraoptimal temperatures, and de novo ABA biosynthesis is required for thermoinhibition (Yoshioka et al. 1998). The ABA levels in seeds of a thermosensitive lettuce accession (Salinas) remain 5-fold higher than those in seeds of a thermotolerant accession (UC96US23) when seeds are imbibed at 35°C in the light. *LsNCED4*, an ortholog of Arabidopsis *NCED6*, is the main regulator of thermoinhibition

in lettuce seeds (Argyris et al. 2008). The central role of *LsNCED4* in regulating thermoinhibition in lettuce seeds is also supported by its unique expression pattern in relation to genotypes and temperatures, and its co-localization in the genome with *Htg6.1*, a significant QTL for high temperature germination, which was identified through genetic analysis of a recombinant inbred line (RIL) population derived from a cross between Salinas and UC96US23 accessions (Argyris et al. 2008, Argyris et al. 2011).

In addition to the enhancement of ABA biosynthesis in seeds, the suppression of an increase in GA levels also causes thermoinhibition of Arabidopsis seeds. Exogenous GA alleviates thermoinhibition of Arabidopsis seeds. Interestingly, GA levels increase at 22°C, but this increase does not occur at 34°C. Alleviation of thermoinhibition by exogenous GA has also been observed in lettuce seeds. In Arabidopsis, the expression of *GA20ox1*, *GA20ox2*, *GA20ox3*, *GA3ox1* and *GA3ox2* is greatly suppressed at high temperatures (Oh et al. 2006). Consistently, *LsGA20ox1* and *LsGA3ox2* are more highly expressed at 30°C than at 33°C in lettuce seeds, and show greater down-regulation in thermosensitive Salinas seeds than in thermotolerant seeds imbibed at high temperatures (Argyris et al. 2008). This indicates that low GA levels are maintained through the repression of GA biosynthesis genes at high temperatures.

Central to our understanding of hormonal regulation during seed germination is the fact that ABA affects GA biosynthesis and deactivation, and that GA can also affect ABA metabolism. This mutual regulation between ABA and GA is the key to understanding the regulation of seed dormancy and germination by other environmental signals such as light. GA biosynthesis and deactivation genes are directly down- and up-regulated by ABA, respectively. On the other hand, red light (~660 nm) down- and up-regulates ABA biosynthesis and deactivation genes in Arabidopsis, respectively (Seo et al. 2006). Therefore, red light reduces ABA levels, which in turn increase GA levels in seeds, and positively affects germination (Seo et al. 2006, Seo et al. 2009). This scheme (light–ABA–GA) is a good example of the comprehensive understanding that has been derived from the recent progress in hormone metabolism research. There are other mechanisms involved in the mutual regulation of ABA and GA (e.g. regulation at the level of signal transduction), which are not mentioned here but are discussed in a recent review paper (Seo et al. 2009).

The effect of germination-promoting chemicals can also be explained by their effect on hormone metabolism. For example, potassium nitrate solution has been used to break seed dormancy in many seed testing laboratories worldwide; however, the mechanisms underlying its positive effects on germination were unknown. Now, we understand that nitrate positively affects ABA deactivation and hence germination in Arabidopsis seeds (Matakiadis et al. 2009).

Through research on ABA and GA metabolism, the genes encoding the rate-limiting enzymes have been identified and this has opened up the possibility of engineering hormone metabolism in seeds to enhance or suppress germination. While manipulation of individual hormone signal transduction proteins may be technically difficult, it is feasible to modify the expression of hormone metabolism genes at the transcriptional level. For example, genes that positively affect germination can be used to enhance germination at suboptimal temperatures. This type of approach could solve problems of low germination at high temperatures, a problem typical of lettuce and spinach seeds. It is also important to develop technology to suppress seed germination because vivipary, precocious germination on the maternal plant, is another serious problem in agriculture. Pre-harvest sprouting (PHS) in cereal crops causes reduction of yield and affects grain quality. The lack of adequate seed dormancy is the major reason for PHS in the field. For example, PHS during wheat production is an example of an agricultural problem caused by the lack of dormancy. Precocious germination of wheat grains triggers α-amylase activity in the aleurone layers, which degrades starch accumulated in the endosperm (Huang and Varriano-Marston 1980). The premature digestion of starch reduces grain quality and causes significant economical losses to farmers. Induction of genes negatively affecting seed germination is expected to enable suppression of PHS. This is an important area of translational seed biology that should be further explored.

While hormone metabolism engineering has not yet been fully explored for PHS prevention, another approach was successfully used to control PHS. In this case, basic knowledge concerning the positive effect of thioredoxin (Trx) on seed germination (Buchanan and Balmer 2005) was translated into potential technology to prevent PHS in wheat (Li et al. 2009). Trx is an enzyme that reduces disulfide (S–S) bonds in diverse seed proteins, including storage proteins, enzymes and enzyme inhibitors, and promotes seed germination through mechanisms not yet identified. Controlled expression of an antisense PTrx *h* gene from sunolgrass (*Phalaris coerulescens*), which is very similar to wheat Trx *h*, using the α-gliadin promoter reduced the expression of Trx *h* in wheat seeds and suppressed PHS (**Fig. 4**). These effects were demonstrated using field-grown wheat (with the aid of a growth chamber for PHS experiments) (Li et al. 2009). This trial moved seed biology research from the laboratory to the field. In China, for example, 83% of the wheat production region is subject to sprouting damage (Xiao et al. 2002). This type of technology will probably showcase successful translational seed biology in areas important for wheat production.

Other strategies to prevent PHS could result from basic research on seed dormancy genes other than hormone metabolism genes and Trx. There are multiple genes associated with seed dormancy (Finkelstein et al. 2008). One of these, *Seed dormancy 4* (*Sdr4*), a rice seed dormancy QTL, has been characterized in the context of PHS phenotypes. In rice, seed dormancy has been lost over the course of domestication. Seeds of the *japonica* cultivar Nipponbare are prone to PHS (*Sdr4-n*), while seeds of the *indica* cultivar Kasalath are relatively resistant to it (*Sdr4-k*). When a NIL was generated by incorporating

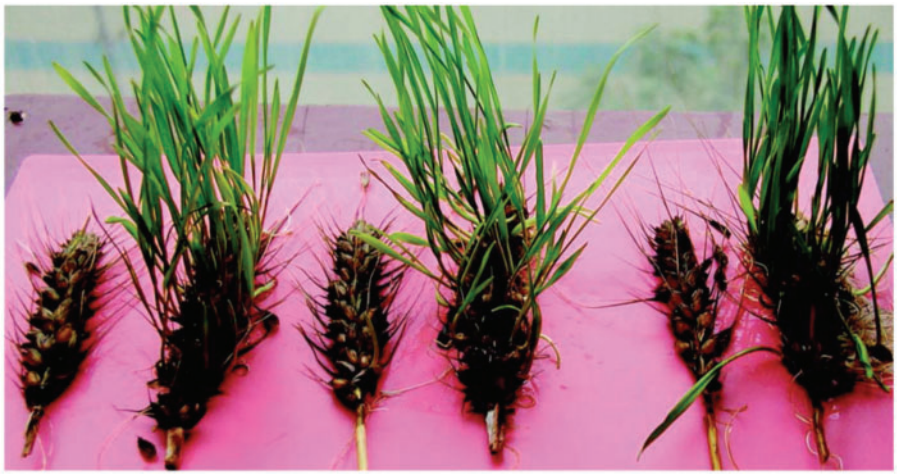

Fig. 4 Prevention of pre-harvest sprouting (PHS) in wheat spikes. PHS occurs when wheat spikes are subjected to humid conditions. PHS was suppressed by down-regulating a thioredoxin (Trx) gene in transgenic wheat. Three sets of transgenic (left) and segregating wild-type control (right) are shown. From Li et al. 2009; *Mol. Plant* 22: 430–441. Copyright © Oxford University Press.

Sdr4-k into the Nipponbare genome, seeds became resistant to PHS (Sugimoto et al. 2010). These results suggest that *Sdr4* is associated with seed dormancy and PHS characteristics, and that this gene has been modified during rice domestication. The promoter region of *Sdr4* contains the seed-specific RY motif, the ABA-responsive element (ABRE) and coupling element (CE) required for the regulation by B3 transcription factors. Consistently, *Sdr4* expression is regulated by the OsVP1 (*Oryza sativa* VP1) rice B3 transcription factor, or rice ABI3 (Sugimoto et al. 2010). Identifying and manipulating orthologs of this gene in other species might facilitate the modification of dormancy and PHS characteristics of seeds. Thus, research on the rice domestication-associated gene *Sdr4* provides useful information for translational biology for seed germination and dormancy, as does *qSH1* for seed shattering issues.

Other applications and perspectives

In this review, we focused on only a small number of the major seed traits important for agriculture, and just highlighted representative cases of successful translational biology. There have been many other significant discoveries in seed biology during the last decades. Recently, another domestication-associated QTL in rice, *WFP* (*WEALTHY FARMER'S PANICLE*) which is important for seed yield, was shown to encode *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14* (*OsSPL14*, also known as IPA1), a target of miR156 (Miura et al. 2010). It was demonstrated that introduction of the high-yielding $OsSPL14^{WFP}$ allele into Nipponbare increased rice yields (Miura et al. 2010). It should also be noted here that substantial progress has been made in the area of nutritional quality and pharmaceutical applications of seeds. With the advanced understanding of biochemical pathways associated with fatty acid synthesis and modifications in seeds, we are now able to modify seed oils in many ways, and are entering the era of 'designer oilseeds' (Napier and Graham 2010), which is another dimension of translational seed biology. For example, it has become possible to produce so-called 'fish oil' in seeds. Omega-3, long chain (~C20) polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the human diet can improve brain and retinal health, and can reduce the risks of coronary heart disease and type II diabetes (Cheng et al. 2009). Traditionally, these fatty acids were supplied from seafood. However, the resources may not be sufficient to support the growing world population. Therefore, alternative sources should be explored. Seeds are an excellent heterologous system to provide fish oils (Cheng et al. 2009, Napier and Graham 2010). Molecular farming to produce insulin, human growth hormone, lysozyme and lactoferrin (iron-binding protein) in seeds has also been established and has advanced in the last decades (Boothe et al. 2010). The knowledge obtained from basic seed biology research, such as the information on oleosins and protein sorting mechanisms, has been translated into applications to produce recombinant proteins in seeds (Boothe et al. 2010). A greater understanding of biochemical pathways and subcellular targeting in seeds, and the identification of genes associated with these pathways and subcellular targeting discovered through basic research, will provide further opportunities for translational seed biology.

As a last note, it should be mentioned that the information obtained from model species was generalized in this review, focusing on some conserved mechanisms among multiple species, to encourage translational seed biology. Mechanisms controlling other traits may differ between species and, for those traits, direct translation between species might be difficult and require more intensive efforts. Other problems, such as genetic stability of transgenes in crop species, also need to be addressed. Nonetheless, our understanding of seed biology has advanced greatly in the last few decades. Community efforts

to translate the knowledge from past discoveries on seeds into practice will help to solve the food and energy issues in the future.

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