The Role of Seed Structure and Oxygen Responsiveness in Pre-Germination Flooding Tolerance of Soybean Cultivars

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Abstract: Flooding during germination often inhibits the germination and emergence of soybean \(Glycine\ max\) \((L.)\ Merr., but little is known about the mechanisms involved in the tolerance of soybean cultivars to the damage caused by the flooding. The objectives of this study were to characterize the germination responses of soybean cultivars to pre-germination flooding and low oxygen conditions, and to identify possible seed traits responsible for the tolerance. A comparison of germination percentages among 18 cultivars under optimal and flooding conditions for 3 d enabled the identification of two tolerant cultivars (Williams and Peking), and two susceptible cultivars (Nakasennari and Enrei), which were used for further analyses. A comparison of the water absorption speed (WAS) in the following seed forms: embryo only (E), embryo with aleurone layer (E + AL), and intact seed with aleurone layer and seed coat (E + AL + SC) revealed that the aleurone layer provides a barrier to water penetration during the first hour of inundation regardless of cultivar. The intact seeds of a tolerant cultivar, Peking absorbed water more slowly than the other cultivars in the first hour of flooding. When the oxygen concentration in the seed container was reduced to 70 mL L\(^{-1}\) for 3 d, the germination percentage of susceptible cultivars was reduced to approximately 70 % whereas that of tolerant cultivars remained high, indicating that responsiveness to low oxygen could also be responsible for pre-germination flooding tolerance of soybean cultivars.

Key words: Aleurone layer, Flooding tolerance, Germination, Low oxygen, Seed structure, Soybean, Water absorption.

In East Asia, the climate during late spring to early summer is characterized by continuous rainfall for several days, making crop fields prone to flooding. Under such conditions, the germination and emergence of crops can be adversely affected, and soybean is especially vulnerable (Arihara et al., 2000). In Japan, rice consumption is decreasing and many rice fields are being converted for growing upland crops including soybean. In the converted fields that are not well drained, poor seedling emergence is the dominant reason for the low yields observed.

Screening cultivars or germplasms for tolerance to pre-germination flooding is one option for addressing this problem. Fausey and McDonald (1983) found that inbred corn cultivars were more tolerant to pre-germination flooding than hybrid cultivars. In soybean, Hou and Thseng (1992) attempted to develop a method for selecting varieties tolerant to pre-germination flooding, and found that continuous flooding of seeds for four days at \(25^\circ\)C allowed tolerant genotypes to be identified. Both additive and dominant genes were found to be controlling the tolerance, and the selection for tolerant genotypes was considered to be feasible (Hou et al., 1995). Despite these findings, the specific traits responsible for tolerance to pre-germination flooding in soybean remain unclear.

The damage caused by pre-germination flooding includes the following: 1) the abrupt inundation of seeds disrupts the membranes of embryo cells, 2) a low oxygen environment in the soil restricts the aerobic respiration of seeds, and 3) toxic substances produced in the soil under hypoxic conditions harm the viability of the seeds. Many previous studies have indicated the importance of the seed coat in protecting seeds from water damage. Powell and Matthews (1978) showed that the rapid early leakage caused when a dry embryo was soaked resulted in the death of cells due to the physical disruption of their membranes. Cell death was restricted to the outer layers of the cotyledon. The flooding damage resulted in a decline in the respiration and transfer of reserved substances, and thereby suppressed the growth rate in the seedlings. Raymond et al. (1981) reported that soybean suffered...
more severely from flooding damage than pea. In their study, the damage was markedly reduced by the use of an osmotic-regulating solution of polyethylene glycol (PEG 6000). They also found that the cells that failed to be stained with TTC were observed only on the periphery of the cotyledon; a similar result was reported by Woodstock and Taylorson (1981). Nordin (1984) demonstrated that the seed coat acted as a semi-permeable membrane, permitting the entry of water and certain solutes while restricting others. Duke and Kakefuda (1981) compared the seeds of four legumes with or without seed coats and found that the embryonic cells of soybean and navy bean seeds without seed coats ruptured when submerged. Matsui et al. (1996) demonstrated, using seeds without a seed coat or aleurone layer, that the aleurone layer significantly alleviated the extent of flooding damage in soybean. A seed with an aleurone layer could act almost as well as an intact seed in preventing the rapid uptake of water, and thereby prevent the rupture of the embryonic cells. They also observed that when the aleurone layer was treated with pectinase and cellulase, a Casparian strip-like tissue was obtained.

Few studies have been reported on the relationship between pre-germination flooding damage and oxygen concentration. Al-Ani et al. (1985) classified soybean into a group of crops more susceptible to low oxygen; their maximum germination occurred at an oxygen concentration close to that of ambient air. The only study that directly linked the pre-germination flooding damage of soybean to a low oxygen concentration inside the seed was carried out by Copeland and McDonald (1995). In an experiment to test the effect of peroxide on seed germination, Langan et al. (1986) showed that the emergence of soybean was slightly enhanced by coating the seed with zinc peroxide. Furthermore, Martin et al. (1991) compared two flooding-tolerant inbred strains of corn with the other two intolerant ones and postulated that the flooding-induced inhibition of germination might have resulted from the accumulation of some volatile metabolite(s) other than ethanol or acetaldehyde. It is therefore likely that multiple factors interact to produce the cultivar’s particular level of tolerance to flooding.

Temperature markedly influences germination under flooding conditions (Wuebker et al., 2001). A 72-h period of flooding is sufficient to produce between-cultivar differences, regardless of temperature (Hou and Thseng, 1992; Wuebker et al., 2001).

Despite these findings, however, little is known about the mechanism(s) involved in the between-cultivar differences in the amount of damage induced by flooding. The objectives of this study were to characterize the germination responses of soybean cultivars to pre-germination flooding and low oxygen conditions, and to identify seed traits responsible for the tolerance.

Materials and Methods

1. Plant materials

The seeds of 18 soybean [*Glycine max* (L.) Merr.] cultivars (19 seed lots), harvested at four different sites in 2002 (Table 1), were used in the preliminary examination of percentage germination rate. A comparison among these 18 cultivars of germination percentages under optimal conditions (GPo) and flooding treatment (GPF) for 3 d enabled us to select two relatively tolerant cultivars (Williams and Peking) and two highly susceptible cultivars (Nakasennari and Enrei). The seeds of these four cultivars, which were harvested from the same field, were used for further analyses. The seeds were stored in plastic bags at 4°C until use. For the anatomical observations, we used the Williams cultivar.

2. Examination of germination percentage

The GPo was measured according to the ISTA rule (1985). Briefly, seeds were folded in two layers of paper towels (230×218 mm, double-layer type, Crecia Company Ltd., Japan) saturated with de-ionized water and placed in a germination chamber (75×45×50 cm). The inside of the chamber was maintained at 100% relative humidity and 25°C, under ambient light. Seeds were considered to have germinated when the radicle was visible. All germinated seeds were counted daily from the third to the seventh day after the seeds had been placed into the chamber. The test was repeated three times, using 30 seeds for each replicate.

The GPF was also tested. A glass box measuring 35×85×85 mm was used as the seed container. Thirty seeds of each cultivar were flooded for 1, 2, 3 or 4 d, by adding 100 mL of de-ionized water to the container. After this treatment, seeds were allowed to germinate in petri dishes (11 cm in diameter) containing a double layer of moistened filter paper. The germination percentage was monitored daily from the third to the seventh day after the end of the respective flooding period. No additional water was added to the petri dishes after the transfer of seeds. To examine the effects of the seed coat on germination, the seed coat was carefully removed, and the seeds were flooded for 3 d, immediately after which the germination percentage was measured. The flooding treatment and consequent monitoring of germination were conducted at 25°C under ambient light conditions.

3. Measurements of water absorption speed and electrical conductivity

Seeds were manipulated in the following three forms; embryo alone (E), embryo with aleurone layer (E+AL), and the intact seed (E+AL+SC). The E and E+AL seeds were obtained by carefully removing the seed coat (SC) with and without aleurone layer (AL), as described by Matsui et al. (1996). The container for
the water absorption speed (WAS) measurement was the same as that used for the germination experiments. Thirty seeds for each replicate were weighed and consequently flooded with 100 mL de-ionized water. The container was controlled at 25°C under ambient light conditions. Seeds were periodically taken out of the water (at 0.5, 1, 2, 3, 6 and 9 h after the initiation of flooding), blotted on filter paper and weighed: the blotting removed excess surface water. The WAS for each interval was calculated as the ratio of the increased seed weight to the original weight.

For the measurement of electrical conductivity (EC), seeds were prepared in the three forms as described above. To compare ECs among cultivars, the weight of 30 seeds of the Enrei cultivar was set as a standard weight for each replicate. In other words, the quantities for each replicate of the other cultivars were adjusted as closely as possible to the standard weight. Seeds of the three forms were flooded in the same way as in the WAS experiments. The EC values were measured with a microprocessor-based pocket EC tester (TDScan 20, Eutech Instruments, Japan) at intervals of 0.5, 1, 2, 3, 6, 9, 12 and 24 h after the initiation of flooding. The results were expressed as cumulative values.

### 4. Responses to low oxygen

Seeds were wrapped in paper towels and wetted with saturated de-ionized water, then placed in germination chambers (75×45×50 cm) at the following oxygen concentrations; 20, 50, 60, 70, 80, 100 and 210 mL L⁻¹. Oxygen concentrations were controlled by mixing N₂ gas with ambient air, which was supplied through an air pump. The flow rate of the gas mixture was maintained at 100 mL min⁻¹. The bottom of the chambers contained a constant volume of water (1 cm depth) to ensure sufficient moisture in the chamber during seed germination. The oxygen treatment was continued for 3 d. Then the seeds were further allowed to germinate under the ambient air conditions, as described in the germination test under optimal conditions, and the germination percentage was recorded. Another hypoxia experiment was conducted by flooding the seeds in the containers, as described in the previous flooding treatment. In this experiment, the flooding water was flushed with 100% N₂ gas or ambient air (210 mL L⁻¹ O₂). The treatment was continued for 3 d, and the germination percentage was measured under the same conditions as described in the germination test under optimal conditions.

| Cultivar  | Harvested site | 100-grain weight | GPo  | 1d  | 3d  | GPo – GPf  |
|-----------|----------------|------------------|------|-----|-----|------------|
| Chamame   | Tohoku University | 32.7             | 65.8±10.7* | 60.8±1.4* | 49.2±8.3* | 16.7       |
| Kuromame  | Tohoku University | 29.9             | 85.0±4.3  | 70.0±4.1  | 56.7±7.2  | 28.3       |
| Kurobe    | Tohoku University | 33.4             | 73.4±3.9  | 60.0±2.3  | 55.0±5.8  | 18.4       |
| Ryuhou    | Tohoku Agr. Res. Center | 28.1   | 92.5±1.7  | 93.3±2.4  | 56.7±3.8  | 35.8       |
| Sakukei 4 | Tohoku University | 35.6             | 83.3±2.7  | 80.8±2.7  | 38.4±1.9  | 45.0       |
| En1282    | Tohoku University | 32.5             | 84.2±1.7  | 79.2±3.6  | 33.3±4.7  | 50.8       |
| Enrei     | Tohoku University | 37.6             | 83.3±8.2  | 87.5±4.9  | 26.7±7.7  | 56.7       |
| Suzuyutaka| Crop Institute  | 23.6             | 90.0±0.0  | 91.7±1.6  | 95.9±1.7  | -5.8       |
| Fukuyutaka| Crop Institute  | 29.7             | 98.4±1.9  | 98.4±1.6  | 97.5±1.7  | 0.8        |
| Harosoy   | Crop Institute  | 16.2             | 98.4±1.9  | 94.2±3.6  | 90.0±2.7  | 8.3        |
| Hatayutaka| Crop Institute  | 30.0             | 99.2±1.7  | 97.5±1.4  | 96.7±2.7  | 2.5        |
| Nakasennari| Crop Institute | 24.4             | 98.4±1.9  | 99.2±1.4  | 55.0±5.8  | 43.4       |
| Enrei     | Crop Institute  | 32.0             | 94.2±1.7  | 93.3±2.4  | 73.3±5.4  | 20.8       |
| Sachiyutaka| Crop Institute | 30.7             | 98.4±1.9  | 97.5±1.4  | 68.4±3.3  | 30.0       |
| Tamahomare| Crop Institute  | 26.0             | 100.0±0.0 | 97.5±1.4  | 42.5±3.2  | 57.5       |
| Lee       | Crop Institute  | 16.8             | 99.2±1.7  | 100.0±0.0 | 97.5±1.7  | 1.6        |
| Williams  | Crop Institute  | 17.8             | 100.0±0.0 | 100.0±0.0 | 98.3±3.3  | 1.7        |
| Peking    | Crop Institute  | 13.1             | 100.0±0.0 | 99.2±1.4  | 98.3±3.3  | 1.7        |
| Tanbaguro | Nagano Agr. Res. Center | 59.5  | 68.4±5.5  | 70.9±5.5  | 0.0±0.0   | 68.4       |

*Mean ± SE of three replicates, each using 30 seeds.

Table 1. Harvested site, 100-grain weight, germination percentage under optimum conditions (GPo), germination percentage after flooding treatment (GPf) for 1 d or 3 d, and the difference in percentages between GPo and GPf for 3 d (GPo – GPf) of 18 soybean cultivars.
5. Seed anatomy
The seeds of the Williams cultivar were manipulated into the three forms as described above (i.e. E, E+AL and E+AL+SC), and fixed in FAA (formalin-acetic acid-alcohol). After being dehydrated through an n-butyl alcohol series, the seeds were embedded in Paraplast Plus (Oxford, USA) and cut into pieces of 10 µm thick sections using a rotary microtome (Reichert Histostat, Germany) equipped with a stainless steel microtome blade (Feather, Japan). The sections were then stained for 8 min in 0.5 mg L⁻¹ (w/v) toluidine blue O (E. Merck, Darmstadt, Germany) solution, and viewed with a fluorescent and optical microscope (Fluophot and Optiphot, Nikon, Japan). Photographs were taken using a microphotograph system (UFX-II, Nikon, Japan) and a digital microscope camera (Polaroid DMC le, Polaroid Co. Ltd.).

To observe the more precise structure of the AL around the hilum and radicle, we coated E+AL Williams seeds with Pt+Pd (Ion Sputter, E-1030 Hitachi, Japan) and examined with a scanning electron microscope (SEM) (S-4700, Hitachi, Japan).

Results

1. Germination percentage under optimum conditions and after the flooding treatment
The GPs of the 18 cultivars ranged from 65.8% to 100.0% (Table 1). Flooding the seeds for 1 d had no significant effect on the germination percentage in most, but not all, cultivars. Flooding the seeds for 3 d enabled us to determine the between-cultivar differences in responsiveness to flooding. The germination percentage after the flooding treatment (GPF) ranged between 0.0 and 98.3%, depending on

| Cultivar       | Germination percentage (%) | GPF (%) |
|----------------|----------------------------|---------|
| Nakasennari    | 55.0±2.9                   | 68.4±1.0* |
| Enrei          | 73.3±2.7                   | 80.0±1.9  |
| Williams       | 98.3±1.7                   | 96.7±1.9  |
| Peking         | 98.3±1.7                   | 98.3±1.7  |

Values are mean ± SE of three replicates, each using 30 seeds. * Significantly different at the 5% probability level.

Table 3. Water absorption speed (WAS) of seeds with or without aleurone layer (AL) and seed coat (SC) in four soybean cultivars. The WAS was measured periodically after initiation of the flooding treatment (see main text for details).

| Flooding period | Cultivar       | E+AL+SC   | E+AL     | E        |
|-----------------|----------------|-----------|-----------|-----------|
|                 |                | g g⁻¹ h⁻¹ | g g⁻¹ h⁻¹ | g g⁻¹ h⁻¹ |
| 0 - 0.5         | Nakasennari    | 0.61±0.07 b | 0.63±0.08 | 0.93±0.03 |
|                 | Enrei          | 0.73±0.06 a | 0.46±0.06 | 0.94±0.05 |
|                 | Williams       | 0.65±0.05 b | 0.53±0.04 | 1.08±0.08 |
|                 | Peking         | 0.14±0.04 c | 0.59±0.06 | 1.02±0.11 |
| 0.5 - 1.0       | Nakasennari    | 0.45±0.02 a | 0.46±0.08 | 0.71±0.02 |
|                 | Enrei          | 0.48±0.02 a | 0.35±0.02 | 0.74±0.07 |
|                 | Williams       | 0.41±0.03 b | 0.36±0.02 | 0.73±0.03 |
|                 | Peking         | 0.16±0.04 c | 0.42±0.06 | 0.86±0.04 |
| 1.0 - 2.0       | Nakasennari    | 0.17±0.01  | 0.17±0.02 | 0.27±0.03 |
|                 | Enrei          | 0.15±0.00  | 0.21±0.03 | 0.20±0.06 |
|                 | Williams       | 0.17±0.02  | 0.14±0.03 | 0.24±0.07 |
|                 | Peking         | 0.14±0.02  | 0.15±0.01 | 0.16±0.01 |
| 2.0 - 3.0       | Nakasennari    | 0.19±0.01  | 0.15±0.01 | 0.11±0.03 |
|                 | Enrei          | 0.24±0.06  | 0.20±0.03 | 0.14±0.05 |
|                 | Williams       | 0.14±0.01  | 0.13±0.03 | 0.06±0.03 |
|                 | Peking         | 0.13±0.06  | 0.14±0.04 | 0.09±0.01 |

Data are shown as means ± SE of three replicates, each using 30 seeds. Values followed by the same letter or no letter within each flooding period for each seed form are not significantly different at the 5% probability level.
the cultivar. The cultivar-based differences between GPf for 3 d and GPo ranged from –5.8 to 68.4%. Of the cultivars tested, Enrei was the only one harvested from two different sites. Because the GPo of Enrei differed between the two sites, it was judged only to be appropriate to use the seeds harvested at the same site for comparisons between cultivars. For further analyses, therefore, four cultivars with high GPo (>90%) which were harvested from the same source (Crop Institute) were selected; the Williams and Peking varieties as the tolerant cultivars and the Nakasennari and Enrei varieties as the susceptible ones.

A comparison of GPf between seeds with and without SC revealed that the GPf of the flooding-tolerant cultivars (Williams and Peking) was high regardless of SC presence or absence, whereas that of the susceptible cultivars (Nakasennari and Enrei) tended to be lower in seeds with SC than in seeds without SC (Table 2).

2. Water absorption speed and electrical conductivity

The water absorption speed (WAS) was higher during the earlier stages of flooding (Table 3). There was a significant difference between the WAS among the cultivars during the first hour of flooding in the intact seed (E+AL+SC), with intact Peking seeds being significantly slower than the other cultivars. The WAS of E+AL seeds was slower than that of E seeds regardless of cultivar type, indicating that the aleurone layer was commonly working as a barrier to water invasion at the initial period of flooding. In the Peking cultivar, the WAS of E+AL+SC seeds was significantly lower than that of E+AL seeds, suggesting that the SC as well as the AL was working as a barrier to water penetration. After 1 h of the flooding treatment, there were no significant differences in the WAS between cultivars.

In all the cultivars, the EC (electrical conductivity) values increased with time of flooding (Fig.1). In the E and E+AL seed forms, the EC values of susceptible cultivars were higher than those of the tolerant cultivars over the period of measurement. Conversely, the tolerant cultivars showed higher EC values in the E+AL+SC seed form than the susceptible cultivars, indicating that more electrolytes were dissolved from the seed coat of the tolerant cultivars than that of the susceptible ones. The E+AL form yielded lower EC values during the measurement period in all the cultivars.

3. Responses to low oxygen concentration

When the oxygen concentration inside the seed chambers was reduced to 60 mL L$^{-1}$ or less, the seeds of all the four cultivars failed to germinate during the 3-d oxygen treatment (Table 4). At O$_2$ concentrations of 100 mL L$^{-1}$ or more, the germination percentages after the 3-d treatment were very high (>90%) regardless of cultivar type. The between-cultivar difference in germination percentage was obvious at O$_2$ concentrations of 70 mL L$^{-1}$; the flooding-tolerant cultivars germinated better than the susceptible ones. At 1 d after the 3-d O$_2$ treatment, the seeds that were exposed to lower O$_2$ concentrations (≤60 mL L$^{-1}$) tended to germinate quickly, however, the germination percentages of the susceptible cultivars nevertheless tended to be lower than those of the tolerant cultivars.

During the 3-d flooding period accompanied with the flushing treatment, germination was observed only in the water flushed with normal air, and the germination percentages 2 d after the initiation of the
were higher in the tolerant cultivars (Table 5). When the flooded seeds were allowed to germinate under optimum conditions, all the cultivars started to germinate, but again the tolerant cultivars germinated better than the susceptible ones regardless of the flooding condition.

4. Seed anatomy

The anatomical characteristics of the outer portions of the seeds are shown in Fig. 2. The hilum of the seed opened from the outside, allowing water to move freely across the porous continuum of the aerenchyma (Fig. 2A and B). The aleurone layer, a single layer of cells, covering the whole surface of the embryo, maintained its integrity (Fig. 2C). Covering its outer layer were some crushed remnants of the parenchyma and endothelium, which were darkly dyed with 0.5 mg L$^{-1}$ toluidine blue O. Beneath its inner layer were the remnants of the endosperm, a type of callose tissue (Brundrett et al., 1988). The tissues adjacent to the outer and inner sides of the aleurone layer tightly adhered to it.

Discussion

Although previous studies suggested that an abrupt water uptake would be a major cause of damage in the embryo and thereby reduce the germination percentage of soybean seed, the physiological and anatomical bases for the underlying mechanism were unknown. Kannenberg and Allard (1964) suggested that the pigmented seed of the lima bean, which contains almost 15 times more lignin content in the seed coat than non-pigmented seeds, germinate more vigorously, most probably because of the lesser mechanical injuries sustained during the harvesting operation. Raymond et al. (1981) shared a common view on the soybean. McDonald et al. (1988)
pre-germination flooding tolerance in soybean demonstrated that the soybean seed coat retards water uptake, controls the direction of water penetration into the embryo, and serves as a reservoir of water for the hypocotyl-radicle axis. In the present study, however, removal of the seed coat did not increase the WAS in three out of four cultivars tested, and the existence of seed coat markedly reduced the WAS only in the Peking cultivar (Table 3).

The germination percentage of soybean seeds was negatively correlated with the amount of sugars exuded from seeds (Zheng and Watabe, 2000). In this study, the electrical conductivity (EC) was used as an indicator for electrolyte leakage from seeds. In the E and E+AL seed forms, the EC values of the susceptible cultivars (Nakasennari and Enrei) were higher than those of the tolerant cultivars (Williams and Peking) over the period of measurement (Fig. 1). The difference in the EC values between seeds with (E+AL+SC) and without (E+AL) seed coat was not marked. This evidence indicates that the aleurone layer, rather than the seed coat, plays a major role in preventing the dissolution of ingredients from the embryo regardless of cultivar type. The EC value of E seed form was markedly higher in the susceptible cultivars than in the tolerant cultivars, suggesting that the susceptible cultivars lost more electrolyte from the embryo than the tolerant cultivars. Further study is needed to clarify whether this electrolyte leakage is associated with the physical characteristics of the membrane of embryo cells.

The outer portion of the soybean seed consists of several layers, including a palisade layer, an hourglass cell layer, the partially crushed parenchyma (aerenchyma), the crushed remnants of the parenchyma and endothelium, an aleurone layer and the crushed remnants of the endosperm (Fig. 2 A). The hilum is an open space surrounded by parenchyma, and it is likely that water can enter more easily from this space. This anatomical characteristic of the soybean seed might explain why the SC was incapable of retarding a sudden influx of water, with the exception of the Peking cultivar. The aleurone layer, a complete single-cell layer lying adjacent to the crushed remnants of the parenchyma and endothelium and the remnants of the endosperm, might block water coming into the embryo tissues. We hypothesize that at the beginning of flooding, water enters the hilum and then quickly moves into the aerenchyma layer, probably completely saturating the entire layer within 15-30 min, then passes through the aleurone layer fairly slowly (Table 3). However, this scenario needs to be verified by further study.

Thorne (1981) and Yaklich et al. (1992) indicated the importance of cellular structure and functioning of aleurone layer in providing photosynthate to the embryo during the seed filling stage of soybean. They observed a dramatic increase during ripening, in the
thickness of the cell wall and accumulation of several storage materials in the aleurone layer, including lipid, protein and starch. It is likely that this thickened layer is capable of retarding an abrupt increase in water. Matsui et al. (1996) highlighted the important role of the aleurone layer in preventing a rapid water invasion into the embryo during the germination of the soybean seed. Our results were consistent with their finding (Table 3). However, it was not clear that the between-cultivar differences in tolerance to pre-germination flooding were associated with the differences in the characteristics of the aleurone layer.

Matsui et al. (1996) reported that a Casparian strip-like tissue was obtained from the aleurone layer following treatment with pectinase and cellulase. We observed transverse sections of the aleurone layer but found no typical Casparian strip. The genesis of the aleurone layer in soybean has been a controversial issue (Esau, 1997; Miller et al., 1999) and its function remains unknown.

Germination is an integrated process consisting of many metabolic events (Bewley, 1997). Oxygen concentration is one of the most important factors affecting the germination percentage under hypoxic conditions. The tolerant cultivars exhibited higher germination percentages than susceptible ones at a range of critical O2 concentrations immediately after the 3-d hypoxic treatment (Table 4). The germination percentage 1 d after the 3-d hypoxic treatment also tended to be higher in tolerant cultivars than susceptible ones exposed to a range of O2 concentrations less than 70 mL L⁻¹ (Table 4). These results suggest that the cultivar difference in responsiveness to low O2 concentration might be another physiological trait affecting the tolerance to flooding.

Despite several previous studies, the relationship between hypoxia/anoxia and seed germination remains unclear (Botha et al., 1992; Copeland and McDonald, 1995). Morinaga (1926) first noticed the differing oxygen requirements among plant species and it has subsequently been found that these differences extend to the process of germination. The cells and seeds of soybean are more susceptible to their oxygen environment than those of other species (AL-Ani et al., 1985; Mohanty et al., 1993). The published literature on differences between soybean cultivars to flooding during germination is extremely limited (Yagasaki et al., 1994; Hou et al., 1995). Van Toai et al. (1988) found that flood-tolerant corn lines could germinate at a lower oxygen concentration than susceptible lines, which might be probably related to the ability of the tolerant lines to produce metabolic energy under oxygen deficient conditions. Further studies on the oxygen-regulated mechanisms of germination are necessary to elucidate the physiological bases for differences between cultivars in their tolerance to pre-germination flooding.

The cultivars used in this study significantly differed in seed size; Enrei > Nakasennari > Williams > Peking. Larger seeds are likely to require more oxygen for respiration during germination, which can accelerate hypoxic condition induced by flooding, leading to an inferior germination of the seeds. This possibility needs to be investigated by further study.

In conclusion, the aleurone layer appears to play a crucial role in the tolerance to pre-germination flooding by blocking abrupt water invasion into embryo, irrespective of cultivar. More precise studies on developmental processes and functions of the layer should lead to clarification of the mechanism controlling germination under flooding condition. For the genetic improvement of flooding tolerance, the retardation of seed water absorption by modifying the structure of seed coat as well as the reinforcement of tolerance to low oxygen availability might be feasible targets.

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