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Cation Permeability in Soybean Aleurone Layer

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The permeation of water and ions into bean seeds is essential for processing and cooking of beans. The permeability of cations, K, Na, Ca, and Mg ions, into soybean seed tissue, especially aleurone layer, during water uptake was investigated to characterize the ion permeation into soybeans. Aleurone layers and seed coats contained relatively high concentration of endogenous K and Ca ions, and endogenous Ca ion, respectively. The amounts of Ca ion entered seed coats and aleurone layers were greater than those of other ions, respectively. Ion contents per dried aleurone layer and dried seed coat were almost same from each other. The release of Ca and Mg ions toward the inside tissue from the aleurone layer was observed during water uptake. The cell wall column of aleurone layers adsorbed more K and Na ions than Ca and Mg ions. These results suggest that the Ca ion entered soybean seeds is actively transported from the seed coat to the embryo through the aleurone layer. The active migration of Ca to soybean embryos would be greatly usefull for producing foods enriched with Ca in processing and cooking of soybeans.

Introduction

The water uptake and ion permeation in bean seeds are very important factors for processing and cooking of beans. Several groups have studied the water sorption of soybeans and the binding capacity of some kinds of ions to soybeans[1)-5)] ; however, the detail of ion permeability into the soybean tissue is unknown. Soybean seed coat and adjacent tissues consist of cuticular, palisade, hourglass, spongy parenchyma, aleurone, and degenerated albumen cell layers. The soybean seed has appreciably developed aleurone layer compared with the other beans[6)-7)]. The soybean aleurone layer contains glycine-rich proteins (GRPs) in the cell wall[8)-10)] and seems to participate in controlling water uptake by preventing rapid water sorption[11)-12)].

In this paper, we report the permeability of several cations into the soybean seed tissue, especially aleurone layer, by comparison of cation permeation into seed coats and aleurone layers.

Materials and Methods

1. Materials

Soybean seeds (*Glycine max* L. cv. Enrei) produced in Fukui prefecture in 1996 were used. Standard solutions of sodium, potassium, magnesium and calcium for a flame atomic absorption spectrophotometry were purchased from Nakalai tesque (Kyoto, Japan). All other chemicals used in this study were commercial products of analytical grade.

2. Observation of soybean seed coat and aleurone layer by scanning electron microscope

Soybean seeds were immersed in distilled water at 25℃ for 1 hr or 6 hr. After water uptake, embryos coated with only the aleurone layer were prepared by removing seed coats. The embryos and whole seeds were lyophilized, respectively. The surface of seed coat and aleurone layer, and the vertical section were observed by a Hitachi scanning electron microscope (type S-530) at 20kV.

3. Cation uptake into soybean tissue

Soybean seeds were immersed in the saline solution which consists of 0.1M NaCl, 0.1M KCl, 0.1M $CaCl_2$, and 0.1M $MgCl_2$. The seeds in solution were shaked at 25℃ reciprocally (120rpm). After constant time, the seed coat and aleurone layer were separated. Three hundreds mg dry weight of seed coats and 60mg dry weight of aleurone layers were collected from 20 seeds and the cation concentration was determined. On the other hand, soybean seeds were immersed in distilled water for 30 min at the same condition as mentioned above and the endogenous cation concentrations was determined. The inside of aleurone layer and surface of embryo after soaking in the saline solution were rinsed with 1% HCl solution and the cation concentration in the rinsing solution was determined.

4. Preparation of cell wall column from soybean aleurone layer and cation binding to the column

Aleurone layers collected from 50 seeds were homogenized with distilled water and were centrifuged at 1,000×g for 10min. The precipitate was washed with distilled water 2 times and thus the cell wall was obtained. It was packed into a column (1.0×6cm). Endogenous cations were released from the column with 18ml of 1% HCl and the column was washed using distilled water until the eluent became neutral. Fifteen ml of the saline solution which consists of 0.1M KCl, 0.1M NaCl, 0.1M $MgCl_2$ and 0.1M $CaCl_2$ was applied on the column at flow rate 0.2ml/min. The column was washed with 50-fold volume of distilled water. The adsorbed cations were eluted with 6-fold volume of 1% HCl.

5. Cation analysis

Lyophilized tissues were homogenized with 1% HCl for 30min to release binding cations, K and Na, from the tissue. The homogenate was centrifuged at 1,000×g for 10 min. The supernatant was used as the ion extract. The extract was diluted to an appropriate concentration with 1% HCl and was used for analysis. On the other hand, lyophilized tissues used for measurement of Mg and Ca ions were ashed by heating at 500℃ for 2hr. The ash was dissolved in 1% HCl. Mg and Ca ions were determined in the presence of 1% lanthanum chloride to avoid interferences with other substances. These cations were measured by atomic absorption flame emission spectrophotometer (Shimadzu AA-6400F, Japan).

Results and Discussion

1. Structural change in soybean aleurone layer by water uptake

Figures 1-A, B, and C show cross sections of soybean seed coat and aleurone layer after 0, 1, and 6 hr of water uptake, respectively. The thickness of seed coat and aleurone layer were little before soaking in water and the occurrence of tissues other than the palisade layer was not obviously observed. The aleurone layer was in contact with the spongy parenchyma layer before soaking in water and it was impossible to distinguish both tissues. However, the aleurone layer and the seed coat tissues, palisade, hourglass, and spongy parenchyma layers, showed obvious tissue structure by swelling after soaking. There was not a great difference between the aleurone layers after 1 and 6 hr of water uptake.

As shown in Fig. 2, although pits in the surface of seed coat were very small in the dry bean (A), the size was enlarged by the passage of water uptake (B and C). On the other hand, the change in surface of aleurone layer was scarcely observed during water uptake

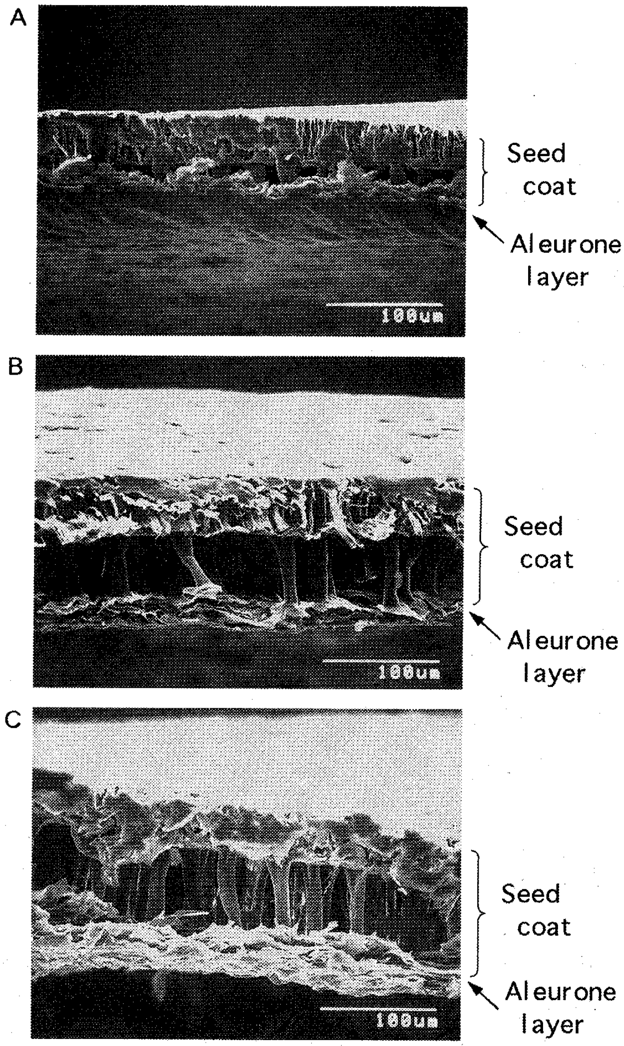

Fig. 1. Change in cross-sectional structure of seed coat and aleurone layer during soaking in water (×300)
A, 0hr; B, after 1hr; C, after 6hr

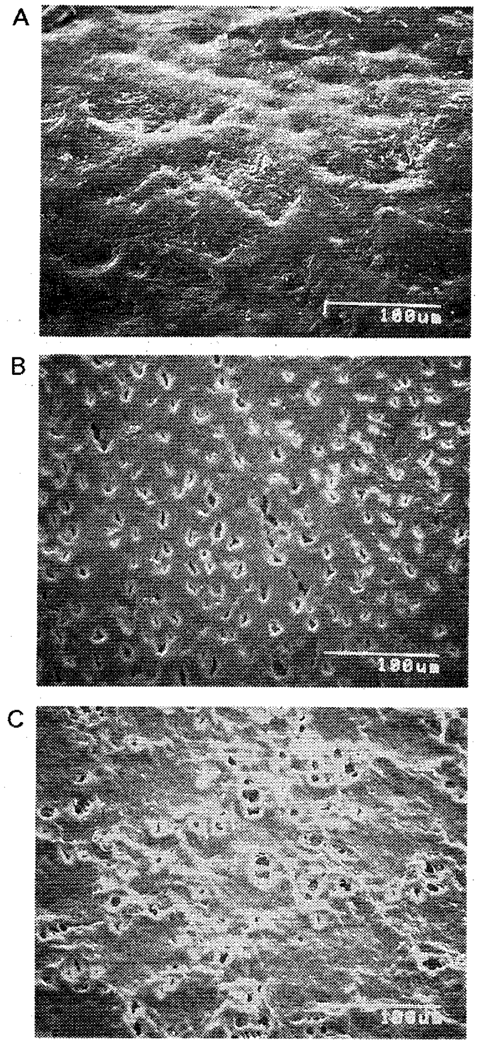

Fig. 2. Change in surface structure of soybean seed coat during soaking in water (×300)
A, 0hr; B, after 1hr; C, after 6hr

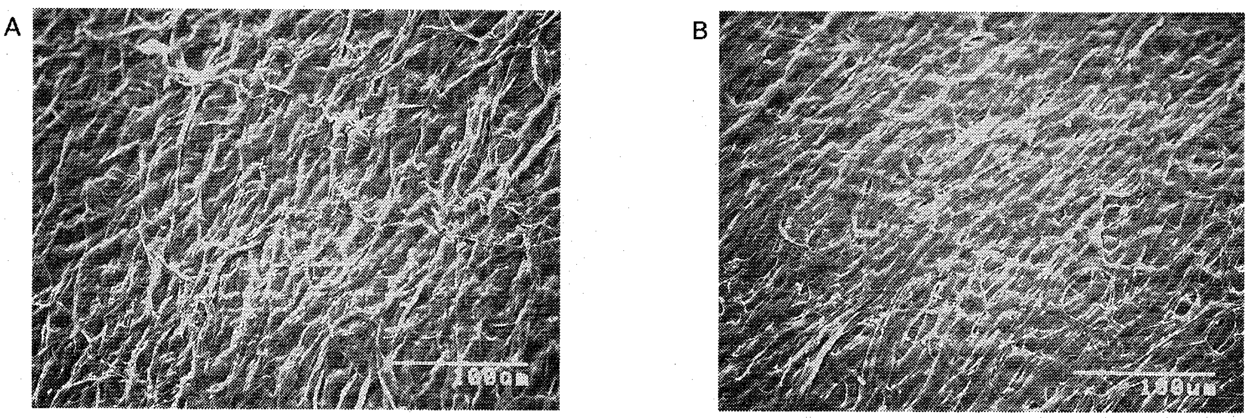

Fig. 3. Change in surface structure of soybean aleurone layer during soaking in water (×300)
A, after 1hr; B, after 6hr

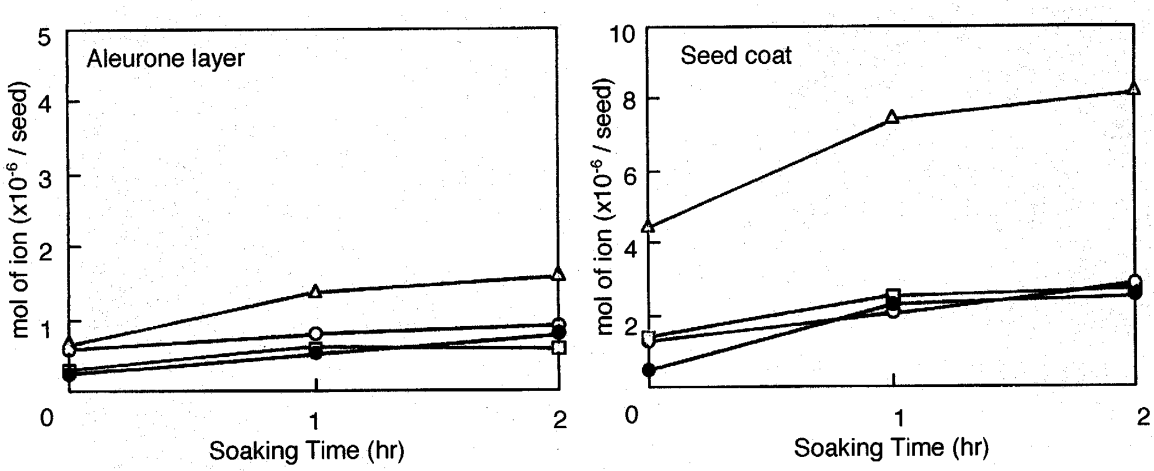

Fig. 4. Cation uptake by soybean aleurone layers and seed coats
○, K; ●, Na; △, Ca; □, Mg

Table 1. Cation contents in seed coats (SC) and aleurone layers (AL)

Ion	Tissue	Soaking time (hr)	Endogenous ($\times 10^{-4}$mol/tissue g)	Increased
K	SC	0	1.08	0
	SC	1	–	0.50
	SC	2	–	1.08
	AL	0	2.39	0
	AL	1	–	0.77
	AL	2	–	0.91
Na	SC	0	0.42	0
	SC	1	–	1.36
	SC	2	–	1.55
	AL	0	1.31	0
	AL	1	–	1.30
	AL	2	–	1.53
Ca	SC	0	3.52	0
	SC	1	–	2.03
	SC	2	–	2.75
	AL	0	2.75	0
	AL	1	–	2.15
	AL	2	–	3.17
Mg	SC	0	1.14	0
	SC	1	–	0.74
	SC	2	–	0.95
	AL	0	1.15	0
	AL	1	–	0.96
	AL	2	–	1.35

(Fig. 3-A and B). Matsui *et al.* reported that the soybean aleurone layer would suppress the rapid water sorption in seeds[11]. Therefore, from these results (Fig. 1, 2 and 3) it was confirmed that the rate of water uptake into the aleurone layer is slow compared with that into the spongy parenchyma layer.

2. Cation uptake in soybean seed coats and aleurone layers

The uptake of cations, K, Na, Ca, and Mg, into soybean seed coats and aleurone layers during water uptake was shown in Fig. 4. and Table 1. Seed coats contained relatively high concentration of endogenous Ca ion, while aleurone layers contained relatively high concentration of endogenous K and Ca ions. The rapid uptake of cations into aleurone layers was observed at the initial stage of water uptake. In particular, Ca ion of divalent cations markedly entered both tissues of seed coats and aleurone layers compared to other ions. Although the total amount of ions entered aleurone layers was little compared with the amount of ion entered seed coats (Fig. 4), the amount of ions per aleurone layer dry weight was almost same as that per seed coat dry weight.

3. Release of cations from soybean aleurone layer

The amounts of cations occurred between the aleurone layer and the embryo during water

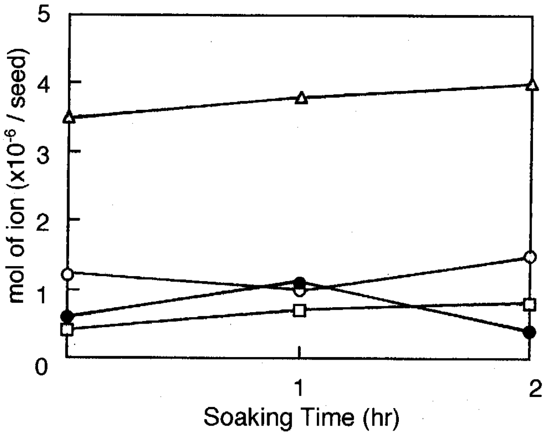

Fig. 5. Release of cations from soybean aleurone layers
○, K; ●, Na; △, Ca; □, Mg

uptake were shown in Fig. 5. The Ca ion exhibited a largest amount among 4 kinds of cations endogenously occurred in the space. The release of Ca and Mg ions toward the inside tissue from the aleurone layer was observed for 2 hr of water uptake, whereas that of K and Na ions were scarecely observed. The permeability of each cation through the aleurone layer seems to depend on the ion species.

4. Cation binding capacity of cell wall column of soybean aleurone layer

The cation binding capacity of the cell wall column prepared from soybean aleurone layers was listed in Table 2.

Table 2. Cation–binding capacity of cell walls column from soybean aleurone layers

Ion	mol of ion per g of aleurone layer	
	Endogenous	Adsorbed
K	1.3×10^{-4}	1.4×10^{-4}
Na	2.3×10^{-4}	1.8×10^{-4}
Ca	9.1×10^{-4}	0.5×10^{-4}
Mg	1.9×10^{-4}	0.8×10^{-4}

The most abundant endogenous cation in the aleurone cell wall was Ca. The cell wall column adsorbed larger amount of monovalent cations, K and Na ions, than that of divalent cations, Ca and Mg ions. The high uptake of Ca into aleurone layers and the weak binding of Ca to aleurone cell walls seem to be in conflict with each other. Bush *et al.* recently reported the diversity of calcium–efflux transporters in wheat aleurone cells[13]. Therefore, the high uptake of Ca into aleurone layers seems to be a symplastic movement of Ca, whereas the weak binding of Ca on aleurone cell walls appears to be an apoplastic movement of Ca.

5. Cation binding to cell wall material

A lot of papers on the binding of cations to cell wall material, especially dietary fiber, were recently reported. Weber *et al.* investigated the binding capacity of 18 kinds of fiber source for Ca *in vivo*[4]. They concluded that Ca binding capacity was not related to phytic acid, soluble and insoluble fiber, and water holding capacity. Idouraine *et al.* reported the binding capacity of 16 kinds of fiber source for Mg, Zn, and Cu *in vitro*[14]. They revealed that correlations and intercorrelations between the amount of minerals bound and protein, acid detergent fiber, and lignin contents of acid–washed fiber sources were low. In addition, they reported the binding capacity of wheat bran, rice bran, and oat fiber for Ca, Mg, Cu, and Zn *in vitro*[15]. On the other hand, several workers reported some physiological functions of dietary fiber[16)-19]. Ward *et al.* investigated that the effect of cell wall and hull fiber from canola and soybean on the bioavailability for rats of minerals[20]. Further, several groups attempted extrusion cooking using potato peel, rice bran, *etc.*[21)-24]. It may be expected that the dietary fiber is used for the bioavailability by the binding capacity for minerals and the development of new foods. We will hereafter report the characterization of mineral–binding capacity of dietary fiber in soybean aleurone layers because the cell wall material in the aleurone layers exhibits the binding capacity for monovalent cation.

6. Permeability of cations through soybean aleurone layers

Although the mechanism of migration of Ca ion from soybean aleurone layer to the embryo is not enoughly understood, the results obtained from this investigation suggest that the Ca ion entered soybean seeds is actively transported from the seed coat to the embryo through the aleurone layer in spite of slow water sorption into the aleurone layer. The active migration of Ca to soybean embryos would be greatly usefull for producing foods enriched with Ca in processing and cooking of soybeans.

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