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ARTICLE

Phenolics in the Seed Coat of Wild Soybean (*Glycine soja*) and Their Significance for Seed Hardness and Seed Germination

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Hardseededness in annual wild soybean (*Glycine soja* Sieb. Et Zucc.) is a valuable trait that affects the germination, viability, and quality of stored seeds. Two *G. soja* ecotypes native to Shandong Province of China have been used to identify the phenolics in the seed coat that correlate with the seed hardness and seed germination. Three major phenolics from the seed coat were isolated and identified as epicatechin, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside. Of the three phenolics, only the change of epicatechin exhibited a significant positive correlation with the change of hard seed percentages both under different water conditions during seed development and under different gas conditions during seed storage. Epicatechin also reveals a hormesis-like effect on the seed germination of *G. soja*. Epicatechin is suggested to be functionally related to coat-imposed hardseededness in *G. soja*.

KEYWORDS: Annual wild soybean; phenolics; hardseededness

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the world's most important crops. It is important not only as the world's largest oil and protein food source but also as a potential renewable supplemental energy source in the face of today's exacerbating energy crisis (*1*). The modern cultivated soybean was domesticated from its annual wild relative *Glycine soja* Sieb. et Zucc. in China (*2*). The annual wild soybean is still widespread in China today, which is considered as a potential gene resource for future soybean improvement in coping with world climate change for food security (*3*). One of the potentially useful traits of the wild soybean is the presence of hard seeds, which is a type of seed dormancy, known as "hardseededness", commonly occurring in legume species (*4*). Hardseededness is an adaptive character for the annual plants to survive long periods in wild adverse environments that can be attributed to natural selection during evolution. Hardseededness in soybeans can protect seeds against deterioration and make seeds maintain high quality and viability through lengthy storage (*5*); therefore, it is valuable for food economy in general and for germplasm preservation in particular.

Unfortunately, hardseededness is usually considered as an undesirable characteristic, as the high level of resistance to deterioration is accompanied by resistance to germination. This trait is also undesirable if seeds are to be consumed. Probably, just because it is considered undesirable for agricultural cultivation and food processing (*6*), hardseededness in cultivated soybeans has been greatly reduced through artificial selection and, as a result, storage tolerance of cultivated soybeans has largely degenerated (*5*). Reduction of soybean seed quality during storage leads to enormous economic losses each year around the world (*7*).

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In addition, seeds are fundamentally important to people, not only because they provide an important food but also because they constitute the chief method of plant propagation. Seeds of many species lose viability after short periods of storage, making their species prone to extinction and causing extensive losses. Thus, ex situ preservation with hard seeds is considered as a preferred strategy for their germplasm preservation (*8*). Whereas domesticated soybeans are generally soft-seeded, hardseededness is well preserved in wild soybeans by natural selection (*9*). Recognizing the value of hardseededness in storage tolerance and germplasm preservation in soybean, some researchers suggested that it could be reintroduced into domesticated soybeans via crossbreeding and other genetic technologies (*10*) and create lines with seed coats that are fairly permeable and reasonably strong. Hence, hardseededness is important to both scientific and industrial communities.

Hard seeds are also known as "stone" or "impermeable" seeds. Whether a seed is impermeable (hard) or permeable (soft) is determined by the seed coat (*11*), different with physiological dormancy of certain species where the embryo is controlled by phytohormones such as abscissic acid and gibberellic acid (*12*). Therefore, hardseededness was also termed as coat-imposed dormancy, or physical dormancy (*4*), where the seed coat inhibits germination by its impermeability to water or/and oxygen. The barrier to permeation of water and/or oxygen in the hard seeds should be two-fold (*13*). One part of the barrier is due to the continuous very hard multiple layer of seed coat structures. The second part of the barrier is due to the presence of chemicals in a continuous layer of cells in the seed coat. It has been proposed that the presence of phenolic compounds is particularly important for seed coat impermeability (*8*, *13*, *14*).

Understanding the chemical basis for hard seed impermeability is an essential first step in developing strategies to utilize such

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seeds in breeding programs and to plan suitable genetic modifications. In the present study, we attempted to examine the chemicals in the seed coat of wild soybean with an emphasis on the phenolic compounds and to identify the phenolics that correlate with the seed hardness and seed germination.

MATERIALS AND METHODS

Seed and Site Characteristics. Seeds of two *G. soja* ecotypes were collected from natural populations at two different sites in Shandong province of China. The two ecotypes were named DS (Dongying and saline soil) and QN (Qingdao and nonsaline soil), respectively. DS ecotype was growing in drought saline habitats and presented a little smaller but relatively wider range of seed size, whereas QN ecotype was growing in rainy nonsaline habitats and presented a little bigger but relatively narrower range of seed size.

Cultivation Experiments. Seeds of two *G. soja* ecotypes collected in 2004 were scarified and sown in big pots (50 cm in diameter and 60 cm in depth) filled with garden soil during the normal growing seasons, and seedlings were grown under a plastic rain shelter, at Medicinal Plant Garden of Qingdao University, Shandong province, China. A total of 200 replicate pots per ecotype, with 5 plants per pot, was maintained. All of the plants were equally watered twice a week prior to drought treatment. The full bloom stage of both ecotypes occurred in mid-August, whereas seed ripened stage was q5 the end of September. Drought stress was induced for treated plants by withholding irrigation beginning at full bloom stage; control plants were continuously maintained under well-watered conditions until seed harvest. After the drought treatment period, on a separate set of treated and nontreated (control) plants, seeds were harvested at physiological maturity 49 days after the imposition of water stress.

Seed Storage Experiments. Seeds of two *G. soja* ecotypes collected in 1999 were stored in paper bags under laboratory conditions (relative humidity 35−45% at 18−25 °C) and used in the experiment of long-term monitoring of hard seeds percentages and seeds germination percentages. Seeds collected in 2004 were packed in big polyvinyl chloride bags filled with air, pure oxygen, and pure nitrogen, respectively, and used in the experiment of determining of storage effect on chemical constituents in seed coats.

The gas filling method was as follows: Air from the bags was removed with a syringe needle attached to a vacuum system, and then the required gas, N_2, O_2, or air, was used to fill bags through the same needle. The procedure was repeated three times to ensure the removal of air traces from the bags, and this was immediately followed by sealing of the access flush point. The relative humidity in all of the bags was controlled at 40%. The packs were placed in plastic storage boxes and stored at laboratory temperature (18−25 °C). The PVC sheets were impermeable to the gases used in the experiment.

Extraction, Isolation, and Identification. The seed coats were dissected manually from seed and frozen in liquid nitrogen. Freeze-dried seed coats were ground to a fine powder. The powders were extracted three times with 50% acidified methanol (containing 1% HCl) by 50 min of sonication in an ultrasonic bath at room temperature. The ratio of solvent to seed coats was 20 mL to 1 g of pure seed coat powder. The crude extracts were centrifuged and filtered to remove the impurities.

The methanol/water crude extract was washed three times with equal volumes of *n*-hexane to remove liposoluble substances, and the remaining fraction was further extracted three times with equal volumes of ethyl acetate. The ethyl acetate fraction was chromatographed in a column of 80 cm × 3 cm i.d. silica gel 60 (Merck, Darmstadt, Germany) and eluted with a gradient of CH_2Cl_2 and MeOH (0→100% MeOH). Five fractions were collected and combined according to their thin-layer chromatography analysis, and the largest fraction was repurified in a column of 20 cm × 1 cm i.d. silica gel 60 (Merck) with CH_2Cl_2/MeOH (9:1) as an isocratic solvent system. After the pure fraction eluted from silica gel column was concentrated and then dried, compound **1** in yellowish needlelike crystals was finally obtained and used for identification.

The methanol/water-soluble fraction was chromatographed in a column of 70 cm × 3 cm i.d. Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and eluted with a gradient of 1% HCl and MeOH (0→30% MeOH). Two red pigments containing fractions were isolated from the Sephadex LH-20 column chromatography. Each fraction was further purified in a column of 30 cm × 3 cm i.d., 50 μm, Lichroprep RP-18 (Merck) with a gradient of 1% hydrochloric acid and MeOH (0→40% MeOH). After concentration and drying, compounds **2** and **3** in amorphous red powders were finally obtained and used for identification.

The three purified compounds above-mentioned were dissolved in 1 mL of 0.1% DCl/CD_3OD and transferred into a 3 mm NMR tube. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL Eclipse 600 spectrometer (JEOL, Tokyo, Japan), and chemical shifts were given on a δ (ppm) scale with TMS as an internal standard (see the Supporting Information).

Phenolics Analysis by HPLC. The fine seed coat powders (0.1 g) were extracted three times with 3 mL of 1% HCl/60% CH_3OH at room temperature. The combined extracts were made up to 10 mL. After centrifuging and filtering through a 0.45 μm membrane filter, 10 μL samples were injected into the HPLC column. HPLC was conducted using a Shimadzu chromatographic system (Shimadzu, Tokyo, Japan). The column used was a 250 mm × 4.6 mm i.d., 5 μm, Nucleosil 100-5 RP-18, with a 4 mm × 4 mm i.d. guard column of the same material (Macherey-Nagel, Duren, Germany). The mobile phase was composed of 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). The gradient conditions were as follows: 10 min, 15% B; 20 min, 25% B; 30 min, 40% B; and then holding for 10 min before returning to the initial conditions. The flow rate was 0.8 mL/min. Epicatechins were detected at 280 nm and anthocyanins at 520 nm. The column oven temperature was set to 25 °C. The contents were calculated by HPLC comparison with calibration curve for purified compound. For each ecotype five different seed samples were analyzed.

Imbibition and Germination Assay. To test the seed longevity, seeds collected in 1999 were taken out of storage on a fixed date yearly for imbibition and germination test with intact and scarified seeds. Scarification was performed by removing carefully a small portion of seed coat by rubbing each seed with a small grater. Three batches of 50 seeds each were placed on two layers of filter paper in a Petri dish (9 cm in diameter) containing 12 mL of distilled water and incubated in dark growth chambers at 25 °C for 7 days. The numbers of imbibed and germinated seeds were counted at the same time daily. Seeds were considered as imbibed when the seed size had increased to at least 2 times their original size and as germinated when the radicles had protruded through the seed coats. Seeds that did not imbibe after 7 days were regarded as hard seeds. Percentages of imbibed seeds, hard seeds, and germinated seeds were calculated.

To test the effects of coat chemicals on seed germination, each isolated phenolic was dissolved in water at a series of concentrations and applied to four sheets of filter paper in a 9 cm Petri dish and dried, respectively, and then the filter paper was replaced in the Petri dish and moistened with 12 mL of water, resulting in final concentrations of 0 (control), 1, 5, 10, 50, and 100 mM. Thirty scarified seeds were placed on two of the filter papers and covered with the other two in a Petri dish. The incubations were conducted in dark growth chambers set at 25 ± 1 °C. Twenty-one replicate Petri dishes were set for each concentration. Scoring was conducted daily until the seventh day. Three replicates of each concentration were scored once to avoid interference of air entering on seed germination. The final germination percentage and the germination index were calculated.

Germination or imbibition percentage was calculated as [(number of germinated or imbibition seeds)/number of sampled seeds] × 100. Hard seed percentage = 1 − imbibition percentage. Germination index was calculated using the formula

$$\frac{7n_1 + 6n_2 + 5n_3 + 4n_4 + 3n_5 + 2n_6 + 1n_7}{\text{total days of test} \times \text{total grains}}$$

where n_1, n_2, ..., n_7 are the numbers of seeds that germinated on the first, second, and subsequent days until the seventh day, respectively.

Statistical Analysis. All experiments were done at least in triplicate, and data were analyzed using the software SPSS 10.0 for Windows. The significance analysis of differences was performed by *t* test at the 95% significance level ($P < 0.05$). Correlation analysis was performed to determine the correlation coefficient at a 95% significance level ($P < 0.05$). The statistical significance of the results is indicated in the text.

RESULTS AND DISCUSSION

Changes of Hardness and Germination of *G. soja* Seeds with Storage Time. **Figure 1** illustrates the changes in hard seed percentage and germination percentage of intact seeds from

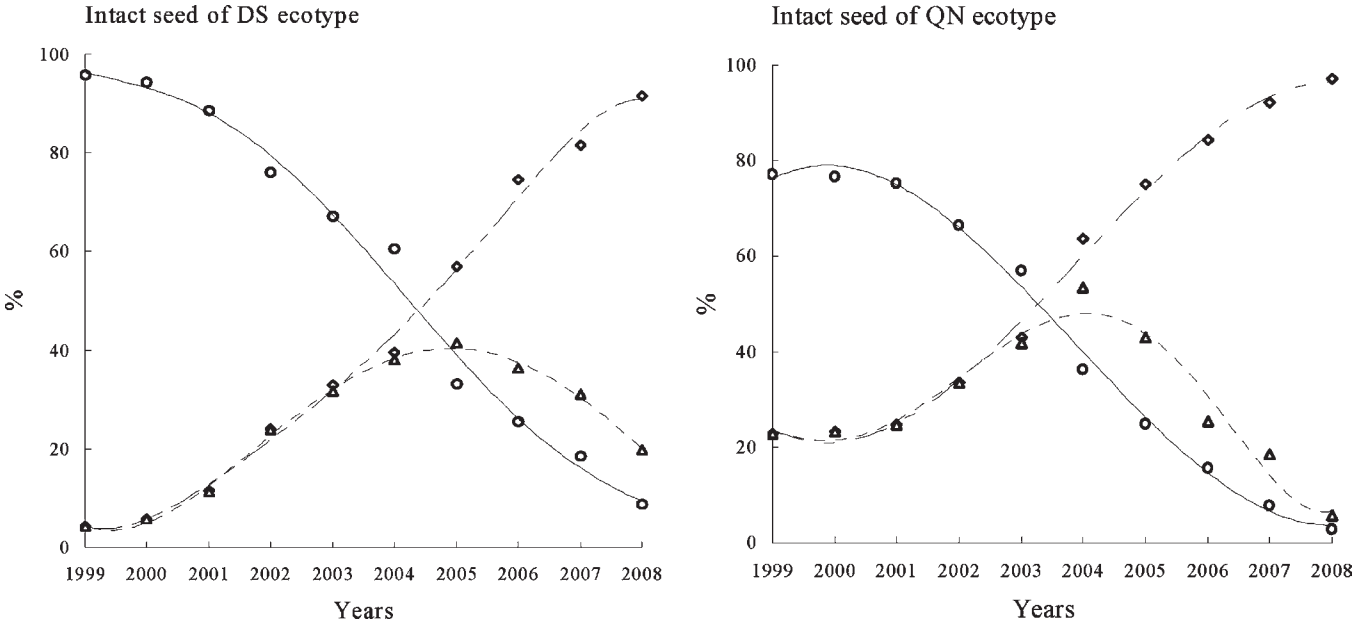

Figure 1. Comparison of the percentages of imbibition (◇), hardness (○), and germination (△) of intact seeds during storage between DS ecotype and QN ecotype of *G. soja*.

two *G. soja* ecotypes (DS and QN) during 10 years of storage. Results indicated that there was a large proportion of hard seeds in *G. soja* when the seeds were fresh, but the hard seed percentages of fresh seeds were different between the two ecotypes; DS from a dry climate was 96%, whereas QN from a rainy climate was 77%. Results also indicated that the hard seed percentage of *G. soja* decreased gradually year by year during seed storage (i.e., imbibition percentage increased gradually year by year), but the decreasing curves of hard seed percentages were different between DS and QN; the DS varied from 96 to 9%, whereas the QN varied from 77 to 2%. This means that after 9 years of storage both DS and QN had a small fraction of seeds that were still hard, although there were some differences between the two ecotypes. In addition, we noted that the remnant hard seeds in the experiment were even much smaller in size and even more difficult to germinate than big ones (data not shown). Results also indicated that the germination curves of intact seeds of the two ecotypes had similar characteristics: the curves presented a gradual increase with storage time, to a peak, which was followed by a gradual decrease afterward as storage continued. However, the peak times of the two ecotypes were different: the sixth year after collection (2005) in DS and the fifth year after collection (2004) in QN, respectively.

The similar germination behaviors of the two *G. soja* ecotypes observed in our experiment (**Figure 1**) are probably very close to reality in the wild. As a wild annual plant species, seeds are the only link from one generation to the next, because all maternal plants of annual species die each year. The survival of wild annual plant species is critically dependent on the endurance and appropriate timing of seed germination because synchronized germination could lead to a sudden extinction due to a single extreme drought or late-season chill in wild states. In wild conditions, hard seeds can be viable in the soil for long periods and can germinate at feasible times when seeds become permeable. From an ecological standpoint, hardseededness in *G. soja* is thought to have great value in maintaining the populations of these plants under seasonally unfavorable climatic conditions. The little difference of germination behavior between the two *G. soja* ecotypes observed in our experiment (**Figure 1**) may be attributed to their different proportions of hard seeds that resulted from their different environmental conditions in two naturally occurring sites.

Figure 2 illustrates the changes in germination percentage of the scarified seeds from two ecotypes (DS and QN) during 10 years of storage. Results first indicated that scarification of fresh seeds from two ecotypes determined full germination (100%), which means that failure of germination of *G. soja* under favorable conditions must be attributed only to seed coat. Results also indicated that the germination percentage of the scarified seeds decreased gradually year by year when the storage time was prolonged; the DS ecotype varied from 100 to 28%, whereas the QN ecotype varied from 100 to 9%. This means that after 9 years of storage both DS and QN ecotypes had a small fraction of seeds that were still viable, which can be attributed to the protection of the hard seed coat because the change of germination percentage of the scarified seeds was concordant with the hard seed percentage of the intact seeds (**Figure 1**). However, we also noted that some scarified seeds could not germinate again due to the loss of seed vigor after long time storage, which could probably be attributed to the change of seed coat impermeability.

Identification and Quantitation of Phenolics in the Seed Coat of *G. soja*. From ^{1}H NMR/^{13}C NMR data, compounds **1**, **2**, and **3** obtained from *G. soja* seed coats were identified as a monomeric flavan-3-ol (epicatechin, **1**) and two anthocyanins (cyanidin 3-*O*-glucoside, **2**; delphinidin 3-*O*-glucoside, **3**), respectively, as shown in **Figure 3**. The NMR data were in good agreement with those reported in previous studies (*15*, *16*). All three phenolics were first found in *G. soja*, whereas cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside have already been identified in black soybeans (*G. max*) (*17*).

The three identified phenolics contents in the seed coats of the two *G. soja* ecotypes (DS and QN) from different growing environments are presented in **Table 1**. Comparison of the two *G. soja* ecotypes from the wild reveals epicatechin contents were significantly higher in DS than in QN, wheras the two anthocyanins contents were slightly lower in DS than in QN. Comparison of the two treatments (water-stressed and well-watered) showed that epicatechin contents in the two *G. soja* ecotypes were both significantly higher in water-stressed than in well-watered,

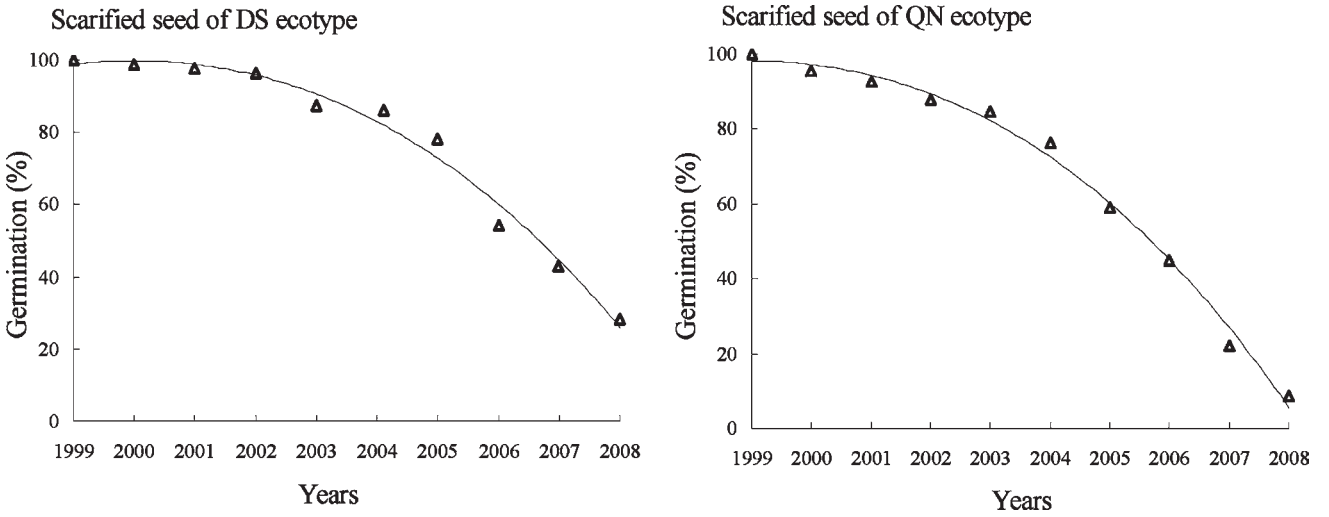

Figure 2. Comparison of the germination percentage (△) of scarified seeds during storage between DS and QN ecotypes of *G. soja*.

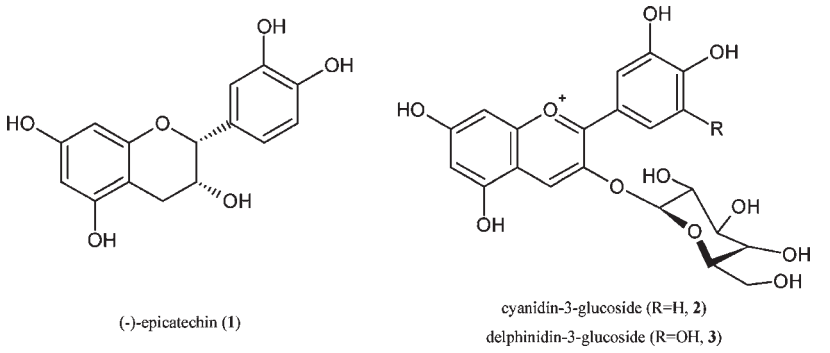

Figure 3. Chemical structures of the phenolics in the seed coat of *G. soja*.

Table 1. Content of Major Phenolics in the Seed Coat of *G. soja*[a]

		content (mg/g of dry coat weight)			
ecotype	conditions	epicatechin	cyanidin 3-*O*-glucoside	delphinidin 3-*O*-glucoside	total
DS	from wild	2.36 ± 0.34 (37.0%)	3.50 ± 0.85 (54.9%)	0.52 ± 0.10 (8.1%)	6.38 ± 0.70 (100%)
	water-stressed	2.60 ± 0.15 (49.3%)	2.45 ± 0.11 (46.5%)	0.22 ± 0.02 (4.2%)	5.27 ± 0.17 (100%)
	well-watered	1.52 ± 0.08 (29.0%)	2.99 ± 0.18 (57.1%)	0.73 ± 0.03 (13.9%)	5.24 ± 0.25 (100%)
QN	from wild	1.00 ± 0.25 (18.0%)	3.79 ± 0.92 (68.2%)	0.77 ± 0.23 (13.8%)	5.56 ± 0.87 (100%)
	water-stressed	2.08 ± 0.08 (44.9%)	2.23 ± 0.18 (48.2%)	0.32 ± 0.03 (6.9%)	4.63 ± 0.23 (100%)
	well-watered	1.10 ± 0.10 (19.2%)	3.77 ± 0.21 (65.9%)	0.85 ± 0.05 (14.9%)	5.72 ± 0.33 (100%)

[a] Results are expressed as mean ± SD (n = 3).

whereas the two anthocyanins contents in the two *G. soja* ecotypes were both significantly lower in water-stressed than in well-watered conditions, which indicated that major phenolics in the two *G. soja* ecotypes (DS and QN) wereboth significantly affected by water conditions. The same results are illustrated in **Figure 4B**.

Changes of the Phenolics Contents in Seed Coat of *G. soja* under Different Water Conditions. The percentages of hard seeds in the two *G. soja* ecotypes (DS and QN) under water-stressed and well-watered conditions are shown in **Figure 4A**. It is clear that water-stress increased the percentages of hard seeds in the two ecotypes (DS and QN) of *G. soja*. The percentages of hard seeds were 51% (DS) and 115% (QN) higher in water-stressed plants compared to well-watered plants.

The contents of phenolics in the seed coats of *G. soja* (DS and QN) under water-stressed and well-watered conditions are shown in **Figure 4B**. Results indicated that water-stress increased the contents of epicatechin but decreased the contents of cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside, whereas adequate water increased the contents of cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside but decreased the contents of epicatechin in the two *G. soja* ecotypes (DS and QN). The contents of epicatechin were 71% (DS) and 89% (QN) higher in water-stressed plants compared to well-watered plants. The contents of cyanidin 3-*O*-glucoside were 22% (DS) and 69% (QN) higher in well-watered plants compared to water-stressed plants. The contents of delphinidin 3-*O*-glucoside were 232% (DS) and 166% (QN) higher in well-watered plants compared to water-stressed plants. The correlation between the hard seed percentages and the contents of phenolics above-mentioned were determined. Hard seed percentages were significantly positively correlated to contents of epicatechin (r = 0.835, P < 0.05) but negatively correlated to contents of

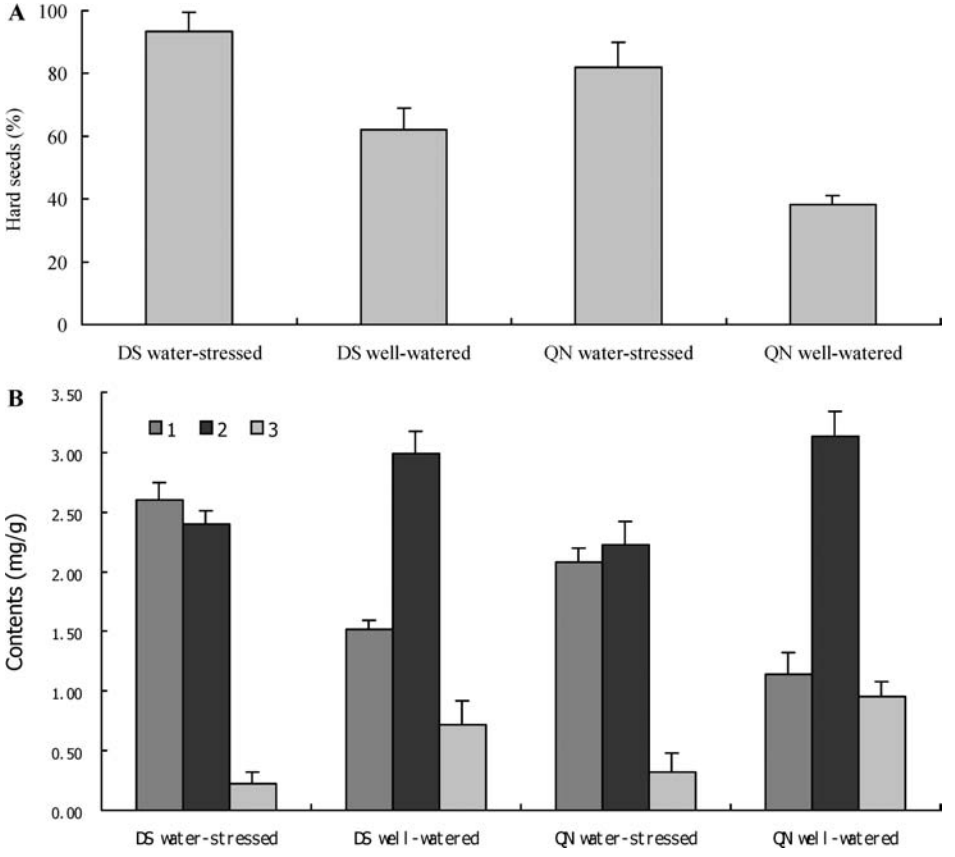

Figure 4. Comparison of the hard seeds percentages (**A**) and the phenolics contents (**B**) in the seed coats of *G. soja* (DS and QN ecotypes) under water-stressed and well-watered conditions: epicatechin (**1**), cyanidin 3-*O*-glucoside (**2**), delphinidin 3-*O*-glucoside (**3**).

cyanidin 3-*O*-glucoside ($r = -0.653$, $P < 0.05$) and delphinidin 3-*O*-glucoside ($r = -0.732$, $P < 0.05$).

Soybean hardseededness is considered to be a quantitative trait that is influenced by genetic and environmental factors (*18*). Nooden et al. (*19*) even reported that the production of soybean hard seeds was affected by drought experienced by the mother plant. We observed that hard seeds of two *G. soja* ecotypes seem to be related to the water conditions of their native area (**Figure 1**), and we further observed that the drought treatments during seed development indeed enhanced the percentage of hard seeds in *G. soja* (**Figure 4A**). Moreover, the seeds in *G. soja* formed under water-stressed conditions had more (−)-epicatechin and less anthocyanins than seeds formed under well-watered conditions (**Figure 4B**); drought appears to be one of the environmental factors that induces (−)-epicatechin biosynthesis. This would indicate that the formation of hard seeds of *G. soja* is related to the increase of epicatechin and the decrease of anthocyanins in its seed coat.

Epicatechin is a precursor of proanthocyanidins in an alternative pathway of anthocyanins biosynthesis (*20*). Xie et al. (*21*, *22*) reported that anthocyanidin reductase (ANR) catalyzed the conversion of anthocyanidins to (−)-epicatechin and that flavonoid 3-*O*-glucosyl transferase (FGT) catalyzed the conversion of anthocyanidins to anthocyanins. There remains some doubt whether drought promotes ANR but inhibits FGT activities that resulted in the accumulation of epicatechin in the seed coats of *G. soja*, and this should be of interest for further investigation.

Changes of the Phenolics Contents in Seed Coat of *G. soja* under Different Storage Conditions. Results indicated that the contents of phenolics in the seed coat of *G. soja* changed with storage time when seeds were stored under N_2, O_2, and air conditions (**Figure 5A−C**). Epicatechin contents decreased significantly over time in samples of O_2 packages (from 2.6 to 1.2 mg/g) and air packages (from 2.6 to 1.6 mg/g) compared with samples of N_2 packages after 5 years (differences were significant, $P < 0.05$), which indicated that epicatechin contents were significantly affected by air and O_2. Contents of cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside decreased slightly over time in samples of air and O_2 packages compared with samples of N_2 packages after 5 years (differences were not significant, $P > 0.05$). In all, aerobic storage conditions (air and O_2) accelerated greater changes of epicatechin than cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside in seed coats during seed storage.

Results also indicated that hard seed percentages in *G. soja* changed with storage time when seeds were stored under N_2, O_2, and air conditions (**Figure 5D**). The hard seed percentages decreased significantly over time in samples of O_2 packages (from 96 to 45%) and air packages (from 96 to 55%) compared with N_2 packages after 5 years (differences were significant, $P < 0.05$). The correlation between the hard seed percentages, and the contents of phenolics were determined. Hard seed percentages were positively correlated to the contents of epicatechin ($r = 0.677$), but there was no significant correlation with the contents of cyanidin 3-*O*-glucoside ($r = 0.076$) and the contents of delphinidin 3-*O*-glucoside ($r = 0.057$).

Obviously, of the three phenolics we identified in seed coats of *G. soja*, only epicatechin exhibited a significant correlation with reduced hard seed percentages during aerobic storage (**Figure 5**). As epicatechin was reported to display the property of autoxidation with O_2 (*23*−*25*), we deduced that the role of epicatechin probably is to impede the entry of O_2 and thus to protect the embryo against oxidation to maintain seed vigor in *G. soja*. When the slow but persistent oxidation with constant presence of O_2 on the surface of seeds would eventually decrease the compound to a threshold, the hardseededness may be broken, allowing

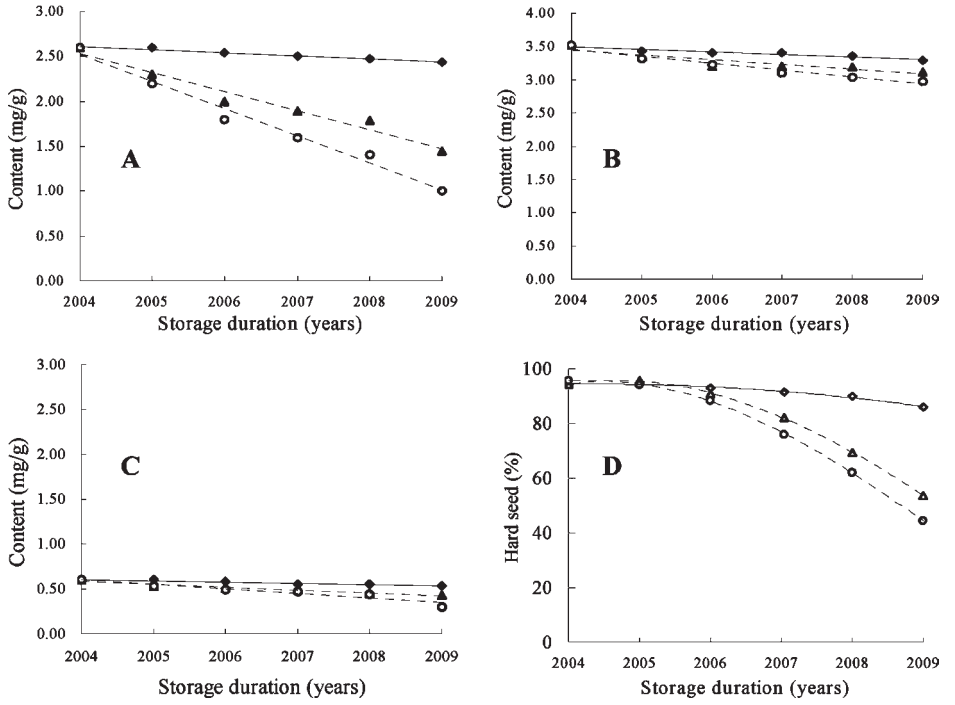

Figure 5. Comparison between the contents of epicatechin (**A**), cyanidin 3-*O*-glucoside (**B**), and delphinidin 3-*O*-glucoside (**C**) in the seed coats and the percentage of hard seeds (**D**) in *G. soja* (DS ecotype) under N_2 (◇), air (△), and O_2 (○) conditions during seed storage.

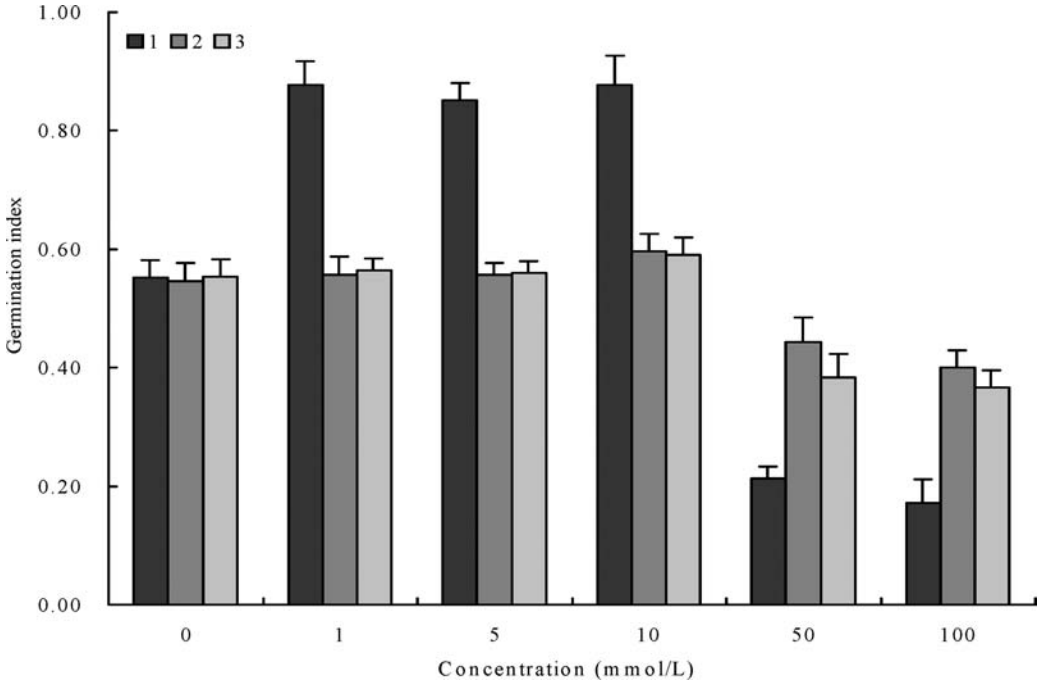

Figure 6. Effects of epicatechin (**1**), cyanidin 3-*O*-glucoside (**2**), and delphinidin 3-*O*-glucoside (**3**) from the seed coats on seed germination index in *G. soja*.

germination to occur when conditions are suitable. Therefore, we deduced that oxidation of epicatechin were correlated with release of hardseededness in *G. soja*. These results illustrated that epicatechin was related not only to the formation and but also to the breakage of hard seeds in *G. soja*. As for the two anthocyanins (cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside), also abundantly found in normal seeds (soft-coated) of black soybeans (*G. max*), their role in seeds needs further study.

Effects of the Isolated Phenolics on Seed Germination of *G. soja*. The effect of the three isolated phenolics on the germination index of scarified *G. soja* seed was examined (**Figure 6**). They showed different effects on seed germination between the monomeric epicatechin and the two anthocynins. Epicatechin illustrated autoinhibitory effects in higher concentrations (50 and 100 mmol/L) but autopromoting effects in lower concentrations (1, 5, and 10 mmol/L), whereas both cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside illustrated autoinhibitory effect only in higher concentrations (50 and 100 mmol/L) on seed germination of *G. soja*. The inhibitory effects of epicatechin were stronger than the two anthocynins in higher concentrations (50 and 100 mmol/L). In addition, we noted that the effects of the three isolated phenolics on seed germination of *G. soja* were not in a dose-dependent manner.

Our results demonstrate that all three phenolics inhibited germination of scarified *G. soja* seeds at high concentrations (**Figure 6**), presumably because they limited oxygen supply to the embryo, mimicking the germination inhibitory property of hard seed coats. Surprisingly, epicatechin also induced germination at lower concentrations, which reveals a hormesis-like effect. Whereas the reason for the hormesis is not clear (*26*), the opposing actions of epicatechin may effectively render it toxic to germination, which may be the ultimate cause for the poor germination rate in wild soybean hard seeds.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data of phenolics from the seed coat of *G. soja* (^{1}H, 600 MHz; ^{13}C, 150 MHz, CD_3OD). This material is available free of charge via the Internet at http://pubs.acs.org.

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