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Variation in pre-harvest sprouting resistance, seed germination and changes in abscisic acid levels during grain development in diverse rice genetic resources

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Abstract

Among domesticated traits, pre-harvest sprouting (PHS) caused by the early breakage of dormancy leads to severe economic losses. Therefore, regulating PHS is important for cereal crop improvement against changes in climate. In this study, we surveyed naturally occurring variations in seed germination in diverse rice germplasm for the available resources of this trait, and investigated the changes of abscisic acid (ABA) levels during grain development by the distinguished PHS-resistant groups. We discovered wide variations in germination among the 205 rice accessions examined and found that 90 accessions are resistant (germination <20%) to PHS. Tropical and subtropical accessions, which are subjected to long wet periods, are more resistant to PHS than the other accessions. We detected an increase in germination of detached seeds from the panicle compared with intact seeds in panicle at harvesting time. This might be attributed to a weakening of the mechanical barrier that prevents water imbibition and radical emergence. ABA levels were maximal at 10 d after flowering and decreased thereafter. Interestingly, PHS-susceptible accessions maintained higher or similar ABA levels compared with PHS-resistant accessions, suggesting that the key factors for seed dormancy and its breakage are ABA perception and signal transduction rather than total ABA content. The diversity of germination ability detected in this study could be sustainably used for crop improvement and to help unveil the genetic and physiological basis of this quantitative trait.

Keywords: ABA, dormancy, genetic resources, germination ability, rice

Introduction

During the domestication of crops from their wild progenitors, several traits that are useful for cultivation have been selected: reduced grain shattering and awn length, increased panicle branching and increased spikelet number per panicle. Among the domesticated traits, seed dormancy causes plants to arrest their maturation status for variable periods of time, allowing them to survive various durations of exposure to hostile environmental conditions and to germinate under favourable conditions.

Dormancy is thought to prevent pre-harvest sprouting (PHS), whereas the early breakage of dormancy causes PHS, which reduces grain yields and grain quality in cereal

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crops (Bewley and Black, 1982; Li *et al*., 2004). In rice, PHS frequently occurs in Southeast Asia due to the long rainy periods in early summer and autumn (Wan *et al*., 2006), with hybrid rice in South China showing higher than average PHS ratios (Guo *et al*., 2004). Since PHS causes severe economic losses by reducing the production and quality of grain, improving PHS resistance has long been one of the main breeding targets for cereal crops worldwide (Zhang *et al*., 2014).

Seed dormancy can be caused by an embryo-outside effect or an embryo-imposed effect. Among the surroundings of embryo, the seed coat acts as a physical barrier against water imbibition and radical emergence. Seed pigmentation is associated with seed dormancy in cereal crops such as rice, wheat and proso millet (Khan *et al*., 1997; Himi *et al*., 2002; Gu *et al*., 2005a; Sweeney *et al*., 2006). Embryo-imposed dormancy is developmentally regulated; the phytohormones abscisic acid (ABA) are required for elaborate developmental regulation and play key roles in seed dormancy acquisition (Cutler *et al*., 2010; Raghavendra *et al*., 2010). To date, the major genes found to be associated with seed dormancy and germination are related to ABA biosynthesis, catabolism and signal transduction. *AtABI3* of *Arabidopsis*, which is orthologous to *ZmVP-1* in maize, *OsVP1* in rice, and *TaVp-1* in wheat, functions in the global regulation of seed maturation by participating in ABA signal transduction (Hattori *et al*., 1994; Nakamura and Toyama, 2001; De Laethauwer *et al*., 2012).

Among cereal crops, rice, which has a relatively small genome size of approximately 300 Mb, is widely used as a model plant for genomic studies. Using newly detected genes from rice is an excellent approach for finding functional genes in grass crops such as wheat (hexaploid) and barley via comparative genomics (He *et al*., 2007; Liu *et al*., 2012). Zhang *et al*. (2014) isolated *TaSdr*, a gene associated with seed dormancy in wheat, and developed a functional marker in wheat via a comparative study based on the rice *Sdr4* gene, which is positively regulated by *OsVP1* (Sugimoto *et al*., 2010).

To date, most seed dormancy and germination studies have been conducted using the limited available rice genetic resources. In this study, we surveyed naturally occurring variations in seed germination ability in diverse rice genetic resources. In addition, we surveyed the changes in ABA content during seed maturation to investigate their association with seed dormancy.

Materials and methods

Plant materials

In total, 205 rice accessions (Table 1 and Supplementary Table 1) were selected based on geographical origin and ecotype among the conserved accessions in RDA GeneBank at the National Agrobiodiversity Center, National Institute of Agricultural Science (NAC), Rural Development Administration (RDA), Republic of Korea. These accessions included 109 *japonica*, 79 *indica* and 17 *Tongil* rice (Kim *et al*., 2014) accessions and were grown using conventional cultural practices at the experiment field of Natonal Agrobiodiversity Center, Jeonju, in 2015. Their ecotypes were confirmed by genotyping using six microsatellite markers (RM235, RM242, RM267, RM23, RM211 and RM270; data not shown), which exhibit distinct allele distribution patterns in *indica, Tongil* and *japonica* rice (Qi *et al*., 2009).

Table 1. Information about the rice genetic resources investigated in this study

Total: 205 accessions
PHS resistant[a]: Sub-total 90 accessions
– *indica*: 43 accessions (East Asia 3, South and Southeast Asia 37, others 3)
– *japonica*: 34 accessions (East Asia 22, South and Southeast Asia 4, others 8)
– *Tongil*: 13 accessions (East Asia 3, South and Southeast Asia 9, others 1)
Moderately susceptible and susceptible to PHS[b]: Sub-total 115 accessions
– *indica*: 36 accessions (East Asia 19, South and Southeast Asia 15, others 2)
– *japonica*: 75 accessions (East Asia 44, South and Southeast Asia 4, others 27)
– *Tongil*: 4 accessions (East Asia 4)

[a] PHS: less than 20% sprouting at harvesting time.
[b] PHS: greater than 20% sprouting at harvesting time.

Characterization of seed germination ability

Seeds and panicles were harvested from five plants at 42 d after flowering (DAF) and the moisture content was adjusted by drying at 15°C (RH 10%) during 7 d. The susceptibility for PHS value was surveyed using three freshly harvested panicles, which were incubated at 25°C (RH 100%) for 7 d, after which the number of germinated seeds on each panicle was recorded and expressed as a percentage of the total grain number per panicle. The lower the PHS value, the lower is the susceptibility and the higher is the PHS resistance. Accessions with a PHS value below 20% were classified as ‘resistant’. Seed germination at harvesting time (GHT) was determined by threshing panicles and planting three replications of 50 seeds (fruits in hulls) onto moistened Whatman filter paper (10 ml of distilled water) in Petri dishes. They were incubated at 25°C (RH 100%) based on International Seed Testing Association (ISTA) guidelines and germinated seeds (radicle and coleoptile emerged from the hull) were counted daily for a period of 10 d. The cumulated number was expressed as per cent of seeds planted. Germination after an after-ripening process (GAR) was determined by storing dry seeds at 25°C for 3 weeks before germination was performed as described above. Germination after a dormancy breakage (GDB) was determined after a dry storage at 50°C for 7 d before germination was performed as described above. Germination index (GI) was calculated as described by Basra *et al.* (2005):

$$\mathrm{GI} = \sum_{i=1}^{10}\left(\frac{\mathrm{N}_i}{\mathrm{D}_i}\right),$$

where N_i is the mean number of germinated seeds on the *i*th day and D_i is the number of days after the beginning of the germination test.

Determination of changes in ABA content

To reduce the effects of environmental factors on phenotypic variation among the tested rice accessions, including differences in PHS and hormonal contents, 14 samples (eight *japonica* accessions and six *indica* accessions; Supplementary Table 1) with similar flowering times (early August) were selected from among the 205 rice genetic resources. The rice grain parts were sampled at the following stages: +0 DAF, +3 DAF, +6 DAF, +10 DAF, +16 DAF, +20 DAF and +42 DAF.

ABA extraction and purification were performed as described by Bollmark *et al.* (1988) with some modifications. Samples including 10 dehulled grains of the 14 rice accessions at seven ripening stages (+0, +3, +6, +10, +16, +20 and +42) were ground in liquid nitrogen and combined with 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluene as an antioxidant. The extracts were incubated at 4°C for 4 h, and, after centrifugation (5000 ***g***, 15 min, 4°C), the supernatants were filtered through pre-washed Chromosep C18 columns; pre-washing was conducted using 10 ml 100% (v/v), followed by 5 ml 80% (v/v) methanol. An approximately 5 ml purified fraction containing ABA, gas and other plant hormones was dried under N_2 and dissolved in 2 ml of TBS (Tris - buffered saline) medium for analysis by enzyme-linked immunosorbent assay (ELISA). ABA concentrations in extracts of dehulled rice grains were determined by ELISA using a Phytodetek ABA Test Kit (Agdia, USA). The ELISA procedures were conducted according to the manufacturer's instructions with three replicate experiments.

Data analysis

The arcsine transformation of PHS values and germination (%) was used for statistical analysis. One-way analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and correlation analysis were conducted using R software (ver. 3.2.3, http://www.r-project.org/); differences were considered significant when the P value was <0.05.

Results

PHS value in diverse rice accesssions

Among the 205 accessions, 90 were classified as PHS resistant, as their PHS value was below 20% (Table 1, Fig. 1). The proportion of accessions with PHS resistance was highest for *Tongil*-type accessions, with a value of 76.4% [13/17 accessions (accs)], followed by *indica* at 54.4% (43/79 accs) and *japonica* at 31.2% (34/109 accs). Focusing on geographical origin, accessions from South and Southeast Asia had relatively high proportion of accessions with PHS resistance of 59.1% (13/22 accs) and 78.7% (37/47 accs), respectively; while only 29% of accessions from East Asia (28/95 accs) and other regions (12/41 accs) were PHS resistant.

Focusing on domestication levels, breeding lines and cultivars showed a low proportion of PHS-resistant accessions of 40.0% (24/60; East Asia, 33.3%; South and Southeast Asia, 66.7%; other regions, 53.3%). On the other hand, weed types and landraces showed a high proportion of PHS-resistant accessions of 55.2% (16/29; East Asia, 50.0%; South and Southeast Asia, 60.0%; other regions, 0%) and 54.1% (20/37; East Asia, 36.4%; South and Southeast Asia, 76.2%; other regions, 0%), respectively (Supplementary Table 1) Among the 79 non-categorized accessions 38.0% (30/79) were PHS resistant.

Focusing on flowering time within the *indica* and *Tongil* types, the accessions that flower in early August had the highest mean PHS value (35.0 ± 33.9%), followed by accessions flowering at the end of July (28.6 ± 33.8%), middle of August (25.8 ± 30.4%) and end of August (11.3 ± 19.5%). By contrast, the *japonica* accessions did not show significant differences in PHS value at the $P < 0.01$ level (Table 2).

PHS values vs. seed GHT

Analysis of variance revealed differences among ecotypes regarding the difference between PHS values (overall mean 31.0 ± 28.8%) and the GHT (51.9 ± 35.6%) ($P < 0.001$) (Fig. 2); the *japonica* ($P < 0.001$) and *indica* ($P < 0.01$) accessions exhibited higher GHTs than PHS values, whereas the *Tongil* group did not. Across all accessions, there was a significant correlation between PHS values and GHT, with a Pearson correlation coefficient value of 0.814 ($P < 0.001$). The GI at 42 DAF was highly correlated with the GHT, with a correlation coefficient of 0.947 ($P < 0.001$).

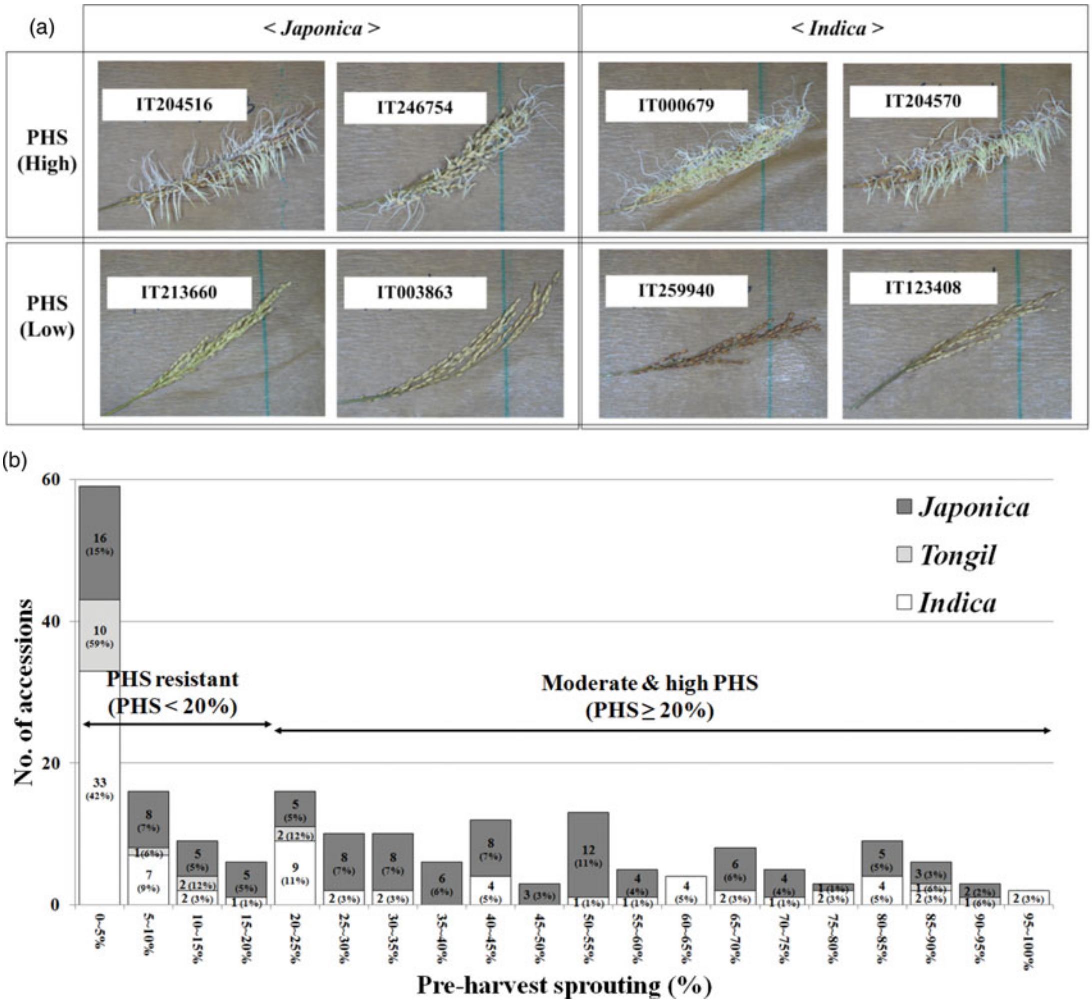

Fig. 1. Investigation of PHS. (a) Representative PHS-resistant and -susceptible accessions; (b) distribution of PHS among 205 rice genetic resources.

Effects of after-ripening and dormancy breakage

The average GI values of the PHS-resistant and non-resistant accessions were 2.8 (±3.4) and 10 (±4.1), respectively. These values increased following after-ripening treatment (10.8 ± 4.6 and 14.6 ± 3.3 for the PHS-resistant group and the PHS non-resistant group, respectively) and further increased by dormancy breakage (18.4 ± 2.9 and 18.7 ± 2.6 for the PHS-resistant group and the PHS non-resistant group, respectively) (Fig. 3). In the PHS-resistant group, the seed germination ability increased by after-ripening treatment and dormancy breakage differently in the three ecotypes. The *japonica* accessions showed relatively low average germination abilities following after-ripening and dormancy breakage, with values of 69.5 (±31.2)% and 91.9 (±15.0)%, respectively, compared with *indica* and *Tongil* accessions, with values of 81.5 (±21.4)% and 97.4 (±4.4)%, respectively ($P < 0.05$; Fig. 3.).

Changes in ABA content after fertilization

The ABA content increased until 10 DAF and decreased thereafter in all 14 accessions examined (Fig. 4). The maximum ABA content was detected during the ripening period (at 10 DAF). During this period, the *indica* accessions had higher average ABA contents (95.9 ± 34.2 mM) than the *japonica* accessions (48.7 ± 10.5 mM) ($P < 0.01$). Among the resistant accessions, the mean ABA content of the *indica* accessions was 106.0 (±26.1) mM, whereas that of the *japonica* accessions was only 41.8 (±7.4) mM. Interestingly, the PHS-non-resistant *japonica* accessions had higher or similar levels of ABA compared with the PHS-resistant accessions, with differences not significant at the P value of 0.05 level, and among the *indica* accessions, there was no significant difference in ABA content at 10 DAF between PHS-resistant and -non-resistant accessions.

Table 2. Average PHS values at harvesting time (42 DAF)

Ecotype	Flowering time	PHS*	Ecotype	Flowering time	PHS
indica and *Tongil*	End of July	28.6 ± 33.8 ($n = 10$)a	*japonica*	End of July	41.3 ± 28.8 ($n = 29$)
	Early August	35 ± 33.9 ($n = 30$)a		Early August	33.4 ± 24.6 ($n = 37$)
	Middle of August	25.8 ± 30.4 ($n = 25$)ab		Middle of August	38 ± 26.1 ($n = 40$)
	End of August	11.3 ± 19.5 ($n = 31$)b		End of August	22.3 ± 31.6 ($n = 3$)

*$P < 0.01$.
a and b were ranked by Duncan's test.

Discussion

Climate change is expected to cause greater fluctuations in rainfall during the ripening periods and harvesting times of various cereal crops, leading to an increase in damage caused by PHS. Various studies, for example involving QTL mapping, gene identification, and the analysis of associated hormones, have been conducted to help overcome these problems in rice, a widely used model cereal crop plant (Hattori *et al.*, 1994; Lin *et al.*, 1998; Dong *et al.*, 2003; Guo *et al.*, 2004; Gu *et al.*, 2005b; Wan *et al.*, 2006; Gu *et al.*, 2010; Hori *et al.*, 2010; Sugimoto *et al.*, 2010; Gu *et al.*, 2011; Kim *et al.*, 2011; Lu *et al.*, 2011; Marzougui *et al.*, 2012; Challam *et al.*, 2013). However, these investigations of valuable alleles were limited in that only a small number of resistant and susceptible accessions were used to uncover various aspects of seed germination and dormancy. Seed germination and dormancy are quantitative traits that retain broadly observed phenotypic variation, the degrees of which vary according to allele type. Therefore, these diverse alleles might serve as valuable resources for regulating seed germination levels, including PHS (Haussmann *et al.*, 2003). The aim of the present study was to survey naturally occurring variations in natural rice populations, so that the resources together with the data can be used in further studies for investigating seed dormancy and germination.

Among the 205 rice accessions examined (109 *japonica*, 79 *indica* and 17 *Tongil*), more PHS-resistant accessions (54.4%) were presented in the *indica* ecotype than the *japonica* type (31.2%). This finding was not unexpected, as most *indica* rice accessions are domesticated and cultivated in tropical and subtropical regions, which have a wet period during ripening and harvesting time. By contrast, *japonica* rice is dispersed throughout south China and has been domesticated in temperate regions, which have relatively dry climates (Khush, 1997; Huang *et al.*, 2012). The selective pressure for PHS resistance is lower in East Asia, which has relatively few environmental constraints, compared with South and Southeast Asian regions. We found that breeding lines and cultivars from East Asia were more susceptible to PHS than those from other regions, with an average proportion of resistant accessions of 33.3%. This finding reflects the notion that selection by humans has placed a relatively low value on PHS resistance in this region compared with tropical and subtropical regions. As a result, unexpected rainfall during ripening and harvesting due to rapid environmental changes can severely damage grain quantity and quality.

Despite the correlation between the germination of grains in the panicle and of detached grains, we detected an increase in germination (%) after separation from the panicle at 42 DAF in both the *japonica* ($P < 0.001$) and *indica* ($P < 0.01$) accessions. Physical dormancy due to the presence of an impermeable seed coat is one cause of seed dormancy in some orthodox seeds, and seed pigmentation is associated with seed dormancy in cereal crops (Werker, 1980; Khan *et al.*, 1997; Himi *et al.*, 2002; Gu *et al.*, 2005a; Sweeney *et al.*, 2006). Kermode and Bewley (1989) reported that the seed detachment from the mother plant in combination with some water loss could elicit germination. In this regard, the increased seed germination after the separation from panicles might be due to the weakening of the mechanical barrier correlated with dehydration in the detachment site of grains. The germination ability of the PHS-resistant group (90 accessions, PHS < 20%) increased following after-ripening treatment, and remained dormancy was nearly absent after the high-temperature dormancy-breaking treatment (Fig. 3). In particular, there was a relatively high increase in germination ability in *indica*-type rice following after-ripening and dormancy breakage compared with *japonica*-type rice. This result indicates that the dormancy of *indica* rice is easily broken, which might be due to its geographically distinct domestication process (Cheng *et al.*, 2003; Vitte *et al.*, 2004; Zhu and Ge, 2005; Huang *et al.*, 2012).

ABA plays a central role in maintaining primary dormancy and germination throughout the plant's lifecycle and affects its seed germination ability and dormancy through active or passive transport (Finch-Savage and Leubner-Metzger, 2006; Finkelstein *et al.*, 2008; Seo and Koshiba, 2011). Similar to previous results (Gu *et al.*, 2010; Kanno *et al.*, 2010), we found that the ABA content was maximal during the middle stage of development (10

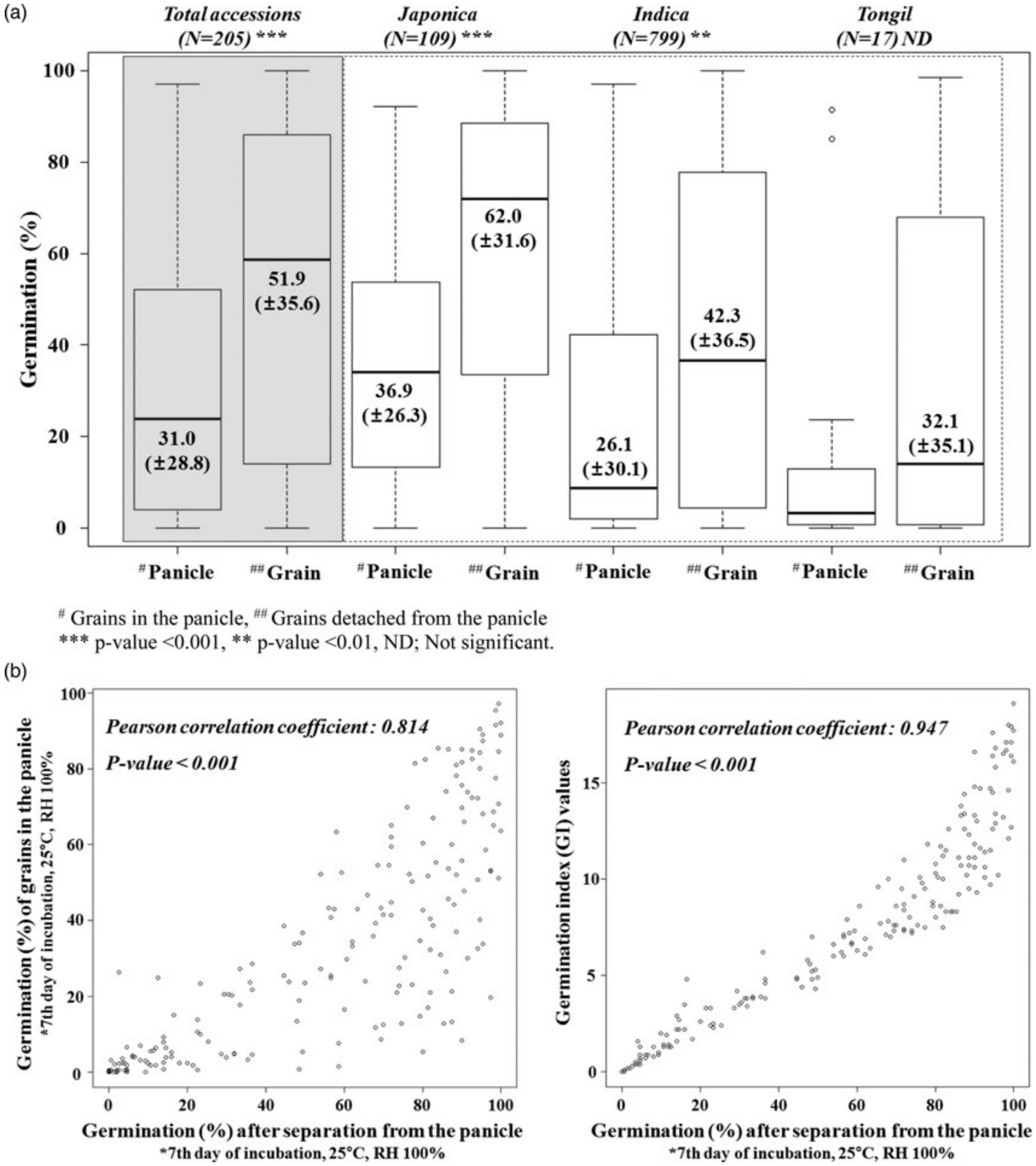

Fig. 2. Comparison of germination ability. (a) Changes in seed germination (%); germination of grains in the panicle and germination after separation from the panicle (b) correlation between PHS, GI, and average germination ratio at harvesting time (7th day of incubation). #Grains in the panicle, ##Grains detached from the panicle. ***$P < 0.001$, **$P < 0.01$, ND, not significant.

DAF) and decreased thereafter in all 14 accessions examined. The *indica* accessions had higher ABA contents than the *japonica* accessions, which is thought to result from natural or human-driven selection to help the plants cope with a long wet period (Khush, 1997). Interestingly, PHS-non-resistant accessions of both the *japonica* and *indica* type maintained high or similar levels of ABA compared with PHS-resistant accessions. In higher plants, ABA concentration and sensitivity to ABA determine the physiological responses mediated by this phytohormone (Seo and Koshiba, 2011). ABA levels are regulated by its biosynthesis and catabolism, and ABA transport, perception and signal transduction are key factors affecting the sensitivity to this hormone. To date, several genes in the ABA biosynthesis and catabolism pathways have been discovered, including NCEDs for ABA biosynthesis during water stress responses (Iuchi *et al.*, 2001; Urano *et al.*, 2009) and CYP707As for ABA catabolism (Kushiro *et al.*, 2004; Umezawa *et al.*, 2006). Furthermore, the discovery of ABA receptors such as OsPYL/RCAR5, the rice

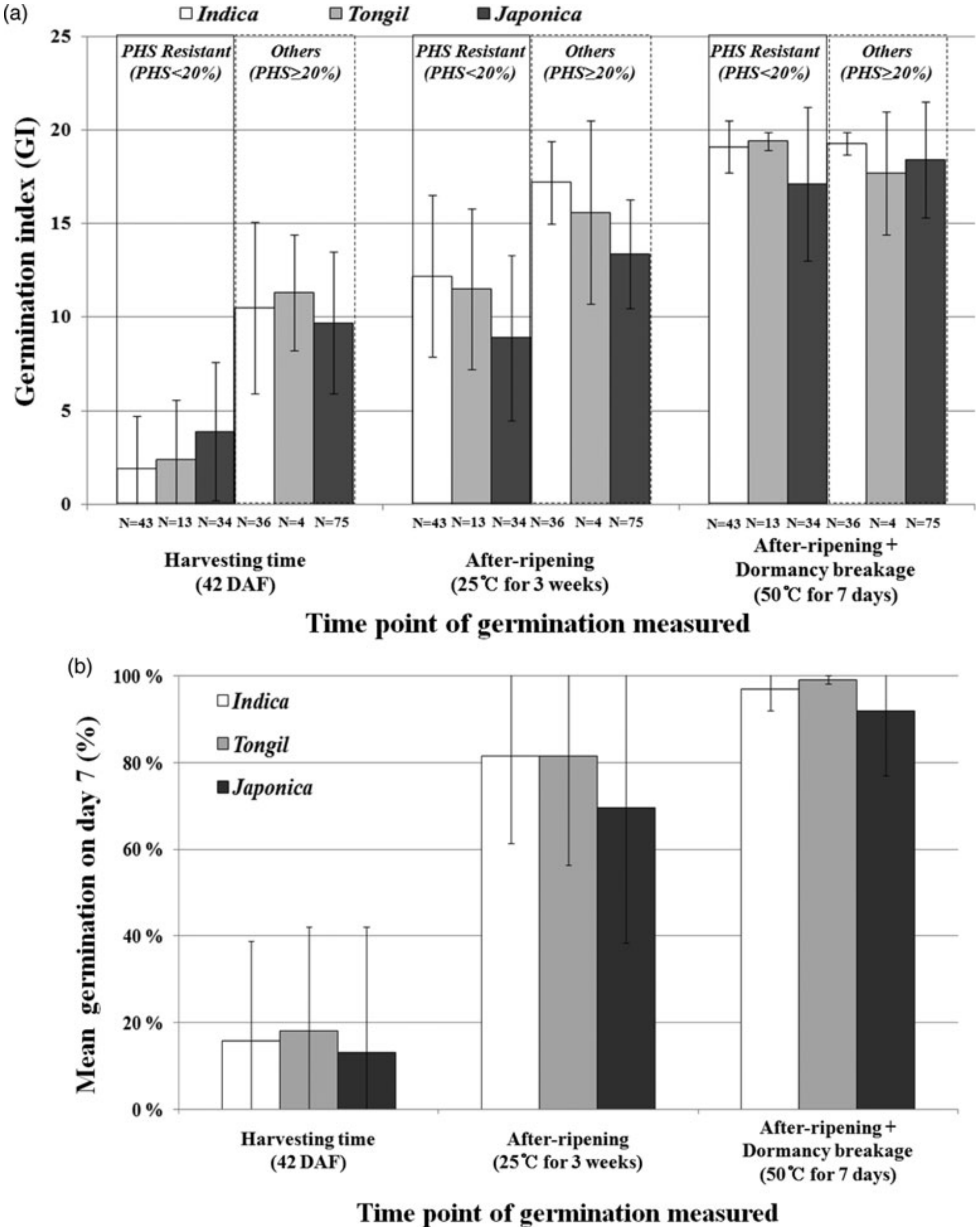

Fig. 3. Changes in seed germination ability after separation from the panicle and dormancy breakage. (a) Changes in average GI values during 10 d of incubation (25°C, RH 100%) in 205 rice genetic resources at harvesting time (42 DAF) and dormancy breakage treatment; (b) changes in average germination ratios on the 7th day of incubation (25°C, RH 100%) at harvesting time and following after-ripening and dormancy breakage for 90 PHS-resistant (PHS <20%) rice genetic resources.

orthologue of an intracellular ABA receptor of *Arabidopsis* (Kim *et al.*, 2011), revealed that ABA is not simply transferred by passive transport (Seo and Koshiba, 2011; Ye *et al.*, 2012). While PHS of less matured seeds could be affected by ABA content in that we measured the PHS level at harvesting time (42 DAF), these findings suggest that the key factors for seed dormancy are the perception and signal transduction of ABA rather than the total content of ABA in a natural population. Indeed, Gianinetti and Vernieri (2007) also reported that there is little correlation between ABA content and seed dormancy.

Based on the results of the present study, we are currently searching for genes associated with seed dormancy affecting PHS at the molecular level. The diversity of seed germination ability in rice genetic resources detected in this study could be sustainably used for crop improvement programmes and for detecting the genetic and physiological basis of this quantitative trait.

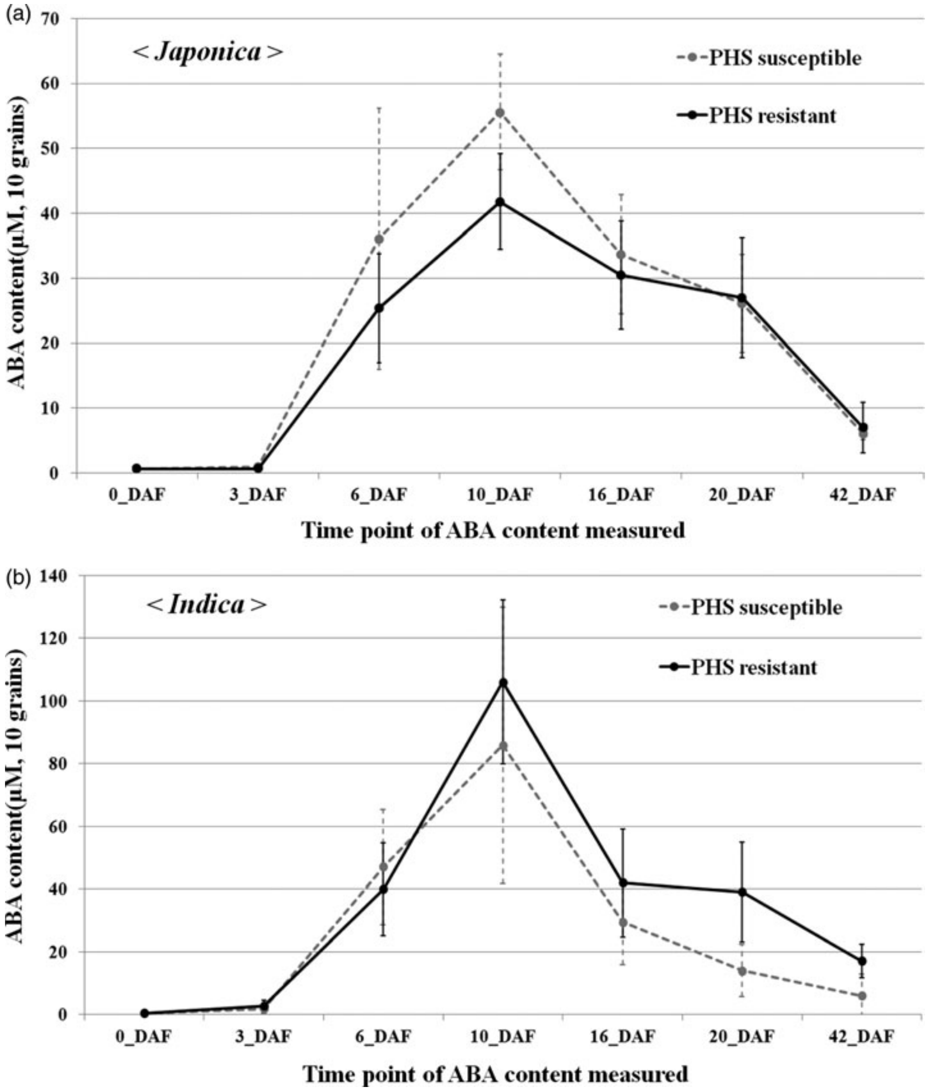

Fig. 4. Changes in ABA content after fertilization in 14 rice accessions. (a) Eight *japonica* accessions; (b) six *indica* accessions.

Supplementary Material

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S1479262116000319

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References

Basra S, Farooq M, Tabassam R and Ahmad N (2005) Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Science and Technology* 33: 623–628.

Bewley JD and Black M (1982) Physiology and biochemistry of seeds in relation to germination. Vol. 2. Viability, dormancy and environmental control, Springer-Verlag, New York.

Bollmark M, Kubát B and Eliasson L (1988) Variation in endogenous cytokinin content during adventitious root formation in pea cuttings. *Journal of Plant Physiology* 132: 262–265.

Challam C, Kharshing GA, Yumnam JS, Rai M and Tyagi W (2013) Association of qLTG3–1 with germination stage cold tolerance in diverse rice germplasm from the Indian subcontinent. *Plant Genetic Resources* 11: 206–211.

Cheng C, Motohashi R, Tsuchimoto S, Fukuta Y, Ohtsubo H and Ohtsubo E (2003) Polyphyletic origin of cultivated rice:

based on the interspersion pattern of SINEs. *Molecular Biology and Evolution* 20: 67–75.

Cutler SR, Rodriguez PL, Finkelstein RR and Abrams SR (2010) Abscisic acid: emergence of a core signaling network. *Annual Reviews Plant Biology* 61, 651–679.

De Laethauwer S, Reheul D, De Riek J and Haesaert G (2012) *Vp*1 expression profiles during kernel development in six genotypes of wheat, triticale and rye. *Euphytica* 188: 61–70.

Dong Y, Tsuzuki E, Kamiunten H, Terao H, Lin D, Matsuo M and Zheng Y (2003) Identification of quantitative trait loci associated with pre-harvest sprouting resistance in rice (*Oryza sativa* L.). *Field Crops Research* 81: 133–139.

Finch-Savage WE and Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytologist* 171: 501–523.

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. *Plant Biology* 59: 387.

Gianinetti A, Vernieri P (2007) On the role of abscisic acid in seed dormancy of red rice. *Journal of Experimental Botany* 58: 3449–3462.

Gu X-Y, Kianian SF and Foley ME (2005a) Seed dormancy imposed by covering tissues interrelates to shattering and seed morphological characteristics in weedy rice. *Crop Science* 45: 948–955.

Gu X-Y, Kianian SF and Foley ME (2005b) Phenotypic selection for dormancy introduced a set of adaptive haplotypes from weedy into cultivated rice. *Genetics* 171: 695–704.

Gu X-Y, Liu T, Feng J, Suttle JC and Gibbons J (2010) The qSD12 underlying gene promotes abscisic acid accumulation in early developing seeds to induce primary dormancy in rice. *Plant Molecular Biology* 73: 97–104.

Gu X-Y, Foley ME, Horvath DP, Anderson JV, Feng J, Zhang L, Mowry CR, Ye H, Suttle JC and Kadowaki K-i (2011) Association between seed dormancy and pericarp color is controlled by a pleiotropic gene that regulates abscisic acid and flavonoid synthesis in weedy red rice. *Genetics* 189: 1515–1524.

Guo L, Zhu L, Xu Y, Zeng D, Wu P and Qian Q (2004) QTL analysis of seed dormancy in rice (Oryza sativa L.). *Euphytica* 140: 155–162.

Hattori T, Terada T and Hamasuna ST (1994) Sequence and functional analyses of the rice gene homologous to the maize *Vp*1. *Plant Molecular Biology* 24: 805–810.

Haussmann BIG, Parzies HK, Presterl T, Susic Z and Miedaner T (2003) Plant genetic resources in crop improvement. *Plant Genetic Resources* 2, 3–21.

He X, He Z, Zhang L, Sun D, Morris C, Fuerst E and Xia X (2007) Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. *Theoretical and Applied Genetics* 115: 47–58.

Himi E, Mares DJ, 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.

Hori K, Sugimoto K, Nonoue Y, Ono N, Matsubara K, Yamanouchi U, Abe A, Takeuchi Y and Yano M (2010) Detection of quantitative trait loci controlling pre-harvest sprouting resistance by using backcrossed populations of japonica rice cultivars. *Theoretical and Applied Genetics* 120: 1547–1557.

Huang X, Kurata N, Wei X, Wang Z-X, Wang A, Zhao Q, Zhao Y, Liu K, Lu H and Li W (2012) A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490: 497–501.

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K and Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *The Plant Journal* 27: 325–333.

Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y and Seo M (2010) Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant and Cell Physiology* 51: 1988–2001.

Kermode AR and Bewley JD (1989) Developing seeds of *Ricinus communis* L., when detached and maintained in an atmosphere of high relative humidity, switch to a germinative mode without the requirement for complete desiccation. *Plant Physiology* 90(2): 702–707.

Khan M, Cavers PB, Kane M and Thompson K (1997) Role of the pigmented seed coat of proso millet (*Panicum miliaceum* L.) in imbibition, germination and seed persistence. *Seed Science Research* 7: 21–26.

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology* 35: 25–34.

Kim H, Hwang H, Hong J-W, Lee Y-N, Ahn IP, Yoon IS, Yoo S-D, Lee S, Lee SC and Kim B-G (2011) A rice orthologue of the ABA receptor, OsPYL/RCAR5, is a positive regulator of the ABA signal transduction pathway in seed germination and early seedling growth. *Journal of Experimental Botany* 63: 1013–1024.

Kim B, Kim D-G, Lee G, Seo J, Choi I-Y, Choi B-S, Yang T-J, Kim KS, Lee J and Chin JH (2014) Defining the genome structure of 'Tongil' rice, an important cultivar in the Korean "Green Revolution". *Rice* 7: 22.

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y and Nambara E (2004) The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO Journal* 23: 1647–1656.

Li C, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J and Cakir M (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice–wheat–barley comparison. *Functional & Integrative Genomics* 4: 84–93.

Lin S, Sasaki T and Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. *Theoretical and Applied Genetics* 96: 997–1003.

Liu Y, He Z, Appels R and Xia X (2012) Functional markers in wheat: current status and future prospects. *Theoretical and Applied Genetics* 125: 1–10.

Lu B, Xie K, Yang C, Wang S, Liu X, Zhang L, Jiang L and Wan J (2011) Mapping two major effect grain dormancy QTL in rice. *Molecular Breeding* 28: 453–462.

Marzougui S, Sugimoto K, Yamanouchi U, Shimono M, Hoshino T, Hori K, Kobayashi M, Ishiyama K and Yano M (2012) Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. *Theoretical and Applied Genetics* 124: 893–902.

Nakamura S and Toyama T (2001) Isolation of a VP1 homologue from wheat and analysis of its expression in embryos of dormant and non-dormant cultivars. *Journal of Experimental Botany* 52: 875–876.

Qi Y, Zhang H, Zhang D, Wang M, Sun J, Ding L, Wang F and Li Z (2009) Assessing *indica- japonica* differentiation of improved rice varieties using microsatellite markers. *Journal of Genetics and Genomics* 36: 305–312.

Raghavendra AS, Gonugunta VK, Christmann A and Grill E (2010) ABA perception and signalling. *Trends in Plant Science* 15: 395–401.

Seo M and Koshiba T (2011) Transport of ABA from the site of biosynthesis to the site of action. *Journal of Plant Research* 124: 501–507.

Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y and Hattori T (2010) Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. *Proceedings of the National Academy of Sciences of the United States of America* 107: 5792–5797.

Sweeney MT, Thomson MJ, Pfeil BE and McCouch S (2006) Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *Plant Cell* 18: 283–294.

Umezawa T, Okamoto M, Kushiro T, Nambara E, Oono Y, Seki M, Kobayashi M, Koshiba T, Kamiya Y and Shinozaki K (2006) CYP707A3, a major ABA 8′-hydroxylase involved in dehydration and rehydration response in *Arabidopsis thaliana*. *Plant Journal* 46: 171–182.

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D and Kobayashi M (2009) Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics. *Plant Journal* 57: 1065–1078.

Vitte C, Ishii T, Lamy F, Brar D and Panaud O (2004) Genomic paleontology provides evidence for two distinct origins of Asian rice (*Oryza sativa* L.). *Molecular Genetics and Genomics* 272: 504–511.

Wan J, Jiang L, Tang J, Wang C, Hou M, Jing W and Zhang L (2006) Genetic dissection of the seed dormancy trait in cultivated rice (*Oryza sativa* L.). *Plant Science* 170: 786–792.

Werker E (1980) Seed dormancy as explained by the anatomy of embryo envelopes. *Israel Journal of Botany* 29: 22–44.

Ye N, Jia L and Zhang J (2012) ABA signal in rice under stress conditions. *Rice* 5: 1.

Zhang Y, Miao X, Xia X and He Z (2014) Cloning of seed dormancy genes (TaSdr) associated with tolerance to pre-harvest sprouting in common wheat and development of a functional marker. *Theoretical and Applied Genetics* 127: 855–866.

Zhu Q and Ge S (2005) Phylogenetic relationships among A-genome species of the genus *Oryza* revealed by intron sequences of four nuclear genes. *New Phytologist* 167: 249–265.