

A COMPARISON STUDY OF PERMEABLE AND IMPERMEABLE SEED COATS OF LEGUME SEED CROPS REVEALS THE PERMEABILITY RELATED STRUCTURE DIFFERENCE

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Abstract

Impermeability of Legume seeds is commonly related to physical dormancy, which is useful for seed preservation but problematical for the soybean food industry. The primary aim of this study was to characterize the structure difference between permeable and impermeable seed coats, revealing the water-controlling structures and demonstrating the water uptake process. Three measurements of water uptake for four Legume species were carried out. The general anatomy of micropyle and seed coat were observed by scanning electron microscopy (SEM). Dye tracking was carried out to investigate the pathway of water movement during imbibition. The mature seed coat of four species contained three layers of cells. SEM observations revealed that the impermeable coat differs from the permeable one. Layer thickness has no direct relationship to the permeability of Legume seeds. The micropyle opening could be observed in permeable seed coats. Osteosclereids could impede the uptake of water; the layer of the osteosclereid near the embryo is hydrophilic and distal side is hydrophobic. The present research provided a variety of information related to the permeability of Legume seeds. The coat structures of permeable and impermeable micropyles are different in several aspects. A novel finding is that the outer surface of the osteosclereid layer is essential to permeability.

Key words: Impermeable seeds; Micropyle; Osteosclereid; Permeable seeds; Seed coat.

Introduction

Legumes are important for maintenance of human health and as crops for sustainable agriculture (Komatsu & Ahsan, 2009). For example, soybean (*Glycine max*) is the world's largest oil seed and legume crop, with very considerable commercial value (Rahim *et al.*, 2017). It is the source of many foods, including soymilk, tofu and miso, and is also the main source of protein in livestock feed supplements. Legume seed coats can be either permeable or impermeable. Impermeable seeds do not imbibe water even after soaking for a considerable time. Under natural conditions, the impermeability could be beneficial to the plant because it can preserve the seeds for a long time (Tyler, 1997). However, impermeability prolongs the hydration step, which is the key processing step in the soybean food industry. Four Legume species have been selected for seeds with the ability to absorb water quickly and evenly. However, some seeds from these species will be impermeable (otherwise called stone or hard seeds), and it has been reported that a large proportion of hard seeds could be produced by species with otherwise desirable agronomic traits (Rolston, 1978). To characterize the permeability and impermeability of soybean seeds is a key step in developing methods to handle different types of seeds for large-scale processes, and to design potential breeding strategies for the future.

In recent decades, several theories were developed to explain why soybean seeds could have different permeabilities to water. Some early studies suggested that this could be due to tightly bound palisade cells (Ballard, 1973; Corner, 1951). Subsequently, attention was paid to the thickness of the seed coat and it was considered that a

thicker coat would result in greater impermeability (Miao *et al.*, 2001; Wyatt, 1977). Intriguingly, some minor discrepancies also were considered to be the causes. Impermeable seed coats were supposed to lack pits compared with permeable ones (Chachalis & Smith, 2001; Hahalis *et al.*, 1996; Harris, 1987; Yaklich *et al.*, 1986). Endocarp deposits could be found in impermeable seed coats (Calero *et al.*, 1981) and it was also noted that dark color was a trait of impermeable seed coats (Wyatt, 1977). By using microscopic analysis, it was found that the hilum and/or micropyle of impermeable seed coats are normally closed (Ballard, 1973; Hyde, 1954; Rolston, 1978). Some features of the palisade cells such as the outer tangential walls and light line have also been considered as causes for seed coat impermeability (Bhalla & Slattery, 1984; Harris, 1987; Serrato-Valenti *et al.*, 1993; Werker *et al.*, 1979). On the other hand, there were several debatable points regarding to the function of some specific structures of seed coat in water uptake, for example, the outer cuticle had been considered important by some researchers (Arechavaleta-Medina & Snyder, 1981; Ragus, 1987), but not by others (Ballard, 1973; Chachalis & Smith, 2001; Werker, 1980). However, when water touches the seeds, the role of the micropyle, hilum and raphe were ignored, especially the micropyle. The permeability of the seed coat is believed to be related to its structure (Gama-Arachchige *et al.*, 2010). The seed coat of a legume consists of the hilum, micropyle, raphe and the extrahilar region. When the funiculus detaches from the mature seed, a scar-like structure appears, known as the hilum. During seed germination, the radicle emerges from the pore of the micropyle. The micropyle is formed during earlier ovule development from the

integuments. The raphe is a ridge on the seed coat, on the other side to the micropyle. Research by Ma *et al.* (2004) has expanded what is known about how the structure of the seed coat affects water uptake, but the details of how this is controlled are still debatable. In permeable seed coats, the micropyle should be the initial site of water entry (Van Staden *et al.*, 1989); however, no report has experimentally demonstrated the relationship between the micropyle and the seed coat in impermeable seeds.

Thus, the purpose of this study is to compare the impermeable and permeable seed coats, as well as the micropyle, and try to provide a basic understanding of the association between seed coat structure and seed permeability. Specific objectives were to answer four questions (1) What are the differences in structure between permeable and impermeable seed coats and micropyle? (2) Are there some water-controlling structures in the impermeable seed coat? (3) Does the micropyle of impermeable seed coats also play a function in water absorption? (4) What is the pathway of water movement during the water absorption of soybean seeds? Previous studies on Leguminosae have investigated the permeability using only one soybean cultivar. To provide a more general view, this study employed four soybean cultivars i.e., four edible legume species to research this topic, trying to help us better understand the mechanism of permeability and offer possibilities for further seed breeding and genetic modifications.

Materials and Methods

Plant material: Seeds of two species, mung bean (*Vigna radiata*) and cow pea (*Vigna unguiculata*) were obtained from plants grown outdoors at the Field Station in Changling county of Jilin province, northeast of China (44°12'11" N, 123°51'02" E), in 2011. The other two commercial species, small red bean (*Phaseolus angularis*) and black soybean (*Glycine max*), were purchased from a seed store. The four species all belong to the Leguminosae (Fabaceae) family. Seeds were handpicked from mature pods, air-dried, and kept under controlled conditions (23°C; 30–50% relative humidity) before use (Meyer *et al.*, 2007). All seeds used for experiments were stored for less than 4 months (Jayasuriya *et al.*, 2007). Seeds were examined microscopically and any seeds with visible defects were discarded. Seeds were soaked in water for 24 hours to distinguish between permeable and impermeable types. Those that did not increase in weight or volume, and had neither cracks nor wrinkles were considered to be impermeable. Otherwise, seeds were deemed permeable. All seeds were air-dried to constant weight for half a month for the subsequent experiments.

Measurement of water uptake: The imbibition test was performed following the method of Ma *et al.* (2004). One thousand seeds of each species were soaked in water for 48 hours at room temperature (25°C) and the increase in weight of both permeable and impermeable seeds was recorded. The test was replicated three times.

Additionally, to determine which part of the permeable seeds, micropyle or seed coat, influenced the ability to take up water, another 50 seeds from each

species were used for the “up-down” test, with three replications. The seeds were put micropyle upward or downward separately on water-holding flower foam. Foam was soaked in water for 1 hours, the seeds were measured placed on top of the foam for 24 hours, and water hydration was recorded.

General anatomy of seed coat: Following immersion in liquid nitrogen for 2-3 seconds, several permeable and permeable seeds of four species were cut in the same manner so that the seed coat, adaxial areas and micropyle could be observed (Fig. 1). For scanning electron microscopy (SEM) observations (Kanwal *et al.*, 2016), all seed samples were dehydrated in absolute alcohol and cleared in xylene. Particularly, impermeable seeds were soaked in the solution of absolute ethyl alcohol and glycerin (1:1) for 5 days to become soft. Scanning electron micrographs were prepared following the method of Gama-Arachchige *et al.* (2010). Briefly, samples were sputter-coated with gold-palladium (15 nm), and scanned with an S-3000N Hitachi scanning electron microscope at an acceleration voltage of 5.0 kV.

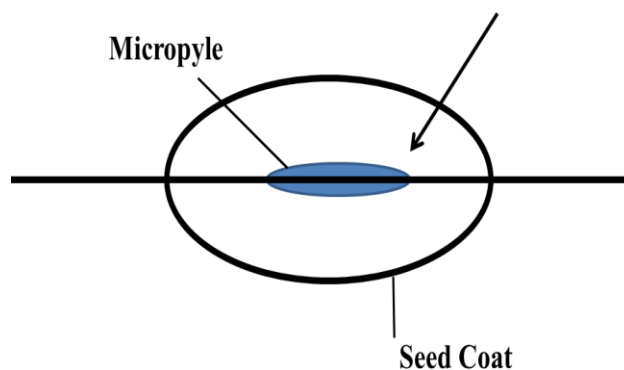


Fig. 1. Direction of cutting (arrow) for examination of seed anatomy.

Water absorbing feature of osteosclereids in permeable seeds: To expose the inner side of the osteosclereids, 10 impermeable seeds were emptied by using a toothdrill to dislodge the embryo under a dissecting microscope. Another 10 impermeable seeds were treated by removing the outside two layers of the seed coat to expose the outer surface of the osteosclereids. Water was then dropped on to both sides of the osteosclereids to study their water-absorbing features.

Dye tracking of the pathway of water imbibition: Dye-tracking experiments (Gama-Arachchige *et al.*, 2010), using mung bean as the plant material, were carried out to track water flow in the seed coat of permeable seeds during the process of imbibition. Methylene blue solution with high concentration was used as dye. After the dormancy was broken by soaking, mung bean seeds were dipped in a concentrated solution of methylene blue. Seeds blotted with tissue paper were removed after the initial 5 minutes and then every 15 min for 4 hr for fluorescence tracking. Seeds were longitudinally cut into two halves at the micropylar and chalazal regions (Fig. 1), and the exposed surfaces were examined using the light microscope with the external light source. The micrographs were taken as the dye travelled.

Table 1. Hydrating percent of seeds by different treatments.

Species	Up (%)	Down (%)	All (%)
Mung bean (<i>Vigna radiata</i>)	10.65 ± 3.67*	91.67 ± 5.23*	99.52 ± 1.67
Small red bean (<i>Phaseolus angularis</i>)	2.39 ± 2.00**	52.24 ± 3.12**	95.47 ± 1.35
Cow pea (<i>Vigna unguiculata</i>)	26.47 ± 1.12*	69.23 ± 1.71**	74.79 ± 3.23*
Black soybean (<i>Glycine max</i> var.)	98.08 ± 1.02**	100 ± 0**	96.88 ± 1.1

Note: *Significant difference at $p < 0.05$, ** Significant difference at $p < 0.01$.

Up or down refers to the orientation of the micropyle. All refers to the total immersion of seeds

Table 2. Thickness of osteosclereid layer between permeable and impermeable seeds of four species.

Species	Thickness of osteosclereid layer (μm)	
	Permeable	Impermeable
Mung bean (<i>Vigna radiata</i>)	15.34 ± 0.82	6.41 ± 0.64**
Small red bean (<i>Phaseolus angularis</i>)	12.82 ± 0.81	13.69 ± 1.44*
Cow pea (<i>Vigna unguiculata</i>)	15.53 ± 0.38	17.39 ± 1.12*
Black soybean (<i>Glycine max</i> var.)	22.22 ± 0.48*	21.25 ± 1.07*

Note: *Significant difference at $p < 0.05$, **Significant difference at $p < 0.01$

Statistical analysis: Hydrating and seed structure data were analyzed by using SPSS (version 11.5, SPSS Inc., Chicago, Illinois, USA). A LSD test was used to determine least significant range between means ($p < 0.05$ and $p < 0.01$). These data were analyzed using SPSS (version 11.5, SPSS Inc., Chicago, Illinois, USA).

Results

Seed hydration: Among the four species, the seeds of mung bean have the greatest water imbibition ability (Table 1). After being soaked in water for 24 hours, only five of 1000 green gram seeds were completely impermeable. In contrast, cow pea is the most impermeable species of the four studied (Table 1). Seeds placed with the micropyle downward have obvious higher hydrating rate compared with seeds placed with the micropyle upward, suggesting the micropyle of the permeable seed coat might have a function in water absorption. In addition, hydrating parts of permeable seeds were wrinkled on the surface (Fig. 2). This result provides evidence that both the seeds coat and micropyle of permeable seeds have the ability to take up water.

Differences in structure of cell layers between permeable and impermeable seeds: The seed coats of mature seeds of all four species consist of three layers of cells. These are epidermis cells (outermost layer), palisade cells and osteosclereids (innermost layer). SEM observations revealed that the thicknesses of these layers differs between permeable and impermeable seeds in the four species studied (Fig. 3). Surprisingly, permeable seeds of mung bean and black soybean have thinner osteosclereids than their impermeable counterparts (Table 2). Impermeable seeds generally contain a thicker palisade layer; the only exception is cow pea (Table 3).

Table 3. Thickness of palisade layer between permeable and impermeable seeds of four species.

Species	Thickness of palisade layer (μm)	
	Permeable	Impermeable
Mung bean (<i>Vigna radiata</i>)	24.67 ± 0.67*	31.35 ± 0.96*
Small red bean (<i>Phaseolus angularis</i>)	44.35 ± 1.63*	66.13 ± 0.72**
Cow pea (<i>Vigna unguiculata</i>)	35.29 ± 0.76*	24.07 ± 1.17*
Black soybean (<i>Glycine max</i> var.)	20.83 ± 0.38*	32.87 ± 0.46*

Note: *Significant difference at $p < 0.05$, **Significant difference at $p < 0.01$

Differences in micropyle morphology between permeable and impermeable seeds: Cow pea was used to investigate the role of the micropyle in seed permeability, as it is the only species whose micropyle could be observed easily. The micropyle in permeable seeds was clearly observed as an open gap, whereas the impermeable one was closed (Fig. 4). These result indicate that transformation of the micropyle might be positively associated with permeability. However, we could not know whether change of morphology in the micropyle caused seeds to become permeable, or vice versa.

Water impediment of osteosclereids: To demonstrate how osteosclereids could have the ability to impede water uptake, we designed two treatments to investigate if the two sides of osteosclereids have different hydrophilicity (see the Materials and Methods section). Water rolling could be observed on the inner side of osteosclereids whereas water spreading could be detected on the other side. The results showed that the layer of osteosclereids near to the embryo is hydrophilic while the part near the palisade cell is hydrophobic.

Dye tracking of water movement: The path of water as it moves through the seed coat and micropyle of permeable seeds was followed using methylene blue dye. After 5 minutes, fluorescence tracking the regions of the micropyle and chalaza turned green, providing evidence that the micropyle was the site of entry of the water (Fig. 5). After 4 hours, water moved into the seed through the micropyle and could be tracked through the seed coat (Fig. 6). Clearly, osteosclereids could impede the water pathway during imbibition (Fig. 6).

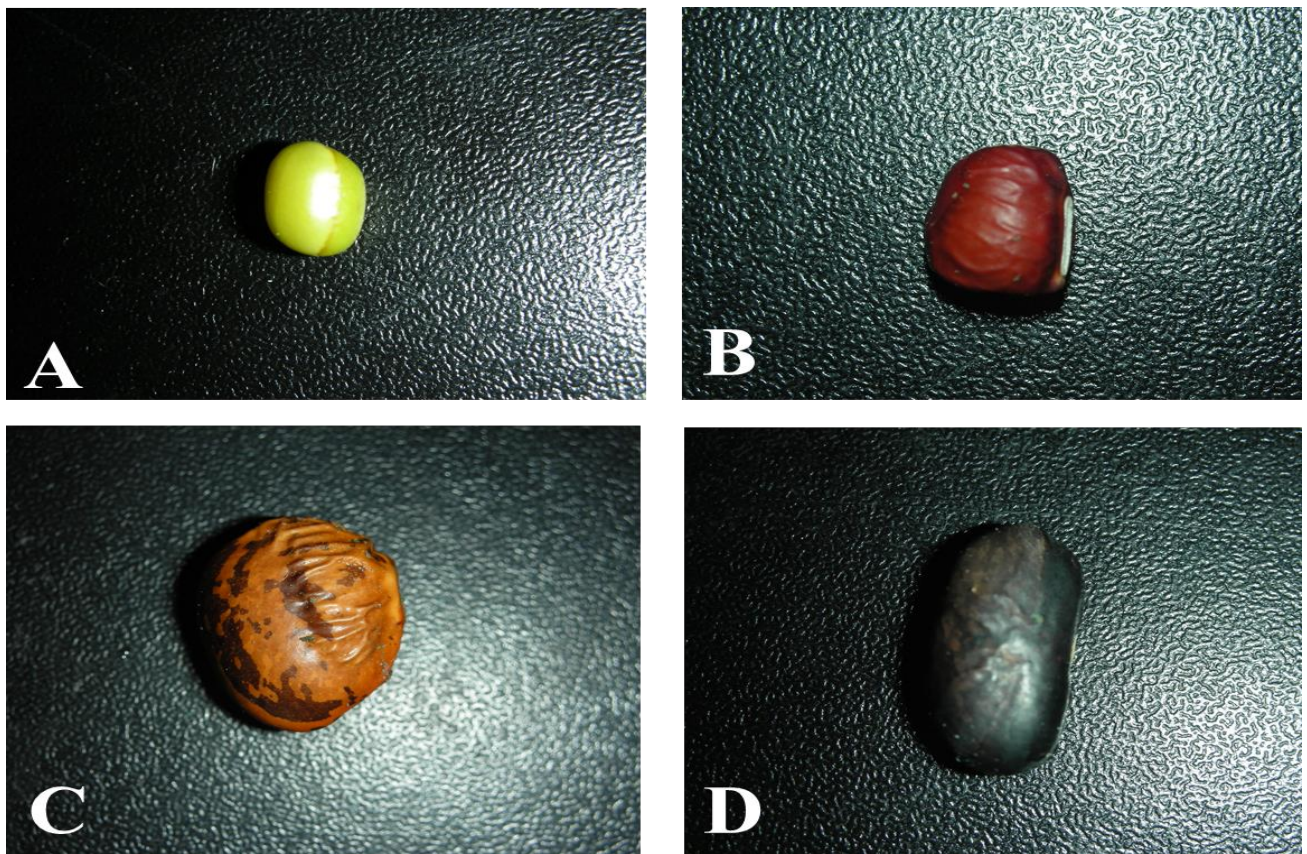


Fig. 2. Surfaces of permeable seed coats. A, mung bean; B, small red bean; C, cow pea; D, black soybean.

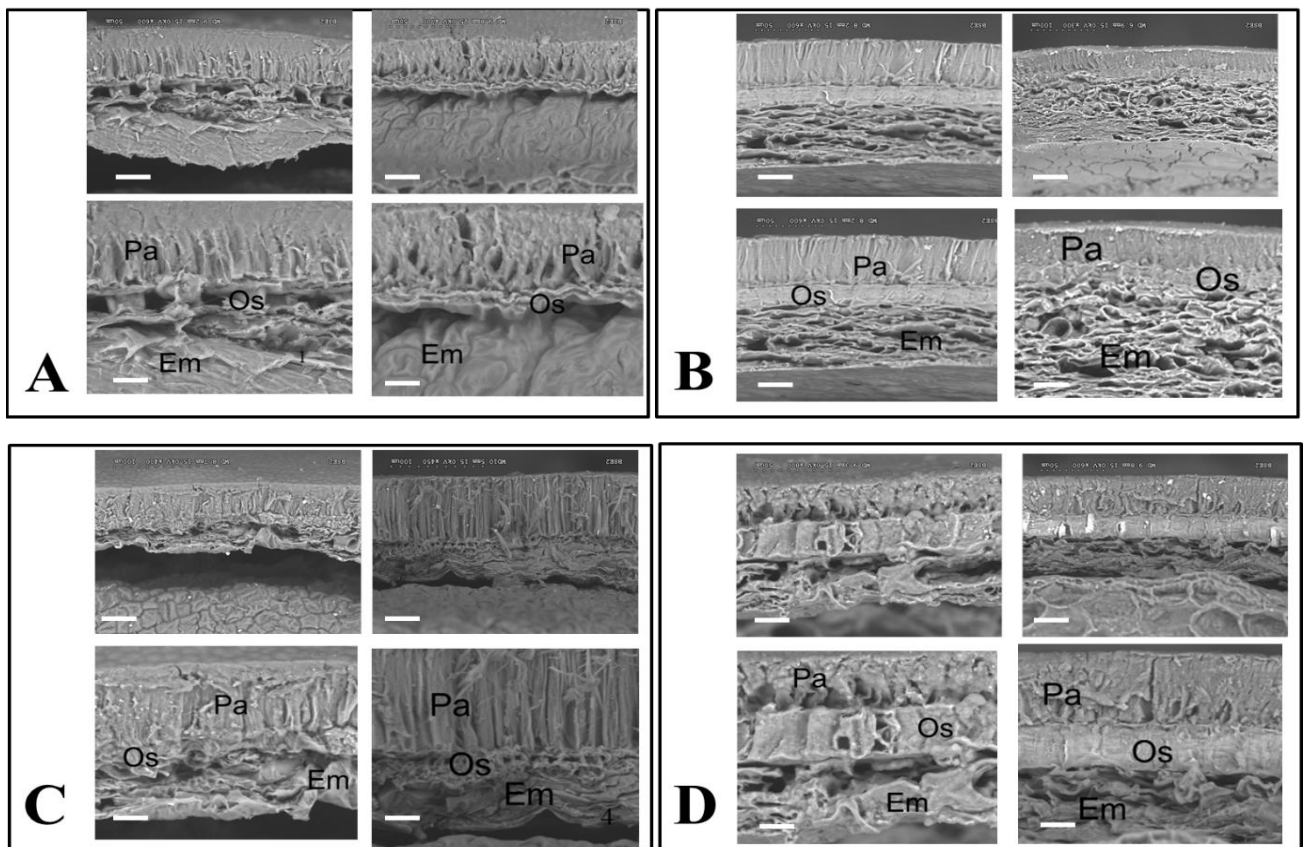


Fig. 3. Longitudinal sections of seeds of four species. A, mung bean; B, cow pea; C, small red bean; D, black soybean. Left, permeable; right, impermeable (A-D). Top: scale bar = 50 μ m; Bottom: scale bar = 100 μ m. Em, embryo; Os, osteoscleroid; Pa, palisade cell.

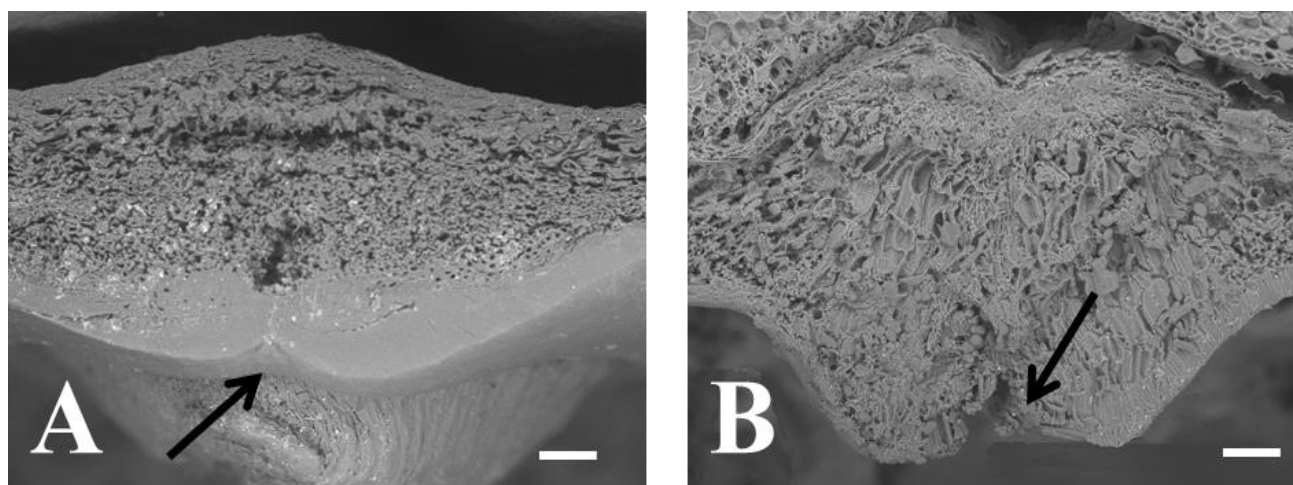


Fig. 4. Longitudinal sections of cow pea. A, closed micropyle (arrow); B, open micropyle (arrow). Scale bar = 50 μ m.

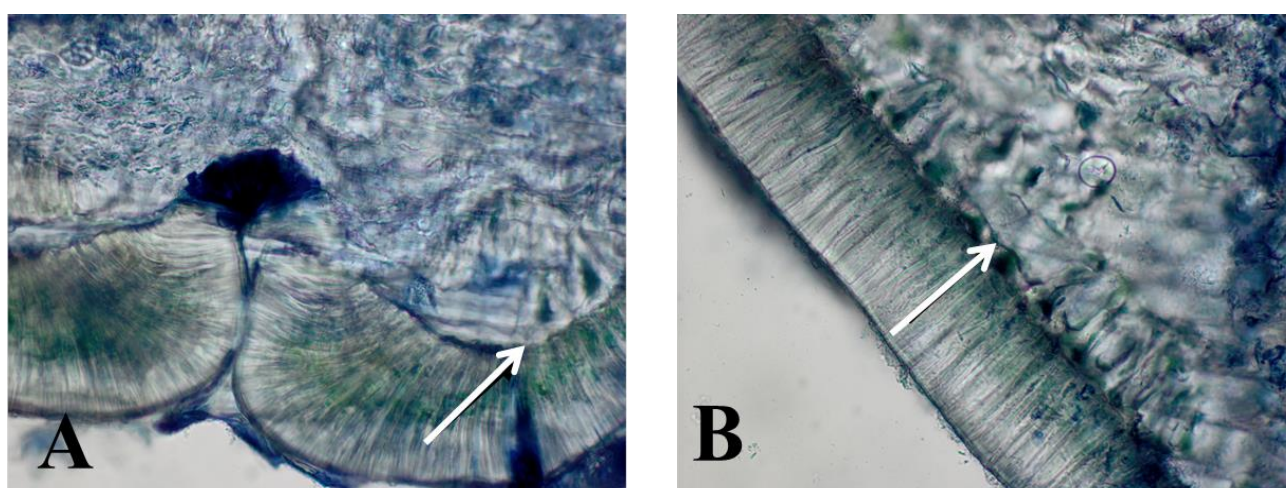


Fig. 6. Water movement pathway. Arrows indicate the osteosclereids. A, micropyle; B, seed coat.

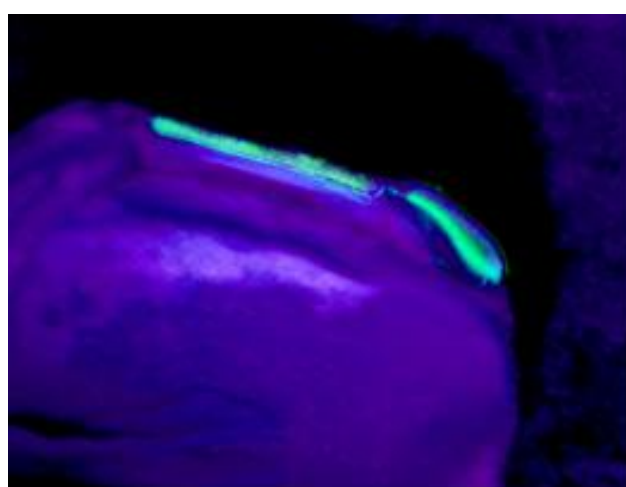


Fig. 5. Fluorescence tracking the in mung bean seed showing micropylar and chalazal regions.

Discussion

In this study, we provide evidence that the coat structure is somewhat different between permeable seeds and impermeable seeds, especially the thickness of several cell layers. For the micropyle, we could observe

that permeable seeds have an open micropyle, implying the transformation of the micropyle might play a role in the change of impermeability. We also identified osteosclereids are one of the coat structures which can impede water uptake. Against our common knowledge, we found that the micropyle of permeable seed coats can still play a role in water absorption function. Finally, by using dye tracking technology, we showed the pathway of water movement during imbibition.

Earlier studies reported that when permeable seeds take up water the seed coat becomes wrinkled on the dorsal side (Ma *et al.*, 2004; Meyer *et al.*, 2007). Subsequent to the wrinkling, the volumes of seeds increased and the expansion force was enough to separate the seed coat from its embryo. Other studies also reported on the polymorphism and anatomical differences between the two types of seed coat (Kelly *et al.*, 1992; Valenti *et al.*, 1989). However, detailed research is absent since no special alterations could be perceived in the impermeable seeds after the water comes in contact with the seed coat. Therefore, the present work used SEM to compare the morphology of the seed coat in permeable and impermeable seeds. As we expected, the thickness of the seeds coat is different between permeable and impermeable seeds. However, according to our data, we

cannot conclude that coat thickness is the crucial reason for seed permeability (Tables 2 and 3). Some investigators proposed the idea that cell layers in the seed coat are essential structures that determine impermeability in hard seeds (Baskin & Baskin, 2001; Baskin *et al.*, 2000; Bevilacqua *et al.*, 1987; Corner, 1951; Graaff & Van Staden, 1983; Hyde, 1954; Van Staden *et al.*, 1989; Werker *et al.*, 1979). In this study, we not only confirmed that osteosclereids could impede water absorption but also clarified that the outer hydrophobic side of osteosclereids should be the key factor for impeding water uptake. These results provide new thoughts about the question of what structure controls seed permeability.

As we know, one or more layers of palisade cells in the seed that are not permeable to water could induce physical dormancy (Baskin *et al.*, 2000). Physical dormancy is present in Angiospermae but not in Gymnospermae (Baskin *et al.*, 2000), including one family of the Monocotyledonae and 16 of the Eudicotidae (Baskin, 2003; Baskin *et al.*, 2006; Horn, 2004; Nandi, 1998). Combinational dormancy has been observed in some families, in which species with physical dormancy and with physiological dormancy are both existed, as well as species that do not exhibit dormancy (Baskin *et al.*, 2000). Only physical dormancy in Dipterocarpaceae, Sarcolaenaceae and Sphaerosepalaceae of the 16 eudicots is determined by seed-coat anatomy (Horn, 2004). (AU: the meaning of this sentence was not clear to me, can the word "Only" be deleted? Can this sentence be deleted, it's not clear how it relates to the study) Even with the favorable environmental conditions, seeds with physical dormancy are not able to imbibe water due to the water-impermeable layer(s) of cells or the featured structures such as the water gaps which serve as environmental "signal detectors" during the process of germination (Baskin *et al.*, 2000; Gama-Arachchige *et al.*, 2010). However, when the closed water gap opens, the physical dormancy is broken, water will be quickly absorbed by a hard seed and germination will ensue (Baskin *et al.*, 2000). For seeds such as soybean, there is no physiological dormancy; but if conditions are favorable, germination will still not proceed if it is prevented by the seed coat. (AU: reworded to prevent similarity, check if the edit preserves your intended meaning) During dormancy, the seeds of four species in this study not only had layers of impermeable cells in the seed coat, but also had impermeable micropyles. These results confirm that micropyle and seed coat could determine the dormancy of most soybean seeds.

To better utilize the permeability of Legume seeds, we propose that future work should focus on the molecular level since permeability or hardness of Legume seeds is influenced by genetic factors in addition to environmental factors (Hill *et al.*, 1986; Keim *et al.*, 1990). The observed differences in seed permeability could be caused by multiple genes and so the permeability was called a quantitative trait (Kilen & Hartwig, 1978; Liu *et al.*, 2007). Disappointingly, no genes affecting seed permeability have been identified so far. A reasonable method could be to use the similar research on *Arabidopsis*. Mutations in several genes of *Arabidopsis* have been shown to influence seed permeability. For example, seeds in which the seed coat proanthocyanidin biosynthesis gene has been knocked out are more permeable than those of wild-type plants (Haughn & Chaudhury, 2005).

Acknowledgements

We are grateful to Professor Carol Baskin, Professor Jerry Baskin and Dr. Gama-Arachchige, Department of Biology, University of Kentucky, for their technical support during the course of experiments. This research was supported by National Natural Science Foundation of China (Grant No. 31600364).

References

- Arechavaleta-Medina F. and H. Snyder. 1981. Water imbibition by normal and hard soybeans. *J. Amer. Oil Chemists Soc.*, 58: 976-979.
- Ballard, L. 1973. Physical barriers to germination. *Seed Sci. & Technol.*, 1: 285-303.
- Baskin, C.C. 2003. Breaking physical dormancy in seeds - focussing on the lens. *New Phytologist*, 158: 229-232.
- Baskin, C.C. and J.M. Baskin. 2001. *Seeds: ecology, biogeography, and evolution of dormancy and germination*: Elsevier.
- Baskin, J.M., C.C. Baskin and K.W. Dixon. 2006. Physical dormancy in the endemic Australian genus *Stylobasium*, a first report for the family Surianaceae (Fabales). *Seed Sci. Res.*, 16: 229-232.
- Baskin, J.M., C.C. Baskin and X. Li. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biol.*, 15: 139-152.
- Beisson, F., Y. Li, G. Bonaventure, M. Pollard and J.B. Ohlrogge. 2007. The acyltransferase GPAT5 is required for the synthesis of suberin in seed coat and root of *Arabidopsis*. *Plant Cell.*, 19: 351-368.
- Bevilacqua, L.R., F. Fossati and G. Dondero. 1987. 'Callose' in the impermeable seed coat of *Sesbania punicea*. *Ann. Bot.*, 59: 335-341.
- Bhalla, P. and H. Slattery. 1984. Callose deposits make clover seeds impermeable to water. *Ann. Bot.*, 53: 125-128.
- Calero, E., S. West and K. Hinson. 1981. Water absorption of soybean seeds and associated causal factors. *Crop Sci.*, 21: 926-933.
- Chachalis, D. and M. Smith. 2001. Seed coat regulation of water uptake during imbibition in soybeans (*Glycine max* (L.) Merr.). *Seed Sci. & Technol.*, 29: 401-412.
- Corner, E. 1951. The leguminous seed. *Phytomorphology*, 1: 117-150.
- Franke, R., I. Briesen, T. Wojciechowski, A. Faust, A. Yephremov, C. Nawrath and L. Schreiber. 2005. Apoplastic polyesters in *Arabidopsis* surface tissues - A typical suberin and a particular cutin. *Phytochem.*, 66: 2643-2658.
- Gama-Arachchige, N., J. Baskin, R. Geneve and C. Baskin. 2010. Identification and characterization of the water gap in physically dormant seeds of Geraniaceae, with special reference to *Geranium carolinianum*. *Ann. Bot.*, 105: 977-990.
- Graaff, J. and J. Van Staden. 1983. The Effect of different chemical and physical treatments on seed coat structure and seed germination of *Sesbania* species. *Zeitschrift für Pflanzenphysiologie*, 112: 221-230.
- Hahalis, D., M. Cochrane and M. Smith. 1996. Water penetration sites in the testa of soybeans (*Glycine max* L. Merrill) during seed imbibition. *The Science of Legumes*, 3: 218-226.
- Harris, W.M. 1987. Comparative ultrastructure of developing seed coats of "hard-seeded" and "soft-seeded" varieties of soybean, *Glycine max* (L.) Merr. *Botanical Gazette*, 148: 324-331.

- Haughn, G. and A. Chaudhury. 2005. Genetic analysis of seed coat development in Arabidopsis. *Trends in Plant Sci.*, 10: 472-477.
- Hill, H., S. West and K. Hinson. 1986. Effect of water stress during seedfill on impermeable seed expression in soybean. *Crop Sci.*, 26: 807-812.
- Horn, J.W. 2004. The morphology and relationships of the Sphaerosepalaceae (Malvales). *Bot. J. of the Linn. Soc.*, 144: 1-40.
- Hyde, E. 1954. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. *Ann. Bot.*, 18: 241-256.
- Jayasuriya, K.M.G.G., J.M. Baskin, R.L. Geneve and C.C. Baskin. 2007. Morphology and anatomy of physical dormancy in *Ipomoea lacunosa*: Identification of the water gap in seeds of Convolvulaceae (Solanales). *Ann. Bot.*, 100: 13-22.
- Kanwal, D., R. Abid and M. Qaiser. 2016. The seed Atlas of Pakistan-XIV. Polygonaceae. *Pak. J. Bot.*, 48(5): 1833-1848.
- Keim, P., B. Diers and R. Shoemaker. 1990. Genetic analysis of soybean hard seededness with molecular markers. *Theor. & App. Genetics*, 79: 465-469.
- Kelly, K., J. Van Staden and W. Bell. 1992. Seed coat structure and dormancy. *Plant Growth Reg.*, 11: 201-209.
- Kilen, T. and E. Hartwig. 1978. An inheritance study of impermeable seed in soybeans. *Field Crops Res.*, 1: 65-70.
- Komatsu, S. and N. Ahsan. 2009. Soybean proteomics and its application to functional analysis. *J. Proteomics*, 72: 325-36.
- Liang, M., E. Davis, D. Gardner, X. Cai and Y. Wu. 2006. Involvement of AtLAC15 in lignin synthesis in seeds and in root elongation of Arabidopsis. *Planta*, 224: 1185-1196.
- Liu, B., T. Fujita, Z.H. Yan, S. Sakamoto, D. Xu and J. Abe. 2007. QTL mapping of domestication-related traits in soybean (*Glycine max*). *Ann. Bot.*, 100: 1027-1038.
- Ma, F., E. Cholewa, T. Mohamed, C.A. Peterson and M. Gijzen. 2004. Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. *Ann. Bot.*, 94: 213-228.
- Meyer, C.J., E. Steudle and C.A. Peterson. 2007. Patterns and kinetics of water uptake by soybean seeds. *J. Exp. Bot.*, 58: 717-732.
- Miao, Z.H., J.A. Fortune and J. Gallagher. 2001. Anatomical structure and nutritive value of lupin seed coats. *Aust. J. Agri. Res.*, 52: 985-993.
- Nandi, O.I. 1998. Ovule and seed anatomy of Cistaceae and related Malvaceae. *Plant Sys. & Evol.*, 209: 239-264.
- Ragus, L. 1987. Role of water absorbing capacity in soybean germination and seedling vigour. *Seed Sci. & Technol.*, 15: 285-296.
- Rahim N., M.K. Abbasi and S. Hamee. 2017. Variability in the growth and nodulation of soybean in response to elevation and soil properties in the Himalayan region of Kashmir-Pakistan. *Pak. J. Bot.*, 49(1): 237-247.
- Ranathunge, K., S. Shao, D. Qutob, M. Gijzen and C.A. Peterson. 2010. Properties of the soybean seed coat cuticle change during development. *Planta*, 231: 1171-1188.
- Rolston, M.P. 1978. Water impermeable seed dormancy. *The Botanical Review*, 44: 365-396.
- Schmutz, J., S.B. Cannon, J. Schlueter, J. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q. Song, J.J. Thelen, J. Cheng, D. Xu, U. Hellsten, G.D. May, Y. Yu, T. Sakurai, T. Umezawa, M.K. Bhattacharyya, D. Sandhu, B. Valliyodan, E. Lindquist, M. Peto, D. Grant, S. Shu, D. Goodstein, K. Barry, M. Futrell-Griggs, B. Abernathy, J. Du, Z. Tian, L. Zhu, N. Gill, T. Joshi, M. Libault, A. Sethuraman, X.C. Zhang, K. Shinozaki, H.T. Nguyen, R.A. Wing, P. Cregan, J. Specht, J. Grimwood, D. Rokhsar, G. Stacey, R.C. Shoemaker and S.A. Jackson. 2010. Genome sequence of the palaeopolyploid soybean. *Nature*, 463: 178-183.
- Serrato-Valenti, G., L. Cornara, M. Ferrando and P. Modenesi. 1993. Structural and histochemical features of *Stylosanthes scabra* (Leguminosae; Papilionoideae) seed coat as related to water entry. *Can. J. Bot.*, 71: 834-840.
- Taylor, G. 2005. Hardseededness in Mediterranean annual pasture legumes in Australia: a review. *Crop & Pasture Sci.*, 56: 645-661.
- Tyler, J.M. 1997. Effect of impermeable seed coat on germination of seed from early maturing soybean. *Seed Technol.*, 19: 45-50.
- Valenti, G.S., L. Melone, M. Ferro and A. Bozzini. 1989. Comparative studies on testa structure of hard-seeded and soft-seeded varieties of *Lupinus angustifolius* L. (Leguminosae) and on mechanisms of water entry. *Seed Sci. & Technol.*, 17: 563-581.
- Van Staden, J., J. Manning and K. Kelly. 1989. Legume seeds-The structure: function equation. *Advances in Legume Biology. Monogr. Syst. Bot. Missouri Bot. Gard.*, 29: 417-450.
- Werker, E. 1980. Seed dormancy as explained by the anatomy of embryo envelopes. *Israel J. Bot.*, 29: 22-44.
- Werker, E., I. Marbach and A. Mayer. 1979. Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. *Ann. Bot.*, 43: 765-771.
- Wyatt, J. 1977. Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat color. *J. Amer. Soc. Hort. Sci.*, 102: 478-480.
- Yaklich, R., E. Vigil and W. Wergin. 1986. Pore development and seed coat permeability in soybean. *Crop Sci.*, 26: 616-624.

(Received for publication 27 May 2016)