Physiological and Molecular Characterization of Natural Genes Controlling Seed Dormancy by Regulating Gibberellin Biosynthesis or Signaling in Rice

Xing-You Gu (Principle Investigator)
Plant Science Department, South Dakota State University
Box 2140C, SNP 248B, Brookings, SD 57007
E-mail:Xingyou.gu@sdstate.edu

1. Objectives

Research in PI's Seed Molecular Biology Lab at SDSU has been focused on genetic, evolutionary and physiological mechanisms directly regulating the natural variation of seed dormancy in grass species, including the major crops rice and wheat. We developed weedy rice (*Oryza sativa*) as a model system, because it has strong seed dormancy, is relatively small in genome size, and shares synteny in gene order and content with the other grass species. Thus, seed dormancy genes cloned from the rice model can be directly used as probes to identify their orthologs in the other grass species, and the knowledge about the dormancy development and release gained from rice can also help understand regulatory mechanisms of seed dormancy and germination in the other cereal crops, such as wheat, barley, oats, sorghum, and rye for which the pre-harvest sprouting has been a worldwide serious problem in crop production.

Both *qSD1-2* and *qSD7-2* are quantitative trait loci (QTL) associated with seed dormancy in rice (Gu et al. 2004a, Ye et al. 2010). Using a map-based cloning approach, we identified a *qSD1-2* candidate gene encoding a Gibberellin (GA) 20-oxidase for GA biosynthesis and also identified a *qSD7-2* candidate gene encoding a predicted protein kinase, which negatively regulated GA signaling for germination (Ye et al. 2013). The plant hormone GA is known to promote germination through GA signaling; however, it is unclear about the regulatory roles of GA or GA signaling in the development of seed dormancy (Steber 2007). Thus, allelic variants identified from the *qSD1-2* and *qSD7-2* loci in PI's lab provide a unique system to elucidate how the GA biosynthesis or signaling pathway evolved in cereal crops to regulate the natural variation in the degree of seed dormancy. The objectives (Obj.) of this project are:
Obj. #1: To confirm the *qSD1-2* and *qSD7-2* candidate genes for the seed dormancy function;
Obj. #2: To identify regulatory roles of the *SD1-2* and *SD7-2* genes in the development or release of seed dormancy; and,
Obj. #3: To characterize the SD7-2 protein for kinase activity and interacting factors in the GA signaling pathway.

2. Rationale

Genotypic variation of seed dormancy retained in natural populations and crop germplasm is of both ecological and agricultural importance. Genetic bases underlying the natural variants have been resolved into QTLs in >10 crop/model plants in the past two decades. Cloning and molecular characterization of the QTL underlying genes will provide in-depth insights into evolutionary and developmental mechanisms of seed dormancy and also provide candidate genes to manipulate germination capability in crop breeding.

Seeds acquire primary dormancy during their development on the mother plant and release the dormancy during a given period of warm dry storage (after-ripening) before germination occurs. Thus, seed dormancy and germination are two sequential biological processes in the life history of flowering plants. It is believed that GA synthesized in embryo promotes germination through GA signaling in the endosperm aleuronic cells of cereal grains (Sun & Gubler 2004,

Steber 2007). However, the promoting effect works for some (e.g., the *qSD1-2* mutant), but not for the other (e.g., the *qSD7-2* variant), genotypes in rice (Ye et al. 2013). Little is known about how GA biosynthesis and signaling pathways evolved to regulate the natural variation in seed dormancy. The natural mutants at *qSD1-2* and *qSD7-2* isolated from weedy rice provided a set of unique genotypes to address this fundamental question. This project will answer: 1) if the *qSD1-2* and *qSD7-2* candidate genes also have an effect on seed dormancy in transgenic or T-DNA insertion lines (Obj. #1); 2) if the *qSD1-2* and *qSD7-2* loci regulate the dormancy development and/or release through the diploid embryo (2n) or the triploid endosperm (3n) tissue (Obj. #2); and 3) if the predicted protein kinase encoded by the dormancy gene *SD7-2* interacts with the core element "DELLA protein" of GA signaling pathway through a phosphorylation mechanism to regulates the dormancy development of release (Obj. #3).

Map-based cloning of a QTL for such a complex trait as seed dormancy is time-consuming. We have cloned genes underlying the *qSD7-1* and *qSD12* seed dormancy QTL from weedy rice; these two genes encode bHLH family transcription factors, but they regulate the types of seed dormancy imposed by the maternal and embryo tissues, respectively (Gu et al. 2011 & 2015, Feng et al. 2014). We are using similar strategies to clone *qSD1-2* and *qSD7-2*. So far, we have obtained *qSD1-2* and *qSD7-2*'s candidate genes and their transgenic plants and/or T-DNA insertion mutants, the most challenging work for QTL cloning. The remaining work to confirm and characterize the candidate genes is straightforward and provides an excellent opportunity to train a doctoral graduate student in seed biology.

3. Background

3.1 Concepts of seed dormancy and germination

Seed dormancy in seed biology is defined as the temporary failure of a viable seed to complete germination under favorable conditions. A seed in grasses refers to a disposal unit, which consists of a diploid embryo (2n, n is the number of chromosomes in a haploid gamete cell) covered by the triploid endosperm (3n) and diploid maternal (2n testa, pericarp, lemma & palea) tissues. Thus, seed dormancy can be embryonic (embryo dormancy), endosperm-imposed, or maternal tissue-imposed (Bewley et al. 2013). All these types of dormancy are acquired during seed development and released after maturation by the exposure of seeds to a set of environmental conditions for a given time period, or after-ripening (Simpson 1990). Marker-assisted genetic approaches combined with a partial after-ripening technique were developed to determine if a gene/QTL is involved in the genetic control of seed dormancy through the component tissues (Gu et al. 2008 & 2015).

Germination is the process that commences with water uptake by dry seeds (imbibition) and terminates with the radicle emergence from the covering tissues. The radicle emergence occurs when the embryo growth potential overcomes the restraint forces imposed by the endosperm and maternal tissues; many cellular and molecular events contribute to the balance between the opposing forces (Nonogaki et al. 2007). Only non-dormant or fully after-ripened seeds can complete germination. GA stimulates germination for some genotypes. GA's role in regulating germination of cereal grains is not clear (Steber 2007).

Lack of seed dormancy causes pre-harvest sprouting (PHS) and viviparous problems in crop production. Both PHS and vivipary refer to germination in the inflorescence, but they differ in timing during seed development (Foley 2002). Vivipary occurs before seed maturation and dry-down. Viviparous mutants were collected as laboratory materials (Durantini et al. 2008) and are absent in natural populations and germplasm collections. PHS happens after seed maturation but before harvest, when moist conditions prevail or untimely rains occur. Many modern cultivars of cereals (barley, oats, rice, rye, sorghum, & wheat) are susceptible to PHS, which causes a loss of

yield and a reduction in grain quality (Ringlund 1993). "The International Symposium on PHS in Cereal Crops" has been focused on genetic solutions to this worldwide problem since 1980s.

3.2 Genetic basis of seed dormancy

Genetic basis underlying the natural variation of seed dormancy have been resolved into QTL in cereal, vegetable and oilseed crops and the model plant *Arabidopsis thaliana* in the past two decades (e.g., Anderson et al. 1993, Ullrich et al. 1993, Lin et al. 1998, Fennimore et al. 1999, Lijavetzky et al. 2000, Alonso-Blanco et al. 2003, Argyris et al. 2005, Gandhi et al. 2005, Masojć et al. 2007, Blaker et al. 2013, Schatzki et al. 2013). Some of the reported QTLs were identified by association with PHS or thermo-inhibitory germination (failure to germinate when seeds are imbibed at warm temperatures) in barley, lettuce and wheat. A QTL is a marked genomic region usually containing many genes that have nothing to do with the quantitative trait. Map-based cloning entails fine-mapping of a QTL region to delimit candidate genes, followed by sequencing, annotation and functional confirmation of selected candidate(s).

Six QTLs for seed dormancy (*DOD1*, *Sdr4*, *qSD7-1 & qSD12*), PHS (*QPhs.ocs-3A1*), or thermo-inhibition (*Htg6.1*) were map-based cloned. The Arabidopsis *DOG1* underlying gene *At5g45830* is expressed specifically in seeds, but unknown for molecular function (Bentsink et al. 2006). The rice *Sdr4* underlying gene *Os07g39700* is also unknown for molecular function and may act downstream of *OsVp1*, an ortholog of the maize mutant *viviparous1* (McCarty et al. 1989), to regulate dormancy development (Sugimoto et al. 2010). Both *qSD7-1 & qSD12* were cloned from weedy "red" rice. *qSD7-1* is involved in the maternal tissue-imposed seed dormancy and encodes a bHLH-family transcription factor, which promoted the expression of the abscisic acid (ABA) biosynthesis gene *OsABA1* (encoding ZEAXANTHIN EPOXIDASE 1) and the hormone accumulation in early developing seeds to induce primary dormancy and also activated the flavonoid biosynthesis pathway to produce red pigment in the lower epidermal cell layer of the pericarp tissue (Gu et al. 2008, 2011 & 2015). *qSD12* was also cloned as a bHLH transcription factor gene, but it is expressed in developing embryos to control embryo dormancy (Gu et al. 2008 & 2015, Feng et al. 2014). The wheat *QPhs.ocs-3A1* locus was cloned as an *MFT* ortholog (a plant growth regulator identified in Arabidopsis and tomato) and is expressed in scutellum and coleorhiza to enhance seed dormancy in low temperatures (Nakamura et al. 2011, Liu et al. 2013). The lettuce *Htg6.1* locus was cloned as the ABA synthesis gene *LsNCED4*; its expression was elevated during late seed development but was not required for seed maturation (Huo et al. 2013). Identifies of the cloned genes indicate that natural variants for seed dormancy include mutants at regulatory genes or a structure gene key to the ABA biosynthesis (*Htg6.1*).

The plant hormone ABA induces seed dormancy while GA counteracts ABA's effect to stimulate germination. The major seed dormancy QTL on chromosome 5H in barley was aligned to the genomic region containing the GA biosynthesis gene *GA20ox1* on chromosome 3 in rice by comparative genomic research (Li et al. 2004). We associated the rice *qSD1-2* seed dormancy locus with a functional mutation of the GA biosynthesis gene *OsGA20ox2* and the mutant effect on reduced germination could be recovered by GA application (Ye et al. 2013). Cloning and characterization of these and other seed dormancy QTLs will help understand how the hormones' effects are achieved in natural variants.

3.3 The GA signaling pathway

Bioactive gibberellins promote stem elongation and germination through GA signaling. The classic GA signaling pathway consists of the GID1 GA receptor, the DELLA core regulator, and the $SCF^{SLY1/GID2}$ F-box-containing protein complex (Fig. 1A, Sun & Gubler 2004, Steber 2007, Sun 2010). Both GID1 and GID2 were identified from the rice dwarf mutants that are insensitive

to GA for leaf elongation and plant height. The DELLA protein is a core negative regulator of GA-responsive traits and its rice orthologous is SLR1, which was identified from the *slender rice 1* mutant. The DELLA or SLR1 protein has two conserved motifs (DELLA & VHYNP) and a poly Ser/Thr region in the N-terminal and the GRAS domain in the C-terminal. To lift the repression of GA responses, GA binds to GID1 to enable the receptor to bind the DELLA motif to form the GA-GID1-DELLA complex, which is required for the F-box protein to target the GRAS domain to trigger DELLA protein ubiquitination and proteolysis by 26S proteasome.

Additional genes are likely involved in GA signaling. The Arabidopsis loss-of-function mutant *SPY* and its rice ortholog *OsSPY* could rescue the reducing effect of GA-deficient mutations on plant height. These genes encode predicted *O*-linked N-acetylglucosamine (*O*-GlcNAc) transferases (OGT) to compete with kinase for phosphorylation sites likely by modifying DELLA proteins (Shimada *et al.* 2006, Silverstone *et al.* 2007). The rice *EL1* was identified from the T-DNA insertion mutant for early flowering (*el1*) and encodes a casein kinase to phosphorylate SLR1 (Dai & Xue 2010). Both SPY and EL1 are negative regulators of early GA signaling by posttranslational modifications to DELLA proteins, which may activate DELLA's repressing activity (Dai & Xue 2010, Sun 2010, Gao *et al.* 2011).

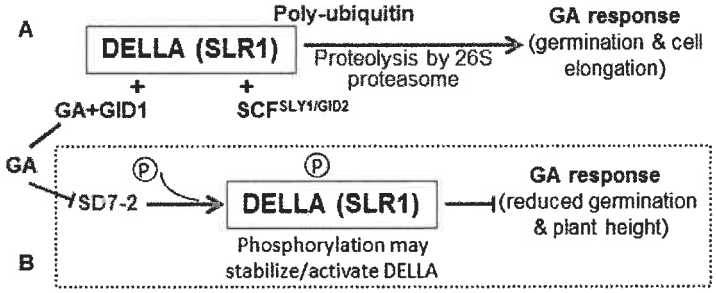

Fig. 1 GA signaling models. **A**, GA-induced proteolysis lifting GA responses. **B**, SD7-2-mediated phosphorylation of the DELLA protein to repress GA responses. SLR1, rice DELLA protein; GID1, rice GA receptor; $SCF^{SLY1/GID2}$, F box-containing protein complex; SD7-2, predicted protein kinase encoded by the *qSD7-2* underlying gene for seed dormancy (Fig. 2B).

The proteolysis-dependent mechanism (Fig. 1A) was proposed mainly based on experimental data from artificial mutants for plant height in Arabidopsis and rice. Arabidopsis mutants of *DELLAs*, *GID1s* and *SLY1-2* also influenced seed dormancy (Penfield *et al.* 2006, Voegele *et al.* 2011, Ariizumi et al. 2013). However, none of the artificial mutants *el1*, *gid1*, *gid2*, *slr1*, and *spy1*) in rice (Iketa *et al.* 2001, Sasaki *et al.* 2003, Ueguchi-Tanaka *et al.* 2005, Shimada *et al.* 2006, Dai & Xue 2010) was reported for an effect on seed dormancy. It was hypothesized that the DELLA' regulatory role in germination may be not as highly conserved as that in stem elongation and may also vary between coat-imposed and embryo dormancies (Steber 2007).

In addition to the proteolysis-dependent mechanism, a proteolysis-independent mechanism of GA signaling was described for the Arabidopsis *sly1-2* mutant (Ariizumi et al. 2013). The *sly1-2* mutant is similar to the rice *gid2* mutant in GA signaling. Unfortunately, both of the rice *gid1* and *gid2* mutants are severely dwarf (10-15 cm in plant height) and could not yield seeds for germination assay in PI's and other labs. In summary, the role of GA signaling in regulation of seed dormancy and germination is not clear in grass species.

3.4 Preliminary data

3.4.1 The alleles of *qSD1-2* and *qSD7-2* from the weedy rice line SS18-2 were introduced into the background of the cultivated rice EM93-1, and both loci associated with seed dormancy and plant height in the isogenic background. The isogenic lines for four homozygous genotypes of *qSD1-2* and *qSD7-2* displayed only three phenotypes of seed dormancy or plant height (Figs. 2A & B). At both QTLs, the alleles that enhance seed dormancy also reduce plant height, or the

alleles that reduce seed dormancy promote plant height. For *qSD1-2*, the mutant allele *sd1-2* enhances seed dormancy and reduces plant height; whereas, for *qSD7-2*, the wild-type allele *SD7-2* enhances seed dormancy and reduced plant height (Ye et al. 2013). GA application rescued inhibitory effects on germination and seedling elongation for *qSD1-2*, but not for *qSD7-2* (Figs. 2C & D). These results indicate that the *qSD1-2* and *qSD7-2* variants represent GA-sensitive and -insensitive mutants, respectively, and also suggest that the phenotypic association between seed dormancy and plant height may arise from pleiotropic effects.

3.4.2 Fine mapping delimited *qSD1-2* to the genomic region containing LOC_Os01g66100:
About 3000 plants from a population segregating only for the *qSD1-2* region were genotyped to select rare recombinants to fine map the QTL. Progeny testing of the recombinants for seed dormancy delimited *qSD1-2* to the genomic segment of 38370-38390 kb on chromosome 1 (data not shown), where there is only the TIGR rice pseudomolecule Os01g66100, which is also the *semi-dwarf1* (*sd1*) locus for plant height. The functional *Sd1* allele encodes GA20-ox2, an enzyme catalyzing the second-to-last step of GA biosynthesis (Ashikari *et al.* 2002, Monna *et al.* 2002, Spielmeyer *et al.* 2002). Sequencing genomic DNAs for the locus from SS18-2 (the weedy rice donor) and EM93-1 (the recipient of the isogenic line) identified 10 point mutations between the two alleles, including a 383-deletion in EM93-1 (Fig. 3). This deletion causes a truncated OsGA20-ox2 resulting a semi-dwarf phenotype and could also account for a functional mutation for the *qSD1-2* seed dormancy locus. This research will confirm the hypothesis.

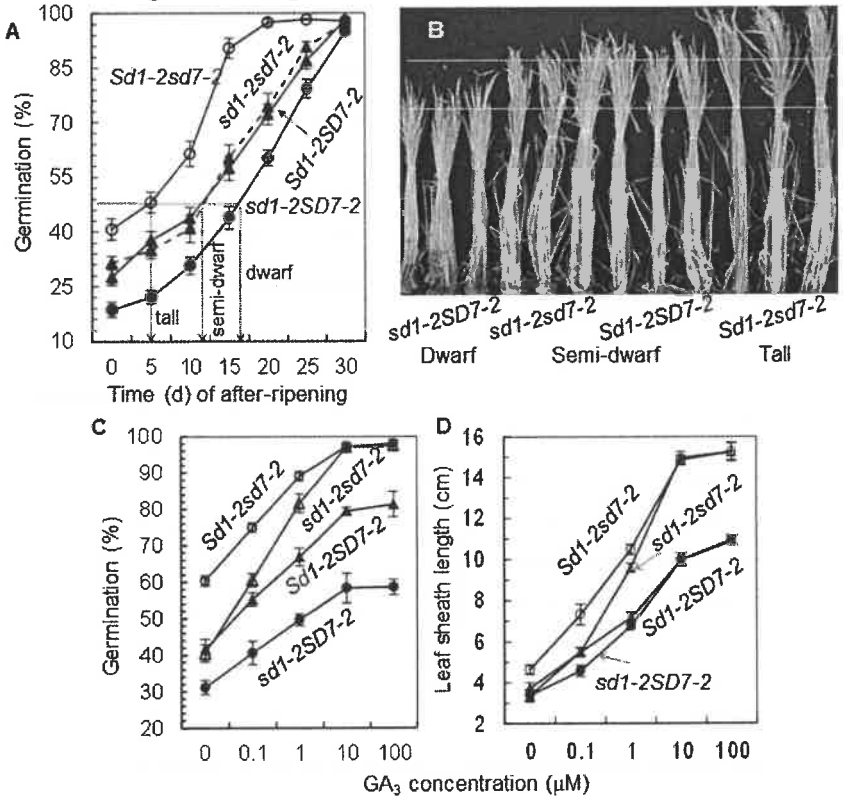

Fig. 2 Genotypic differences among four isogenic lines. **A.** Seed dormancy evaluated by germination. *SD1-2* & *SD7-2* are wild-type and *sd1-2* & *sd7-2* are mutant alleles at *qSD1-2* & *qSD7-2*. **B.** Plant height at maturation. **C.** Germination responses to GA3. **D.** Seedling growth responses to GA3. Seedlings of 7-d-old were grown in GA3 solutions for 15 d. Data shown are leaf sheath lengths of the second leaves. Original data were published in Ye et al. 2013.

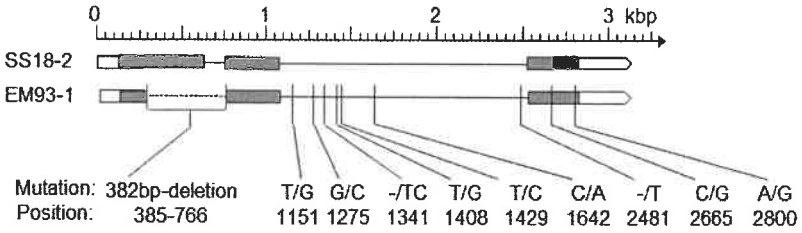

Fig. 3 Allelic difference in *Os01g66100* DNA sequence between the parental lines SS18-2 and EM93-1.

3.4.3 Fine mapping delimited *qSD7-2* to the genomic region containing LOC_Os07g36570:
About 4000 plants from a population segregating only for the *qSD7-2* region were genotyped to select rare recombinants to fine map the QTL. Progeny testing of the recombinants for seed dormancy delimited *qSD7-2* to the genomic segment of 21850-21870 kb on chromosome 7 (data not shown), where there is only the TIGR rice pseudomolecule Os07g36570 that encodes a putative protein kinase (Fig. 4). We screened our BAC library constructed for SS18-2 and sequenced Os07g36570 and its flanking regions from positive clones. We also amplified overlapped DNA segments covering Os07g36570 and its flanking regions from EM93-1 by long-PCR to sequence the mutant allele. Sequence alignment identified 45 (including 4 in the promoter) point mutations between the SS18-2 (SD7-2^D) and EM93-1 (SD7-2^d) alleles (Fig. 4A).

We sequenced full-length cDNAs from 5-d developing seeds. Alignment of the cDNA against the genomic DNA sequence for each of the two parents demonstrated that the *qSD7-2* candidate gene consists of 8 exons and 5′ and 3′ untranslated region (UTRs, Fig. 4A). Annotation of the SD7-2^D and SD7-2^d cDNAs using the Pfam protein families database (Finn *et al.* 2010) detected 4 putative domains, including the one with Ser-, Thr-, and Tyr-protein kinase functions (Fig. 4B). Interestingly, the 10 mutations in the exons do not change the predicted domains in the SD7-2^d allele, suggesting that the functional mutation may be not present in the coding sequence.

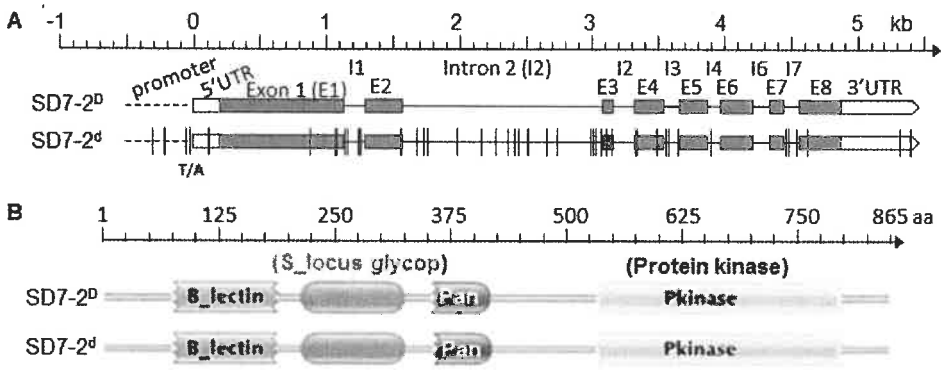

Fig. 4 The *qSD7-2* candidate gene *Os07g36570*. **A**. Gene structure. SD7-2^D and SD7-2^d represent gene alleles from SS18-2 and EM93-1, respectively. Genomic DNAs and cDNAs isolated from the parental lines were aligned to develop the gene model. Vertical bars indicate point mutations. **B**. Protein structure. Amino acid sequences were deduced based on the cDNAs and function domains (color-depicted bars) predicted using the Pfam protein families database (Finn et al. 2009).

We sequenced the promoter region and quantified the transcription level of *Os07g36570* for 21 lines of wild, weedy or cultivated rice and found out that a T/A transversion at -9 bp, coupled with a TA deletion in the TATA box at -27 bp, upstream the 5′UTR greatly repressed the gene expression (Fig. 5). This data suggest that natural mutants of *SD7-2* may arise from altered promoter sequence(s). We are conducting a promoter transcription efficiency analysis to prove the hypothesis.

Geographic distributions of these 21 lines suggest that the *SD7-2* mutant (dormancy-reducing) allele likely originated from South Asia. Of the 6 lines displaying little transcripts for *Os07g36570* (Fig. 5), TKN12-2 belongs to weedy rice from Nepal, three (Kasalath, N22 & Dular) are landraces from India, and two (WJX & EM93-1) are related and also have a pedigree from a Burma landrace. It is noted that EM93-1 was selected by the PI from an F_2 extremely early maturation plant of the cross between WJX and NJ11 (also shown in Fig. 5) (Gu et al. 2004b). Thus, the *SD7-2* mutant is likely limited in a relatively small area in South Asia and has not been widely used for breeding.

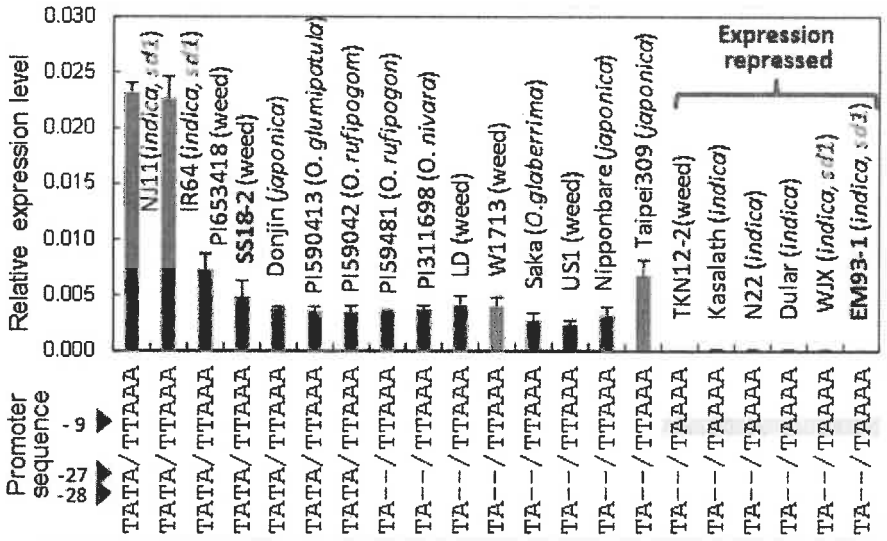

Fig. 5 Genotypic differences in *Os07g36570* promoter sequence & transcription. RNAs from 7-d-old seedlings were quantified by qRT-PCR and transcription data are means± SD of 3 replicates relative to the *Actin* control. NJ11 & WJX are the parents of EM93-1. Arrows point to a transversion or deletion (-) (counted from 5'UTR. The transversion (red color background) repressed the gene transcription.

4. Procedure

4.1 To confirm *qSD1-2* and *qSD7-2* candidate genes for seed dormancy function (Obj.#1)

Seed dormancy is controlled by multiple genes, with each contributing a relatively small effect on the phenotypic variation in germination capability. Thus, we are using to two (T-DNA insertion mutant & gene-overexpression analyses) to confirm functions of the candidate genes.

4.1.1 Mutant analysis: We have obtained T-DNA insertion mutants for *qSD1-2* and *qSD7-2*'s candidate genes from Crop Biotech Institute at Kyung Hee University, South Korea (Jeong et al. 2006). The genetic background of the T-DNA is the *japonica* cv. Donjin. The original T1 seeds were a few and were used to develop T3 lines by single-plant selection to purify the mutant lines.

The T-DNA insertion mutation line 3A-14727 contains a T-DNA insertion site in the 5' UTR of the Os01g66100 locus (*qSD1-2* or *OsGA20-ox2*) (Fig. 6).

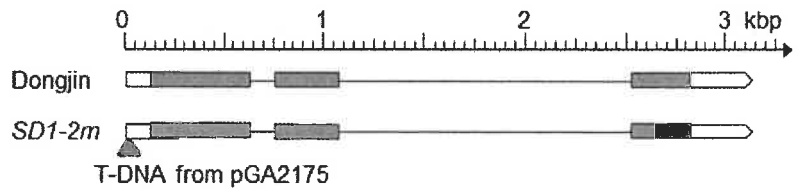

Fig. 6 Graphic representation for a T-DNA insertion mutation (3A-14727) of *Os01g66100* (refer to Fig. 3). Dongjin is the recipient of the T-DNA shown by a red triangle.

The T-DNA insertion lines 3A50043 and 3A13867 have a T-DNA insertion in intron 2 or exon 5 of *Os07g36570* on chromosome 7 (Fig. 7A), and 3A13867 has another insertion in the inter-genic region between *Os03g4200* and *Os03g42110* on chromosome 3 (Jeong et al. 2006). We verified T1 plants for the reported insertion sites by Southern blotting (Fig. 7B) (probed using a 600-bp sequence of the hygromycin resistance gene on the vector pGA2175) and TAIL-PCR analyses. A progeny line homozygous for the intron 2-insertion, designated *SD7-2m1*, was selected as one mutant event for *Os07g36570*. To eliminate the insertion on chromosome 3, 3A13867 was crossed with Donjin and an F_2 homozygous only for the exon 5-insertion, designated *SD7-2m2*, was selected as the other independent mutant event for *Os07g36570* (Fig. 7C). A quantitative real-time PCR (qRT-PCR) analysis detected the expression of *07g36570* in the recipient Donjin, but barely in *SD7-2m1* and *SD7-2m2* (Fig. 7D).

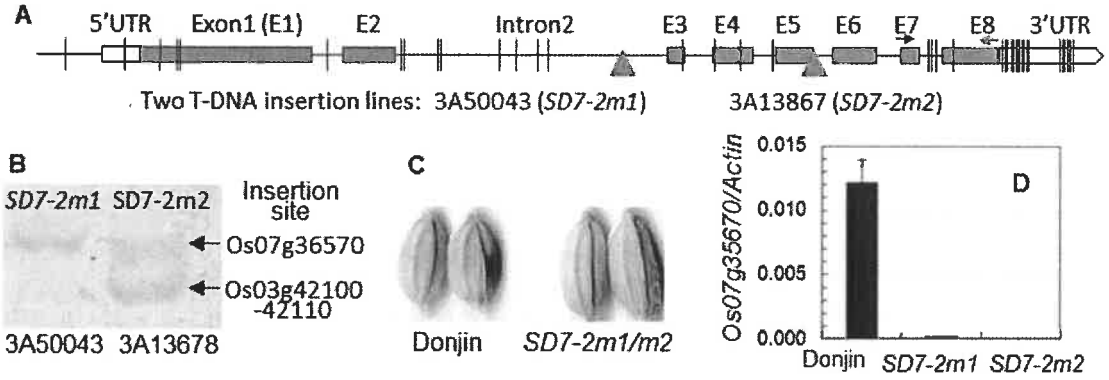

Fig. 7 Two T-DAN insertion mutations of *Os07g36570* in the genetic background of Dongjin. **A.** Graphical representation of the insertion sites. Vertical bars indicate point mutations relative to the SS18-2 allele (Fig. 4A) and triangles indicate 2 insertion sites in lines 3A50043 & 3A13867, respectively. **B.** Southern blotting analysis. The image shows that 3A50043 and 3A13678 have 1 and 2 insertions, respectively. **C.** Image of seed morphology. *SD7-2m1* and *SD7-2m2* are 2 mutant lines arisen from independent T-DNA insertions within *Os07g36570*. *SD7-2m2* was selected from an F_2 plant of the Donjin/3A13678 cross. **D.** Transcription levels of *Os07g36570* in Donjin, *SD7-2m1* and *SD7-2m2*. RNA samples from 14-d seedlings were quantified by qRT-PCR, PCR primers designed based on the exon-7 or -8 sequence indicated by the arrow headed lines in **A**, and the expression levels normalized to the *Actin* control.

The T purified -DNA insertion lines *SD1-2m*, *SD7-2m1* and *SD7-2m2*, together with the Dongjin recipient will be grown in a greenhouse to harvest seeds for seed dormancy assessment using the previously described methods (Ye et al. 2013). Briefly, seeds will be harvested at 40 d after flowering, air-dried in the greenhouse for 3 d, and then stored in a -20 °C freezer to maintain the dormant status. The degree of seed dormancy will be quantified by germination rate. Germination will be conducted for individual plants, each with three replicates, at 30 °C and 100% relative humidity (RH) in dark for 7 d.

We will also sample developing and germinating seeds from the *SD1-2m*, *SD7-2m1*, *SD7-2m2*, and Dongjin at different time points. First, the seed samples will be used to prepare RNAs to quantify for transcription levels of *Os01g66100* and *07g36570* by qRT-PCR. Second, since both *qSD1-2* and *qSD7-2* were associated with GA biosynthesis or signaling, the seed samples will also be evaluated for genotypic differences in hormone (GA, ABA & other growth regulators) profiles. The hormone test will be conducted at Plant Biotechnology Institute, Canada National Research Council. And the third, seed samples at 20, 30 and 40 days post anthesis will be evaluated for moisture content, dry mass, and germinability to determine physiological mechanisms of dormancy development regulated by *SD1-2*, *SD7-2* and their combination.

4.1.2 Gene over-expression analysis for *qSD7-2*'s candidate gene: The natural mutant of *qSD7-2* (EM93-1) is a change-of-functional mutation, as the functional polymorphism is predicted at the promoter sequence of *Os07g36570* (Fig. 5). Thus, we have developed transgenic lines with the coding sequence of *Os07g36570* driven by the overexpression promoter Ubi to provide an additional line of evidence to the function. The recipient of the transgene is the *japonica* cultivar Nipponbare and transgenic lines were developed at Plant Transformation Facility at Iowa State University. We have selected pure lines with a single copy of the transgene, as confirmed by Southern blotting analysis. We will the transgenic lines, together with the Nipponbare recipient, in a greenhouse to harvest seeds for seed dormancy assessment. *qSD7-2* is co-dominant for seed dormancy and plant height. Thus, transgenic effects will be evaluated by the phenotypic difference between the transgenic lines and the recipient Nipponbare.

Discussion: We have all required plant materials for the above-stated experiments. Expected outcomes include additional lines of evidence from the T-DNA insertion and transgenic analyses to prove that both *Os01g66100* (*SD1-2*) and *Os07g36570* (*SD7-2*) have an effect on seed dormancy. We may also provide information on how these two seed dormancy genes affect the GA/ABA balance in developing and germinating seeds.

4.2 To identify regulatory roles of the *SD1-2* and *SD7-2* genes in the development or release of seed dormancy (Obj. #2)

We developed marker-assisted genetic approaches (MAGA) to determine if a gene/QTL controls seed dormancy through the embryo, endosperm, or maternal tissues (Gu et al. 2008 & 2015). Different from the other molecular approaches (e.g., RNA *in situ* hybridization), MAGA is non-destructive, can be applied to a large sample of seeds, and is based on both genotypes and phenotypes (germinability) of individual seeds. We recently improved the method by genotyping the endosperm tissue of individual seeds. In an F_2 seed population, there are three genotypes of embryo (*DD*, *Dd* & *dd*, upper panel of Fig. 8A), which can be distinguished by the size and

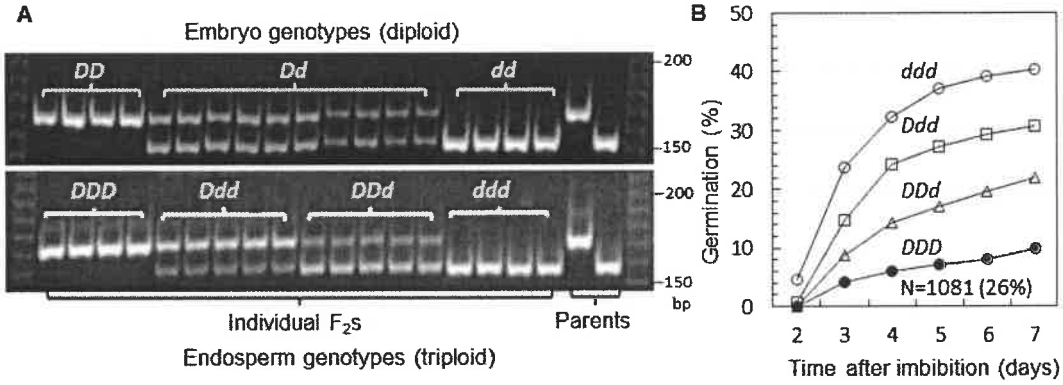

Fig. 8 Segregation patterns for endosperm genotypes. **A**. Gel images showing difference in a co-dominant marker between embryo and endosperm genotypes. A same set of seeds was used to prepare DNA samples from embryonic leaves or endosperm tissues. Note that the two alleles (bands) in the *DDd* & *Ddd* endosperms are unequal in signal intensity. **B**. Distributions of germination for four endospermic genotypes of seeds. Seeds (F_2) from the plant heterozygous for *SD1-2* were partially after-ripened prior to germination, and then cut the endosperm tissue from each of the germinated and non-germinated seeds. N was the total number of seeds used for the germination test; 26% was the germination rate. Letters *D* and *d* in the genotypes indicate dormancy-enhancing and -reducing alleles, respectively, at *SD1-2*. Note that the four genotypes of seeds differed in germination rate.

number of alleles for a co-dominant marker located within or tightly linked to the dormancy gene, and there are four genotypes of endosperm (*DDD*, *DDd, Ddd & ddd*, lower panel of Fig. 8A), which can be distinguished by the presence/absence of the two alternative alleles (for seeds different from each other for germination rate and the genotype with more dormancy-enhancing allele (*D*) was stronger in dormancy, or *DDD>DDd>Ddd>ddd* (Fig. 8B). Thus, we concluded that *SD1-2* is involved in endosperm-imposed dormancy (Gu et al. 2015).

We will use the same methods to determine the type of seed dormancy controlled by *SD7-2*. The F_2 seeds will be developed from a cross between *SD7-2* and *sd7-2* isogenic lines. Newly harvested seeds from the F_1 plants were partially after-ripened for a given period to generate a linkage disequilibrium in the germinated and the non-germinated seed subpopulations. After germination counting at 7 d, the endosperm tissue (~1/3 of a seed without the embryo) will be cut from individual germinated and non-germinated seeds to prepare DNA samples. The samples will be genotyped with a co-dominant marker located within the dormancy gene. The F_2 germinated and non-germinated seeds will be grouped based on endosperm genotypes to calculate their germination rates to confirm the germination patterns (Fig. 8B). However, if the F_2 seeds display only three germination patterns, with the *DDd* and *Ddd* heterozygotes similar in germination rate, the marked gene would control seed dormancy through the embryo tissue, such as *qSD12* (Gu et al. 2008 & 2015). Or, if all the four genotypes are similar in germination rate, the gene would regulate seed dormancy through the maternal tissue(s), such as *qSD7-1* (Gu et al. 2008 & 2015).

Since *SD1-2* and *SD7-2* were associated with GA biosynthesis and signaling, respectively, it is important to know if these two loci interact with each other to regulate the development or release of seed dormancy. To test this hypothesis, we will develop hybrid F_1 plants for both *SD1-2* and *SD7-2* loci. The resulting F_2 seed samples of a few thousands will be genotyped for the endosperm tissue with co-dominant markers for *SD1-2* and *SD7-2*. Data analysis will be conducted using the model presented in Gu et al. 2015.

Discussion: Expected outcomes from the above experiments include new information on types of seed dormancy regulated by *SD1-2* and *SD7-2* in terms of seed component tissues. *SD1-2* and *SD7-2* are annotated as GA biosynthesis and signaling genes, respectively. It is known that the endosperm aleuron cells play a central role in GA signaling for germination of cereal seeds. Our data will show if the GA signaling also contribute to natural variation in seed dormancy.

4.3 To characterize the SD7-2 protein for kinase activity and interacting factors in the GA signaling pathway (Obj. #3)

Os07g36570 is predicted to encode putative KI domain interacting kinase 1 (Fig. 4B). The following experiments are being or will be conducted to determine if the gene product SD7-2 has a kinase activity and to identify SD7-2's interacting factors or phosphorylation substrate(s).

4.3.1 Protein kinase activity assay: We are using a non-radioactive kinase assay to determine SD7-2's kinase activity. A DNA segment corresponding to the predicted protein kinase domain was isolated from the *SD7-2* allele of SS18-2 (Fig. 4B). This segment was ligated into pET29a$^+$ vector. The resulting construct was used to transform Rosetta-gami2(DE3)pLysS strain (EMD Millipore Co.) using the method in Elinarson *et al.* (2007) to express the kinase domain. A purified recombinant-expressed SD7-2 fragment is shown in Fig. 9A. We used the PepTag Assay to determine SD7-2's kinase activity. In this system, the substrate PepTagR C1 Peptide was incubated with the kinase domain (Fig. 9A) to show a phosphorylation reaction. As shown in Fig. 9B, the SD7-2 fragment has a function to phosphorylate the commercial peptide.

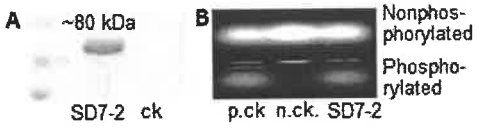

Fig. 9 Detection of SD7-2 kinase activity. A. Gel image showing a segment of the SD7-2 kinase domain. B. Gel image showing phosphorylation activity of the kinase domain. p./n. ck: positive/negative control.

In the next step, we will ligate the *Os07g36570* full-length cDNA into pET32c vector to express SD7-2 in an *E. coli* system. The expressed protein and the non-radioactive protein kinase detection system will be used to assay SD7-2's kinase activity.

4.3.2 Screening of SD7-2's interacting factors: We used a yeast-two-hybrid (Y2H) system to screen for SD7-2's candidate interacting factors. A cDNA library constructed using a RNA sample bulked from 5-, 7- & 10-d developing seeds and the yeast strain Y187 consisted of >1.3 million clones. The *SD7-2* full-length cDNA was ligated into the pGBKT7 vector and fused with the transcriptional binding domain, and the construct used to transform the yeast Y2H-Gold strain to develop a SD7-2 bait strain. The bait strain was mated with the seed cDNA library (prey) to screen for potential interacting factors. The positive clones selected as candidate interacting genes include the DELLA protein coding gene *Slr1*, which is the core element of GA signaling in rice (Sasaki *et al.* 2003, Itoh *et al.* 2005, Dai & Xue 2010). We are using *in-vitro* phosphorylation assay to test interactions between SD7-2 and SLR1 and other candidate interacting factors. The candidate genes will be expressed in *E.coli* or in the eukaryotic system *Pichia pastoris*. Purified proteins will be used as substrates for phosphorylation by SD7-2 using the non-radioactive ProQ diamond detection kit (Taylor et al. 2013). ^{32}P-labeled ATP could be used to improve the accuracy and sensitivity of phosphorylation assessment (Xie et al. 2003).

4.3.3 Analysis of effects of SD7-2 on stability of SLR1 in-vivo. The DELLA protein SLR1 negatively regulates GA responses (Fig. 1). We hypothesize that SD7-2 phosphorylates the SLR1 protein to maintain its stability during the signal transduction. The following experiments are designed to prove the hypothesis. The antibody against the SLR1 protein will be prepared by COSMO BIO USA, INC. Isogenic lines for the dormancy-enhancing ($IL_{SD7\text{-}2}^{D}$) and –reducing ($IL_{SD7\text{-}2}^{d}$) alleles will be planted to harvest new seeds to prepare protein samples. Seed samples will be treated with 10-μM GA3 for 0, 30, 60, 120 and 240-min to collect imbibed seeds and newly germinated seedlings. Total proteins will be extracted from the samples and the protein concentration normalized base on intensity of protein bands. Quantitative western-blotting will be performed for the samples to detect SLR1. The stability of SLR1 protein in the isogenic lines will be quantified based on intensities of the western-blotting signaling. It is expected that SLR1 N-terminus is responsible for GA dependent ubiquitin-degradation and the phosphorylation of SLR1 by SD7-2 may occur in N-terminus to increase the stability of the DELLA protein in GA signaling. Therefore, upon GA treatment, the intensity of quantitative western-blotting for SLR1 should be higher in $IL_{SD7\text{-}2}^{D}$ than in $IL_{SD7\text{-}2}^{d}$.

Discussion: We believe that *SD7-2* is a new, uncharacterized gene involved in GA signaling to regulate the natural variation in seed dormancy. Expected outcomes from the above experiments include information on SD7-2's kinase activity and a list of confirmed and candidate subtracts. Characterization of a new protein kinase gene involves a series of biochemical experiments. The PI is collaborating with Dr. Shuqun Zhang, a professor of biochemistry (an expert in plant protein kinase) at University of Missouri Columbia, to work on the *SD7-2* seed dormancy gene. We will also conduct experiments to elucidate molecular mechanisms for interactions between SD7-2 and key GA signaling elements. These include: 1) to determine SD7-2's effects on the stability of SLR1 *in-vivo* upon GA treatments using the *Actin* promoter::HA-SLR1-GFP

transgenic system (Itoh et al. 2002) and the SD7-2 T-DNA insertion lines; 2) to identify phosphorylation sites in the rice DELLA protein SLR1 by SD7-2 using PCR-directed mutagenesis and *in-vitro* kinase assay; and 3) to identify upstream transcription or regulatory factors of *SD7-2* using Y1H screening.

The PI understands that the ASRF funding will help us develop new data to compete for NSF and USDA-NIFA/AFRI grants to advance the fundamental research on seed dormancy.

4 Budget and budget justification

We request a total of $104860 from ASRF to conduct this proposed research for 3 years from 9/1/2015 to 8/31/2018. The requested funding does not include indirect costs, which will be contributed by South Dakota State University.

Table 1. Summary of budgets for three years.

Item	Year 1 (9/1/15-8/31/16)	Year 2 (9/1/16-8/31/17)	Year 3 (9/1/17-8/31/18)	Subtotal
GRA's salary ($)	24095	24818	25562	74475
GRA's benefit ($)	482	496	511	1490
Tuition remission ($)	3266	3364	3465	10095
Domestic travel ($)	1100	-	1100	2200
Publication ($)	-	1300	1300	2600
Greenhouse rent ($)	1000	1000		2000
Materials & supplies ($)	5000	4000	3000	12000
Total ($)	34943	34978	34939	104860

As shown in Table 1, about 70% of the fund is requested to support a graduate research assistant (GRA) who will work on this project for his/her dissertation leading to a Ph.D. A doctoral GRA's salary at SDSU will be $24,095/year for physic year 2016 and increase by 3% for the next two years. Benefit for the GRA will be 2% of the salary. GRA's tuition remission will be $3266/year for the first year and increase by 3% for the next two years.

The travel fund of $2200 is requested for PI or GRA to attain one academic meeting closely related to the project.

The publication fund is requested to defray the cost of 2 papers (about $1300/paper) developed based on data from this research.

The fund of $1000 per year is requested to rent greenhouse benches at the rate $0.5/ft^2/month to plant segregating populations and advanced generations of T-DNA insertion and transgenic lines for this project in the first two years.

The fund of $5000, 4000, and 3000 is requested to purchase materials and supplies for various experiments of this research in the first, second and third year. The coats include: 1) regular reagents for DNA extraction, regular and real-time PCR (Taq polymerase, dNTP etc.), electrophoresis and imaging for marker genotyping ($2000 for year 1 and ~$1000 for years 2 & 3); 2) the other molecular biology experiments, such as phosphorylation, hormone testing, sequencing (about $1000/year); 3) plastic consumables (tips, tubers & plates) for molecular

experiments (about $1000/year); 4) supplies for seed harvesting, storage and germination (Petri dishes & filter papers) ($500/year); and 5) lab equipment/instrument (PCR machines, electrophoresis instruments, pipettes) maintenance (about $500/year).

References

(* indicates the work was done by the PI's group)

Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M. 2003. Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 164:711-729.

Anderson JA, Sorrells ME, Tanksley SD. 1993. RFLP analysis of genomic regions associated with resistance to pre-harvest sprouting in wheat. Crop Sci 33:453-459.

Argyris J, Truco MJ, Ochoa O, McHale L, Dahal P, Van Deynze A, Michelmore RW, Bradford KJ. 2011. A gene encoding an abscisic acid biosynthetic enzyme (LsNCED4) collocates with the high temperature germination locus *Htg6.1* in lettuce (*Lactuca sp.*). Theor Appl Genet 122:95-108.

Ariizumi T, Hauvermale AL, Nelson SK, Hanada A, Yamaguchi S, Steber CM. 2013. Lifting della repression of Arabidopsis seed germination by nonproteolytic gibberellin signaling. Plant Physiol 162:2125-2139.

Ashikari, M., A. Sasaki, M. Ueguchi-Tanaka, H. Itoh, A. Nishimura et al. 2002. Loss-of-function of a rice gibberellin biosynthetic gene, *GA20oxidase* (*GA20ox-2*), led to the rice 'green revolution'. Breed Sci 52:143-150.

Bentsink L, Jowett J, Hanhart CJ, Koornneef M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc Natl Acad Sci USA 103:17042-17047.

Bewley JD, Bradford K, Hilhorst H, Nonogaki H. 2013. Seeds: physiology of development and germination (3rd ed). Springer, New York.

Blaker K. M., Chaparro J. X., Beckman T. G. 2013. Identification of QTLs controlling seed dormancy in peach (*Prunus persica*). Tree Genetics & Genomes 9:659-668.

Dai C, Xue HW. 2010. Rice early flowering1, a CKI, phosphorylates DELLA protein SLR1 to negatively regulate gibberellin signaling. EMBO J 29:1916–1927.

Durantini D, Giulini A, Malgioglio A, Pilu R, Tuberosa R, Sanguineti C, Gavazzi G. 2008. Vivipary as a tool to analyze late embryogenic events in maize. Heredity 101:465-470.

Einarson MB, Pugacheva EN, Orlinick JR. 2007. Preparation of GST fusion proteins. CSH Protoc. 2007:pdb.prot4738.

*Feng, J, Ye H, Srivastava V, Gu X-Y. 2014. Evolutionary and developmental mechanisms of seed dormancy revealed by map-based cloning of genes underlying a major quantitative trait locus from weedy rice. *The 35th Rice Technical Working Group Meeting*, New Orleans, LA., Feb. 18-21, 2014.

Fennimore SA, Nyquist WE, Shaner GE, Doerge RW, Foley ME. 1999. A genetic model and molecular markers for wild oat (*Avena fatua* L.) seed dormancy. Theor Appl Genet 99:711-718.

Foley M.E. 2002. Weeds, seeds and buds: opportunities and systems for dormancy investigations. Weed Sci. 50:267-272.

Fujino K, Sekiguchi H, Matsuda Y, Sugimoto K, Ono K, Yano M. 2008. Molecular identification of a major quantitative trait locus, *qLTG3-1*, controlling low-temperature germinability in rice. Proc Natl Acad Sci USA105:12623-12628.

Gandhi SD, Heesacker AF, Freeman CA, Argyris J, Bradford K, Knapp SJ. 2005. The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. Theor Appl Genet 111:619-629.

Gao XH, Xiao SL, Yao QF, Wang YJ, Fu XD. 2011. An updated GA signaling 'relief of repression' regulatory model. Mol Plant 4:601-606.

*Gu X-Y, Kianian SF, Foley ME. 2004a. Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (*Oryza sativa*). Genetics 166:1503-1516.

*Gu X-Y, Foley ME, Chen Z. 2004b. A set of three genes regulates photoperiodic response in rice (*Oryza sativa*). Genetica 122:127-140.

*Gu X-Y, Turnipseed EB, Foley ME. 2008. The *qSD12* locus controls offspring tissue-imposed seed dormancy in rice. Genetics 179:2263-2273.

*Gu X-Y, Foley ME, Horvath DP, Anderson JV, Feng J, Zhang L, Mowry CR, Ye H, Suttle JC, Kadowaki K, Chen Z. 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 189:1515-24.

*Gu X-Y, Zhang J, Ye H, Zhang L, Feng J. 2015. Genotyping endosperms to determine genes regulating seed dormancy through the embryo, endosperm or maternal tissues in rice. G3: Genes, Genomics, Genetics 5:183-193.

Huo H, Dahal P, Kunusoth K, McCallum CM, Bradford KJ. 2013. Expression of 9-cis-EPOXYCAROTENOID DIOXYGENASE4 is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. Plant Cell 25:884-900.

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J. 2001. *slender* rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/RHT/D8*. Plant Cell 13:999-1010.

Itoh H, Sasaki A, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Hasegawa Y, Minami E, Ashikari M, Matsuoka M. 2005. Dissection of the phosphorylation of rice DELLA protein, SLENDER RICE1. Plant Cell Physiol 46:1392-1399.

Jeong D-H, An S, Park S, Kang H-G, Park G-G, Kim S-R, Sim J, Kim Y-O, Kim M-K, Kim S-R, Kim J, Shin M, Jung M, An G. 2006. Generation of flanking sequence-tag database for activation-tagging lines in *japonica* rice. Plant J 45:123-132.

Li C, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R, Appels R. 2004. Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. Funct Integr Genomics 4:84-93.

Lijavetzky D, Carolina Martínez M, Carrari F, Esteban Hopp H. 2000. QTL analysis and mapping of pre-harvest sprouting resistance in sorghum. Euphytica 112:125-135.

Lin SY, Sasaki T, Yano M. 1998. Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor Appl Genet 96:997-1003.

Liu S, Sehgal SK, Li J, Lin M, Trick HN, Yu J, Gill BS, Bai G. 2013. Cloning and characterization of a critical regulator for preharvest sprouting in wheat. Genetics 195:263-273.

Masojć P, Banek-Tabor A, Milczarski O, Twardowska M. 2007. QTLs for resistance to preharvest sprouting in rye (*Secale cereale* L.). J Applied Genetics 48:211-217.

McCarty DR, Carson CB, Stinard PS, Robertson DS. 1989. Molecular analysis of *viviparous-1*: an abscisic acid-insensitive mutant of maize. Plant Cell 1:523-532.

Monna, L., N. Kitazawa, R. Yoshino, J. Suzuki, H. Masuda *et al.*, 2002 Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Res 9:11-17.

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H. 2011. A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. Plant Cell 23:3215-3229.

Nonogaki H, Chen F, Bradford KJ. 2007. Mechanisms and genes involved in germination *sensu stricto*. *In*: Bradford KJ & Monogaki H (ed.) Seed Development, Dormancy and Germination. Blackwell Publishing, Oxford, UK. pp. 264-304.

Penfield S, Gilday AD, Halliday KJ, Graham IA. 2006. DELLA-mediated cotyledon expansion breaks coat-imposed seed dormancy. Current Biology 16:2366-2370.

Ringlund K. 1993. The importance of pre-harvest sprouting research. *In*: M. K. Walker-Simmons and J. L. Ried (eds), Pre-harvest Sprouting in Cereals 1992, pp. 3-7. American Association of Cereal Chemists, St. Paul, MN, USA.

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M. 2002. Green revolution: a mutant gibberellin-synthesis gene in rice. Nature 416:701-702.

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong D-H, An G, Kitano H, Ashikari M, Matsuoka M. 2003. Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299:1896-1898.

Schatzki J, Schoo B, Ecke W, Herrfurth C, Feussner I, Becker HC, Möllers C. 2013. Mapping of QTL for seed dormancy in a winter oilseed rape doubled haploid population. Theor Appl Genet 126:2405-2415.

Shimada A, Ueguchi-Tanaka M, Sakamoto T, Fujioka S, Takatsuto S, Yoshida S, Sazuka T, Ashikari M, Matsuoka M. 2006. The rice *SPINDLY* gene functions as a negative regulator of gibberellin

signaling by controlling the suppressive function of the DELLA protein, SLR1, and modulating brassinosteroid synthesis. Plant J 48:390-402.

Silverstone AL, Tseng TS, Swa in SM, Dill A, Jeong SY, Olszewski NE, Sun TP. 2007. Functional analysis of SPINDLY in gibberellin signaling in Arabidopsis. Plant Physiol 143:987-1000.

Simpson G.M. 1990. Seed Dormancy in Grasses. Cambridge University Press. Cambridge, England.

Spielmeyer, W., M. H. Ellis, and P. M. Chandler, 2002 Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci USA 99:9043-9048.

Steber CM. 2007. De-repression of seed germination by GA signaling, pp. 248-263 In: *Seed Development, Dormancy and Germination*. Edited by K. Bradford and H. Nonogaki. Blackwell Publishing, London, UK.

Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y, Hattori T, Yano M. 2010. Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. Proc Natl Acad Sci USA 107:5792-5797.

Sun T. 2010. Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development, Plant Physiol 154:567-570.

Sun TP, Gubler F. 2004. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol 55:197-223.

Taylor I, Seitz K, Bennewitz S, Walker JC. 2013. A simple in vitro method to measure autophosphorylation of protein kinases. Plant Methods 9:22.

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T-Y, Hsing Y-I, Kitano H, Yamaguchi I, Matsuoka M. 2005. *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. Nature 437:693-698.

Ullrich SE, Hayes PM, Dyer WE, Blake TK, Clancy JA. 1993. Quantitative trait locus analysis of seed dormancy in 'Steptoe' barley. *In:* Walker-Simmons, M.K. and J. L. Ried (eds), Pre-harvest Sprouting in Cereals, pp. 136-145. American Association of Cereal Chemists, St. Paul, MN.

Voegele A, Linkies A, Muller K, Leubner-Metzger G. 2011.Members of the gibberellin receptor gene family GID1 (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during *Lepidium sativum* and *Arabidopsis thaliana* seed germination. J Exp Bot 62:5131-5147.

Xie C, Zhang JS, Zhou HL, Li J, Zhang ZG, Wang DW, Chen SY. 2003. Serine/threonine kinase activity in the putative histidine kinase-like ethylene receptor NTHK1 from tobacco.Plant J 33:385-393.

*Ye H, Foley ME, Gu X-Y. 2010. New seed dormancy loci detected from weedy rice-derived advanced populations with major QTL alleles removed from the background. Plant Sci 179:612-619.

*Ye, H, Beighley DH, Feng J, Gu **X-Y.** 2013. Genetic and physiological characterization of two clusters of quantitative trait loci associated with seed dormancy and plant height in rice. G3: Genes, Genomes, Genetics 3:323-331.