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Water Uptake by Seeds in Yellow-seeded Soybean (*Glycine max* (L.) Merrill) Cultivars with Contrasting Imbibition Behaviors

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Abstract : The rate of water uptake by seeds is assumed to be an important factor affecting the susceptibility of seeds to flooding injury, but the traits which could contribute to restricting rapid imbibition by seeds remain undetermined in yellow-seeded soybeans. This study was conducted to determine the possible factors controlling the rate of imbibition in soybeans with yellow seed coats. The imbibition behavior of two yellow-seeded cultivars, Tamahomare and Tachinagaha, with different flooding susceptibilities was examined in relation to initial seed moisture content, the hydration location of the seed surface during imbibition, and the state of the seed coat. Low seed moisture increased the water uptake by Tamahomare intact seeds and even allowed substantially faster imbibition than occurred in its embryos, while in Tachinagaha seed moisture content had little influence on seed imbibition. This rapid imbibition by low-moisture Tamahomare seeds was not due to alterations in the permeability of the seed coat nor in water uptake by the embryo, but rather to increased movement of water along the abaxial sides of the cotyledons. The seed coat of Tamahomare loosened readily upon submergence whilst that of Tachinagaha rather tightly adhered to the embryo, suggesting that the seed coat of Tamahomare adheres very loosely to the embryo and may aid in conducting water laterally between the seed coat and embryo. The degree of adherence of the coat to the embryo and/or the ease with which the coat can be loosened upon hydration appear to play a crucial role in determining the rate of water uptake in yellow-seeded soybeans.

Key words : Flooding injury, Imbibition damage, Seed coat, Seed hydration, Seed moisture content, Soybean.

In many areas of Japan soybean seeds are planted in the summer-rainy season, so that excess rainfall after sowing can often lead to soil inundation and consequently to decreased field emergence of the seedlings, particularly when they are planted in heavy texture soils with poor drainage. Flooding injury in seeds during germination has therefore been recognized as a major constraint on soybean production in Japan.

The mechanism of flooding injury in the seeds of grain legumes has been presumed to be imbibition damage, a physical disruption of seed tissue resulting from a too-rapid uptake of water by seeds placed under excess water (Powell and Matthews, 1978; Woodstock and Taylorson, 1981). Several reports have shown that the incidence of imbibition damage was closely associated with the rate of imbibition of seeds of various cultivars (Powell et al., 1986a; 1986b; Chachalis and Smith 2000; Nakayama et al., 2004). Imbibition damage is greatly alleviated by osmotically reducing the initial rate of water uptake by seeds (Woodstock and Taylorson, 1981; Powell et al., 1986a; 1986b; Nakayama et al., 2005). Since the seed coat plays a crucial role in governing water uptake by seeds during

imbibition (Duke and Kakefuda, 1981; McDonald et al., 1988), the use of seeds with low-permeable seed coats could offer a practical means to prevent flooding injury.

In order to identify the seed coat characteristics that could determine the imbibition properties of seeds, numerous studies have been conducted to date. While yellow-seeded soybean cultivars typically have a seed coat with a rapid permeability characteristic, the pigmentation of the seed coat appears to confer a delayed permeability on the coat and consequently results in a higher resistance of the seeds to imbibition damage (Tully et al., 1981; Kuo, 1989; Hou and Thseng, 1991; Chachalis and Smith, 2000). A similar association between the pigmentation of the seed coat and delayed permeability has also been reported in peas (Powell, 1989), French beans (Powell et al., 1986b) and faba beans (Kantar et al., 1996). The hydrophobic nature of pigmented seed coats, likely due to the presence of phenolic compounds in the epidermis of the seed coat (Marbach and Mayer, 1974; Werker et al., 1979), was presumed to be responsible for the delayed permeability of dark-colored seeds. On the other hand, Chachalis and Smith (2000)

showed that one black-seeded soybean accession whose seed coat appeared to loosely adhere to the embryo imbibed water more quickly than other black soybean accessions, which suggested that the adherence of the seed coat to the embryo, apart from the pigmentation of the coat, could play an important role in controlling water uptake. They also reported in another study on seed coat structure in soybeans (Chachalis and Smith, 2001) that a marked difference between accessions with delayed-permeable and rapid-permeable seed coats was the existence of pores in the surface of the seed coat. Deep and wide open pores were abundant in the seed coats with rapid permeability but were less abundant or absent in seed coats with delayed permeability or impermeable seed coats. Several studies on the seed coat structures of impermeable seeds, so-called hard seeds or stone seeds, have also revealed an association between pores in the seed coats and water uptake by the seeds (Calero et al., 1981; Otobe and Yoshioka, 2008). Recently, Tian et al. (2005) reported that the aleurone layer, rather than the seed coat, provides a barrier to water penetration during imbibition in soybeans. Despite these findings, the seed coat characteristics that could contribute to retarding the rate of water uptake by seeds and consequently to improving the flooding tolerance of seeds remain undetermined in yellow-seeded, agronomically-important soybeans. Further, since pigmented or impermeable seed coats per se are agronomically undesirable traits, transferring the delayed-permeability seed coat characteristics from cultivars/lines with these traits to other yellow-seeded cultivars seems to be problematic.

Previously, we found a cultivar, Tamahomare, which showed particularly high susceptibility to flooding injury among the yellow-seeded cultivars tested (Nakayama et al., 2004). Another yellow-seeded cultivar, Tachinagaha, in comparison to Tamahomare, showed a significant difference in the susceptibility of seeds to flooding injury but an almost identical rate of imbibition under the conditions used in the study. The result was contradictory to the postulated hypothesis of a close association of imbibition damage with the rate of imbibition of seeds. Thus, it appeared to be of interest to further examine these two cultivars, focusing on their imbibition properties of their seeds under various conditions. Additional information obtained on the basis of a comparison between yellow-seeded cultivars could provide some important clues to improved flooding tolerance in soybeans with yellow seed coats. The objective of this study was to characterize the imbibition properties of Tamahomare in comparison with those of Tachinagaha in order to identify the seed coat characteristics that could be responsible for the difference in the susceptibility of the seeds to flooding injury.

Materials and Methods

1. Plant materials

Unless otherwise mentioned, all studies were conducted using seeds of the yellow-seeded soybean cvs 'Tamahomare' and 'Tachinagaha' produced at the experimental field of National Institute of Crop Science, Tsukuba in 2005. Seeds from different seed lots including Tachinagaha seeds produced in 2004 and Tamahomare seeds produced in 2003 at the same location were also used in an experiment in which the effect of the production year on the imbibitional property was examined. The seeds in each seed lot were sieved, and those representing the average seed size of each lot were selected for use. The hundred-seed weights of each seed lot were 28.65, 27.76, 30.57, and 32.71 g dry weight for Tamahomare seeds produced in 2005, Tamahomare produced in 2003, Tachinagaha produced in 2005, and Tachinagaha produced in 2004, respectively. Only seeds without visible damage such as a cracked seed coat were used. Prior to use, the moisture content of the seeds was equilibrated to 8% or 12% (fresh weight basis) by incubating the seeds over dry silica or distilled water at room temperature (about 25°C) for one to two days. For some experiments, entirely or partially decoated seeds were prepared. The seed coats were carefully removed with a razor blade. In the partially decoated seeds, the seed coat was removed only from the dorsal half (opposite the hilar end) of the seed so that the other half, the ventral end including the hilum-micropyle region, which was in direct contact with water during the water uptake studies (see below), was covered by the seed coat.

2. Measurement of water uptake by seeds

The time course of the water uptake by intact seeds with seed coats, entirely decoated seeds (embryos without seed coats), and partially decoated seeds (embryos with ventral seed coats remaining intact) was measured under identical conditions as follows. Ten weighed seeds per replicate were placed on water-saturated cotton (5 mL water g⁻¹ cotton) in a plastic box with their ventral (hilar) end down in direct contact with water and incubated at 25°C. At predetermined intervals (1, 2, 3, 4, 6, and 8 hr) all ten seeds were removed, blotted dry, weighed and immediately returned to the moist cotton to continue imbibition. Changes in weight due to imbibition were expressed as the amount of water absorbed per seed dry weight. The measurement was replicated at least three times. As for the intact seeds, the same time course was also obtained for water uptake from the dorsal end. The hydration was carried out under conditions identical to those used for the ventral hydration (see above) except that the seeds were placed on the moist cotton with their dorsal ends down.

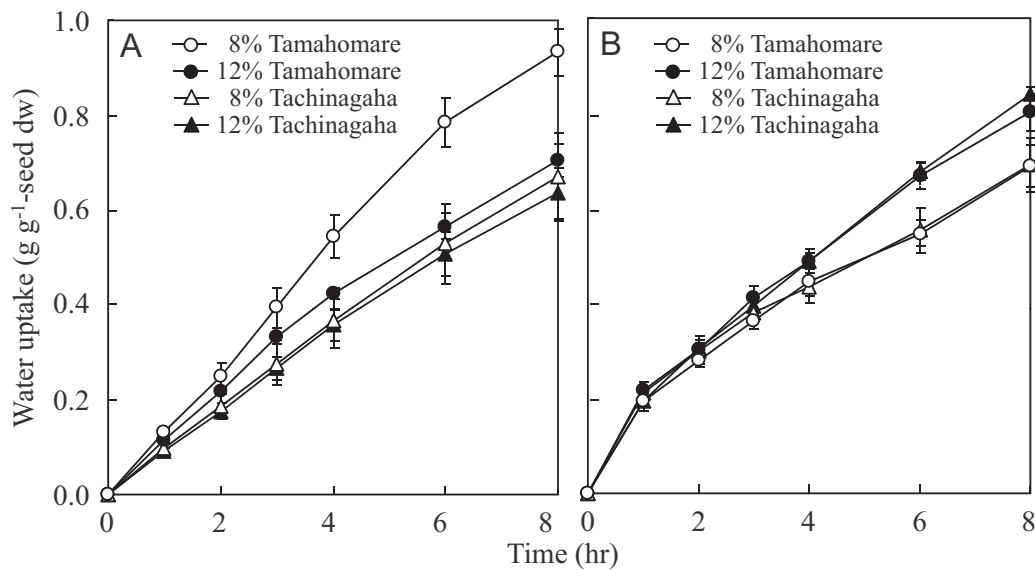


Fig. 1. Time course of water uptake by Tamahomare and Tachinagaha intact seeds (A) and embryos (B) with 8 or 12% initial moisture content. The seeds and embryos were placed on moist cotton with the ventral (hilar) end down in contact with water. Each value is a mean of 10 seeds \pm SE. Measurements were replicated at least three times.

3. Measurement of the movement of water along the abaxial side of the cotyledons

The movement of water along the abaxial side of the cotyledons was determined by placing 30 seeds with their ventral end down on the moist cotton at 25°C. After 1 hr of imbibition, the vertical distance between the hilar fissure and the farthest edge of the wrinkled area of the seed coat formed by water penetration (Meyer et al., 2007), was measured on both abaxial sides of the cotyledons in each seed (see Fig. 4 for reference). The measurement was replicated twice.

4. Loosening of the seed coat after submerging seeds in water

Both Tamahomare and Tachinagaha seeds were subdivided into two types by their seed coat appearance, as seeds with and without a light-colored patch in the seed coats (see Fig. 5A for reference). Both types of seeds were immersed in distilled water for 15 min, and then the extent of wrinkles formed on the seed coat by immersion was compared between the cultivars in relation to the initial seed moisture content.

Results

The time course of water uptake by intact Tamahomare and Tachinagaha seeds, whose moisture content was equilibrated to 8 or 12% (fresh weight basis) before imbibition, was measured with their ventral (hilar) ends in direct contact with moist cotton (Fig. 1A). The 12%-moisture Tamahomare seeds imbibed at a slightly higher rate than the 12%-moisture Tachinagaha seeds, whereas a large and significant increase in the rate of water uptake was noted in the

8%-moisture Tamahomare seeds in comparison with the 8%-moisture Tachinagaha seeds. On the other hand, no significant difference in the rate of water uptake was observed between 8%- and 12%-moisture Tachinagaha seeds. In contrast to the intact seeds, the rate of imbibition of the decoated Tamahomare seeds (embryos) was more rapid at the 12% seed moisture level than at the 8% level, while there was no significant difference in water uptake by the embryos between the cultivars (Fig. 1B). A comparison of the rate of imbibition between the embryos and the intact seeds with the equivalent moisture content revealed that the embryos imbibed more rapidly than did the intact seeds in both cultivars at the 12%-moisture level. In 8%-moisture Tachinagaha seeds, the seed coat slowed down the rate of water uptake during the first 6 hr, after which it had little effect on water uptake; the intact seeds imbibed at the same rate as the embryos after this time point. It should be noted, however, that the 8%-moisture intact Tamahomare seeds showed a higher rate of water uptake than the 8%-moisture embryos after 3 hr of imbibition, which is indicative of a promotive effect of the seed coat on water penetration into the seed at low seed moisture levels.

In order to determine whether this increased rate of imbibition in 8%-moisture Tamahomare seeds reflected varietal characteristics, seeds from seed lots produced in different years were imbibed, and their water uptake was measured (Fig. 2). Similar trends existed within each cultivar across different seed lots, with Tamahomare imbibing more rapidly at low seed moisture levels while Tachinagaha imbibed equally at both moisture levels, suggesting that the rapid imbibition of 8%-moisture Tamahomare was not due

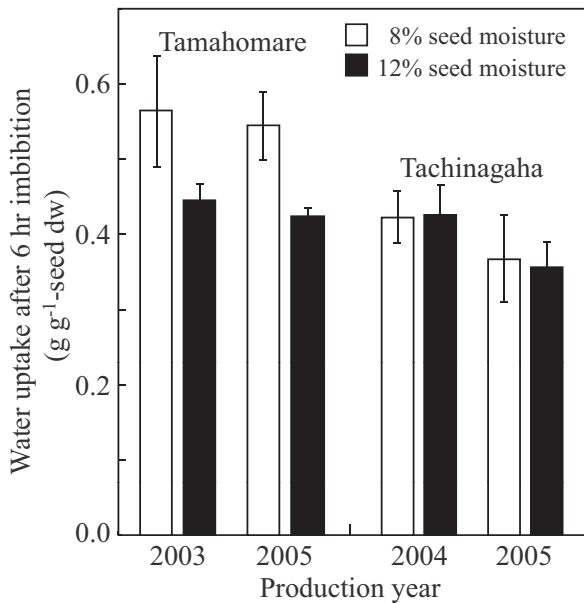


Fig. 2. Effect of production year on water uptake by Tamahomare and Tachinagaha seeds hydrated from the ventral end during a 4 hr imbibition. Each bar is a mean of 10 seeds \pm SE ($n = 3$).

to variations caused by the production year or the handling of this particular seed lot, but rather was due to a varietal feature. We searched for the characteristics of the seeds responsible for the difference in the rate of water uptake between 8%- and 12%-moisture Tamahomare seeds.

The possibility that the low seed moisture level altered the permeability of the seed coat to water, resulting in the rapid imbibition by Tamahomare, was also tested. Since it has been widely accepted that in legume seeds water uptake through the hilum and/or micropyle may be greater than through the

rest of the seed coat, the permeability of the hilum-micropyle region and that of the rest of the seed coat were assessed separately. When intact Tamahomare seeds imbibed through their dorsal ends (opposite to the hilum), 8%-moisture seeds had a slightly slower rate of imbibition than 12%-moisture seeds (Fig. 3A), as in the case of the embryos. As expected, both 8%- and 12%-moisture seeds imbibed more slowly than either the embryos or the intact seeds hydrated from the ventral end. As shown in Fig. 3B, both 8%- and 12%-moisture partially decoated seeds, in which the seed coat was carefully peeled only from the dorsal half so that the ventral end in contact with water during imbibition remained covered by the seed coat, took up water equally, at a rate similar to that obtained for the dorsal hydration of the intact seeds (Fig. 3A). In addition, the partially decoated seeds displayed a substantially slower rate of imbibition than the intact seeds hydrated from the ventral end (Fig. 1A), although they differed only in terms of the presence of the dorsal seed coats.

In the course of these imbibition studies we observed the movement of water around the seed, with water progressing upward along the abaxial sides of the embryo beyond the area exposed to water (Fig. 4). The extent to which the lateral movement of water extended along the abaxial sides was significantly greater in 8%-moisture Tamahomare seeds (Table 1). Both 8%- and 12%-moisture Tachinagaha seeds showed the same extent of the lateral water movement.

Some soybean cultivars, including Tamahomare and Tachinagaha, often produce some seeds with a light-colored patch on their seed coats (Fig. 5A); the color difference between the patch and the rest of the coat is slight but conspicuous. The seed coats isolated from such mottled seeds were shown to be uniform in color

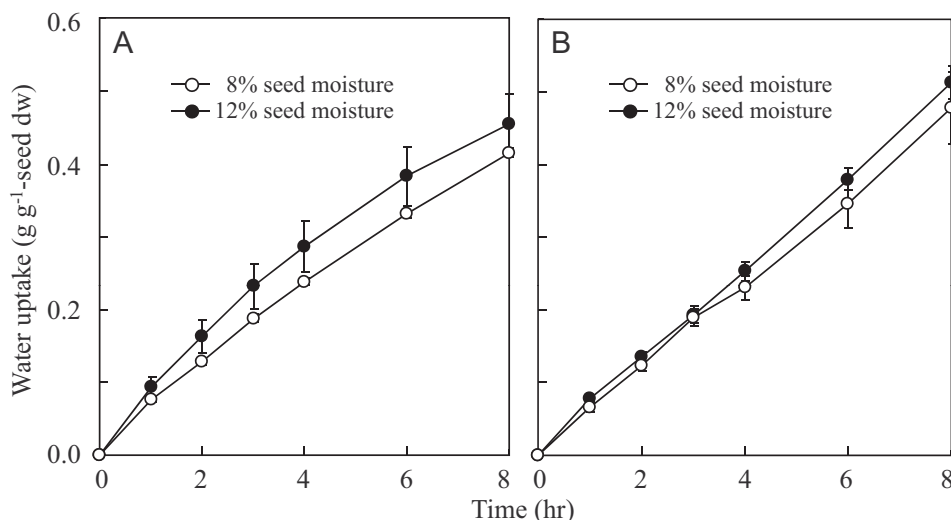


Fig. 3. Rate of water uptake by intact Tamahomare seeds hydrated from the dorsal end (A) and by partially decoated Tamahomare seeds with the ventral coat intact hydrated from the ventral end (B). Each value is a mean of 10 embryos \pm SE ($n = 3$).

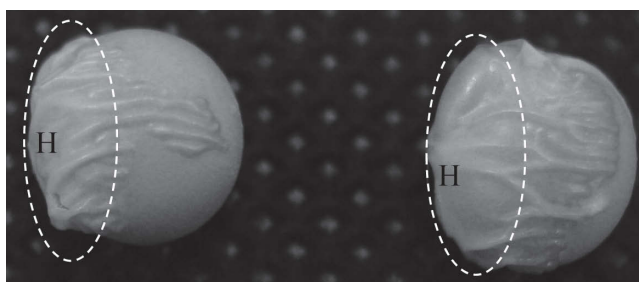


Fig. 4. Tamahomare seeds hydrated from the ventral end on moist cotton for 1 hr. The circled area indicates the area in direct contact with water during imbibition. Wrinkles in the seed coat caused by hydration of the underlying cotyledon extend upward beyond the area exposed to water. H represents the hilum.

Table 1. Extent of water movement along the abaxial side of the cotyledon during the imbibition of seeds for 1 hr through the ventral end. Each value is a mean of 60 seeds (duplicate of 30 seeds). Values followed by the same letter are not significantly different at $P < 0.001$ by Tukey's test.

Initial seed moisture (%)	Water movement along the abaxial side (mm h^{-1})	
	Tamahomare	Tachinagaha
8	6.14 a	4.49 b
12	4.41 b	4.51 b

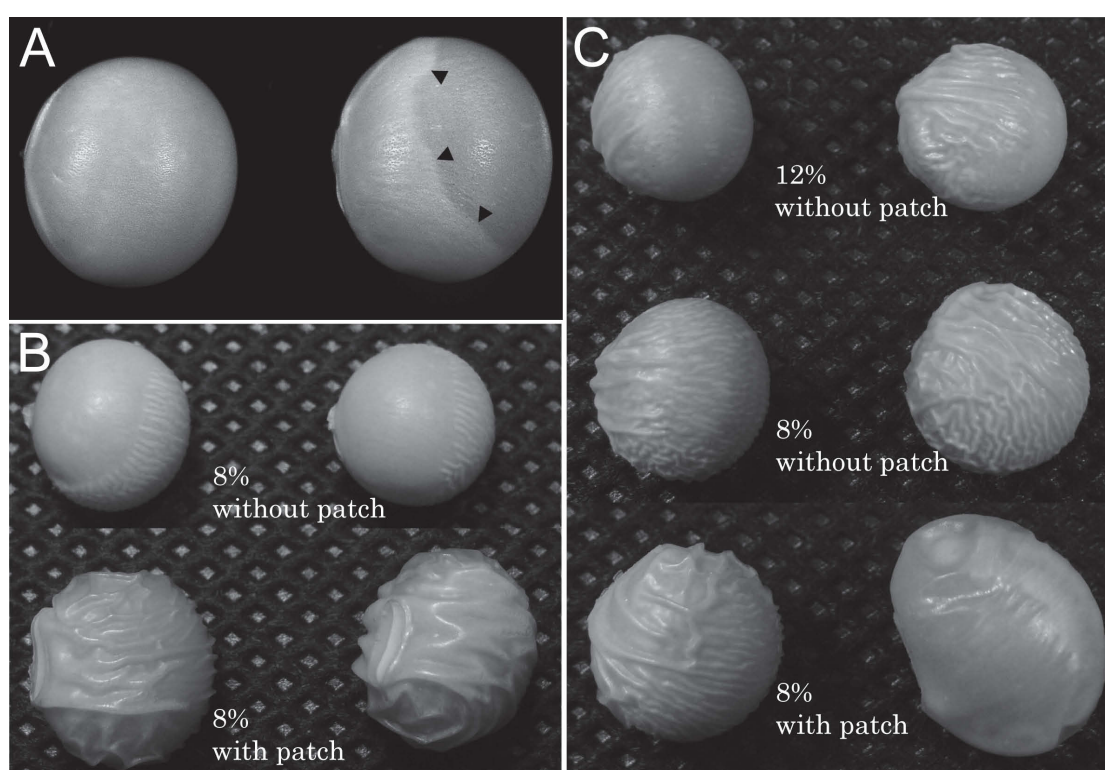


Fig. 5. Loosening of the seed coat after seeds were submerged in water. (A) Dry Tachinagaha seeds with (right) and without (left) a light-colored patch in the seed coat. The border of the patch is indicated by arrowheads. (B) 8%-moisture Tachinagaha seeds without (upper) and with (bottom) the patch after 15 min immersion in water. (C) Tamahomare seeds immersed in water for 15 min. 12%-moisture seeds without the patch (upper) and 8%-moisture seeds without (middle) and with (bottom) the patch.

with no differences according to location (data not shown), suggesting that the patch was not a feature of the seed coat itself but seemed to reflect the state of the adherence of the seed coat to the embryo in a localized region. When the Tachinagaha seeds with and without the patch were immersed in water, the mottled seeds loosened rapidly, resulting in numerous and extensive wrinkles all over the seed coat within 15 min, whereas in those without the patch, the wrinkles formed were localized in the dorsal end (Fig. 5B). In

striking contrast, Tamahomare seeds with or without the patch loosened readily, resulting in wrinkles all over the seed coat, although the wrinkles were less prominent at the 12% moisture level (Fig. 5C).

Discussion

A previous study on flooding injury in soybean seeds (Nakayama et al., 2004) showed that the rates of water uptake by 10%-moisture Tachinagaha and Tamahomare seeds were comparable, but the

susceptibility of seeds to flooding injury varied considerably, being far greater in Tamahomare than in Tachinagaha. The present study showed, however, that the rate of water uptake by Tamahomare seeds was greatly affected by the initial seed moisture content, and that at the 8% seed moisture level the seed coats of Tamahomare seeds did not sufficiently reduce the rate of water uptake, resulting in seeds with intact seed coats showing more rapid imbibition than the embryos with the coats removed (Figs. 1A, B). In Tachinagaha, by contrast, the rate of imbibition was not affected by the initial seed moisture content, a finding that is in good agreement with Obendorf and Hobbs (1970). These results clearly indicate that the seed coats of Tamahomare seeds at low seed moisture levels have a promotive effect on water uptake rather than a protective effect. As it has been previously proposed that the incidence of imbibition damage in seeds is closely associated with the rate of water uptake by the seeds (Powell and Matthews, 1978; Tully et al., 1981; Chachalis and Smith, 2000), this intriguing seed characteristic of Tamahomare seeds upon hydration could partly explain our previous observation that, among yellow-seeded cultivars, Tamahomare seeds have a greater susceptibility to flooding injury (Nakayama et al., 2004).

The rapid imbibition shown by Tamahomare at the low seed moisture level is clearly not due to the characteristics of the embryo (Fig. 1B). Of interest is that 12%-moisture Tamahomare embryos imbibed faster than 8%-moisture embryos, even though the water potential gradient between the embryo and its surroundings should be higher in the drier embryos. A similar observation has been reported by Vertucci and Leopold (1983). Since the low initial seed moisture content had little promotive effect on either the dorsal hydration of the intact seeds or the ventral hydration of the partially decoated seeds (Figs. 3A, B), alterations in the permeability of the seed coat due to the lowering of the seed moisture content also seems unlikely. A possible explanation for the rapid water uptake would be the lateral movement of water along the abaxial sides of the embryo, as shown in Fig. 4 and Table 1. The importance of the seed coat in conducting water circumferentially to the embryo during imbibition has been pointed out in soybeans (McDonald et al., 1988; Chachalis and Smith, 2000; Meyer et al., 2007) and French beans (Powell et al., 1986a). Our observation that the presence of the intact seed coat had a promotive effect on water uptake in Tamahomare at the low seed moisture level provides further support for the occurrence of such a flow of water in the area between the coat and the embryo.

In seeds of certain leguminosae such as peas (Powell and Matthews, 1978) and French beans (Powell et al., 1986a; 1986b), the seed coats are known to play a protective role in preventing the rapid inrush of water

into the seeds during imbibition. The soybean is a leguminous species, and hence promotion of water uptake by the seed coat per se does not occur (Tully et al., 1981; Duke and Kakefuda, 1981). In a very few cases, however, the opposite has been observed, particularly where a loose adherence of the testa to the embryo is observed. Chachalis and Smith (2000) reported that one black-seeded soybean accession with the testa intact took up water more rapidly than its embryo with the testa removed due to an increased water flow through a gap created between the seed coat and the embryo, probably due to the loose adherence of the testa. Powell et al. (1986a) observed that the difference in the rates of imbibition between French bean cultivars resulted from the relative ease with which water could gain access between the seed coat and the embryo, and they concluded that the degree of adherence of the seed coat to the embryo was a major factor determining the formation of the gap and therefore the rates of imbibition of the cultivars.

In addition to the degree of adherence of the seed coat to the embryo, wrinkling (loosening) of the coat upon hydration also has a role in determining the ease with which water enters the seed (Powell, 1989). Since wrinkles are sufficient to separate the coat from the embryo, creating a wide gap between them, while loose adherence of the testa to the embryo could in turn facilitate the wrinkling of the testa, the degree of adherence and the ease of wrinkling of the seed coat interact with each other to determine the extent of lateral water movement through the resultant gap during imbibition.

A dramatic difference in the wrinkle formation on the seed coat was observed between Tachinagaha seeds with and without a light-colored patch (Fig. 5B). In the seeds without the patch the wrinkles in the coat were localized in the dorsal end, which is consistent with the general observation on soybean seeds submerged in water (McDonald et al., 1988; Meyer et al., 2007), whereas in the mottled coats this was not the case and the wrinkles were more numerous and extensive, involving the whole seed within 15 min of immersion (Fig. 5B). Since the patch in the coat seems to reflect the state of the adherence of the coat to the embryo in a localized region, these findings suggest that close adherence of the coat to the embryo could contribute to preventing the coat from rapid loosening. In striking contrast, Tamahomare seeds even without the patch loosened immediately after the seeds were immersed in water, suggesting that the seed coat of the Tamahomare seeds, compared to that of Tachinagaha seed, probably adheres very loosely to the embryo or has a greater tendency to loosen upon hydration, or both. Further, in Tamahomare seeds without the patch, the extent of the wrinkle formation on the coat was less prominent at 12% moisture level than

at 8% level (Fig. 5C). Although it was reported that the seed moisture content had little influence on the rate of imbibition by the seeds (Obendorf and Hobbs, 1970) and has not been demonstrated so far that the seed moisture content extends its influence on the adherence of the coat to the embryo and/or the degree of the wrinkling of the coat, our results showed that this could happen in some soybean cultivars. These seed coat characteristics of Tamahomare seed might aid in allowing water access between the coat and the embryo as evidenced by Table 1 and thus allow more water to penetrate into the seed of this cultivar at low seed moisture levels.

The intact seeds took up water much faster from the ventral end than from the dorsal end (compare Fig. 1A and Fig. 3A), a finding that supports the previous hypothesis that the hilum-micropyle region is a major route of water entry into the seed (McDonald et al., 1988; Pietrzak et al., 2002). On the other hand, it is also of interest that the ventral hydration of the partially decoated seeds (Fig. 3B) was substantially slower than that of the intact seeds. The permeabilities to water of the hilum-micropyle region and the dorsal region were found to be almost identical through up to 6 hr of imbibition (Figs. 3A, B). Hence, the hilar hydration depends little on the permeability of the hilum-micropyle region itself and is largely dependent on the presence of the seed coat, that is, on the transmission of water to the embryo via the gap between the coat and the embryo. Chachalis and Smith (2000) reported that the hilum-micropyle region played little or no role in water uptake compared to other regions of the seed coat, a finding that is inconsistent with previous studies. The data presented herein would help resolve the inconsistency among the previous studies.

Powell (1989) reported that in pea seeds the adherence of the seed coat was associated with the color of the seed coat. On the other hand, Chachalis and Smith (2000) observed marked differences among black-coated soybean accessions in the rate of imbibition, probably resulting from the differences in the adherence of the seed coat to the embryo. The present study also showed that differences in the adherence of the seed coat may exist among yellow-seeded cultivars. These findings emphasize that the adherence of the seed coat to the embryo is not associated exclusively with the pigmentation of the seed coat, at least in soybeans. Therefore, focusing on seed coats that adhere tightly to the embryo and/or loosen only slightly might provide insight into the improved tolerance of seeds to flooding injury among agronomically important yellow-seeded soybeans.

In conclusion, the degree of the adherence of the coat to the embryo and/or the degree of the wrinkling of the coat upon hydration appear to play a crucial role in determining the rate of water uptake in yellow-

seeded soybean cultivars. Although the present study has not estimated the topographical presence or the progress of water during imbibition, further study in which such methods are applied should provide sound observations of “where and how much” water is present in the seed during imbibition. In our future study we will concentrate on measuring the degrees of adherence and the wrinkling using developing methodologies and demonstrating their clear association with the rate of water uptake.

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