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GENERAL MORPHOLOGY AND RELATION BETWEEN POTASSIUM AND POLLEN IN JAPANESE ANGELICA (A*RALIA ELATA* L.)

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Abstract

Japanese angelica (*Aralia elata* L.) pollens were collected from the mature central open spikelet having visible anthers and were used for morphological observation. Scanning electron microscope revealed two distinctive types of pollen. One was comparatively larger in size and regular in shape (fertile) while the other was smaller and irregular in shape (sterile). Scanning electron microscope showed that pollen has clearly visible four apertures. It was hypothesized that potassium (K) may be involved in pollen swelling and the mechanisms underlying the rapid imbibition of water. Scanning electron microscopy with EDX (Energy dispersive X-ray) attachment was used to observe K at aperture area of pollen. It was found the K intensity were higher when the beam was passed through the aperture whereas intensity of K peaks reduced when the beam passed through the area that is between apertures. These results support our hypothesis that if K drives rapid imbibition of water then it should be located in the aperture area of pollen. These results demonstrate that a likely relationship exists between K located at the aperture and the swelling of pollen.

Introduction

Potassium (K) has an essential role in plant water relation as well as in enzyme activation, protein synthesis, photosynthesis and other function (Marschner, 1995). It has been reported that pollen germination and pollen tube growth has significantly regulated by the transport of inorganic ions such as Ca and K (Feijo *et al*., 1995; Tayler & Hepler, 1997). It is also known that K is required for both pollen germination and tube growth (Brewbaker & kwack, 1963; Feijo *et al*., 1995). Obermeyer & Blatt (1995) reported that inward K current in a non germinating pollen grain may play a role in initiating the osmotic water influx required for pollen germination.

Rehman *et al*., (2004) reported that mature barley (*Hordeum vulgare* L.) pollen swell in a fraction of a second upon hydration and the presence of potassium (K) at the aperture area of pollen was considered responsible for the rapid hydration of pollen. Fan *et al.*, (2001) also elaborated the physiological importance of K in *Arabidopsis* pollen germination and tube growth and K influx may play a role in the regulation of pollen turgor pressure. The presence of potassium (K) at the aperture area of pollen was previously detected by energy dispersive X-ray analysis (EDX) technique in barley (Rehman *et al*., 2004).

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These previous studies strongly suggest that potassium (K) may be essential component involved in the processes of pollen germination and tube growth and that the regulation of the K may play a regulatory role in pollen germination and tube growth. Therefore, present experiment was designed to investigate (1) general morphology of Japanese angelica *(Aralia elata* L.) pollen, (2) Does K have any relation with pollen swelling and the mechanisms underlying the rapid imbibition of water.

Materials and Methods

Japanese angelica *(Aralia elata* L.) was grown in experimental farm of Sunchon National University, Korea. Pollens were collected from the mature central open spikelet having visible anthers and were used for morphological observation. Light and Scanning electron microscope was used to study the general morphology of pollen. Scanning electron microscope (SEM) (JEOL JSM-6400) was also used to differentiate between cytoplasmic fertile and sterile pollen. Scanning electron microscope (SEM) (JEOL JSM-6400) with energy dispersive X-ray analysis (EDX) attachment was used to verify the K presence at the aperture area in *Aralia elata* pollen as described by Rehman *et al*., (2004). The SEM also had a scanning electron micrograph facility that was used for the surface examination of pollen. A thin layer of carbon was coated on pollen surface before EDX study (Rehman *et al*., 2004).

Results

Scanning electron microscope revealed two types of pollen (Fig. 1). One was comparatively larger in size and regular in shape while the other was smaller and irregular in shape. Regular shape pollens were cytoplasmic fertile while the pollens of irregular type were sterile. To check the result of sterile pollens, light electron microscope was used to observed immature seed pod and internal section of seed pod (Fig. 2). Internal section of seed pod shows that some of the pod doesn’t have seed may be due sterile pollen. Light and scanning electron microscope showed that pollen have clearly visible four apertures. Two apertures are laying one side of pollen and two on the other side. The central area between the apertures has ridges like appearance (Fig. 3A). Fig. 3B shows the bunch of pollen stick with each other (placed in different angles) just like a ball.

Scanning electron microscopy with EDX attachment was used to observe K at aperture area of pollen. It was found the K intensity were higher when the beam was passed through the apertures whereas intensity of K peaks reduced when the beam passed through the area that is between apertures (Fig. 4A). Verticals lines show the chart of potassium (K) peaks after the X-ray beam was passed across the aperture (Fig. 4A). SEM micrograph indicates the pollen aperture area corresponding with chart of K traces (Fig. 4B). Higher peaks at the aperture indicated that K was concentrated in these areas whereas in between apertures there was no or lesser K (presence of peaks due to high topography). These results show that K is located in aperture area even when pollen is in a dehydrated state.

Discussion

One of the most important functions of pollen aperture is to regulate the water balance of the pollen when it is subjected to changes in humidity (Shukla *et al*., 1998). Japanese angelica has four clearly visible apertures (Fig. 3A) and, therefore, it should be the way to regulate the water uptake.

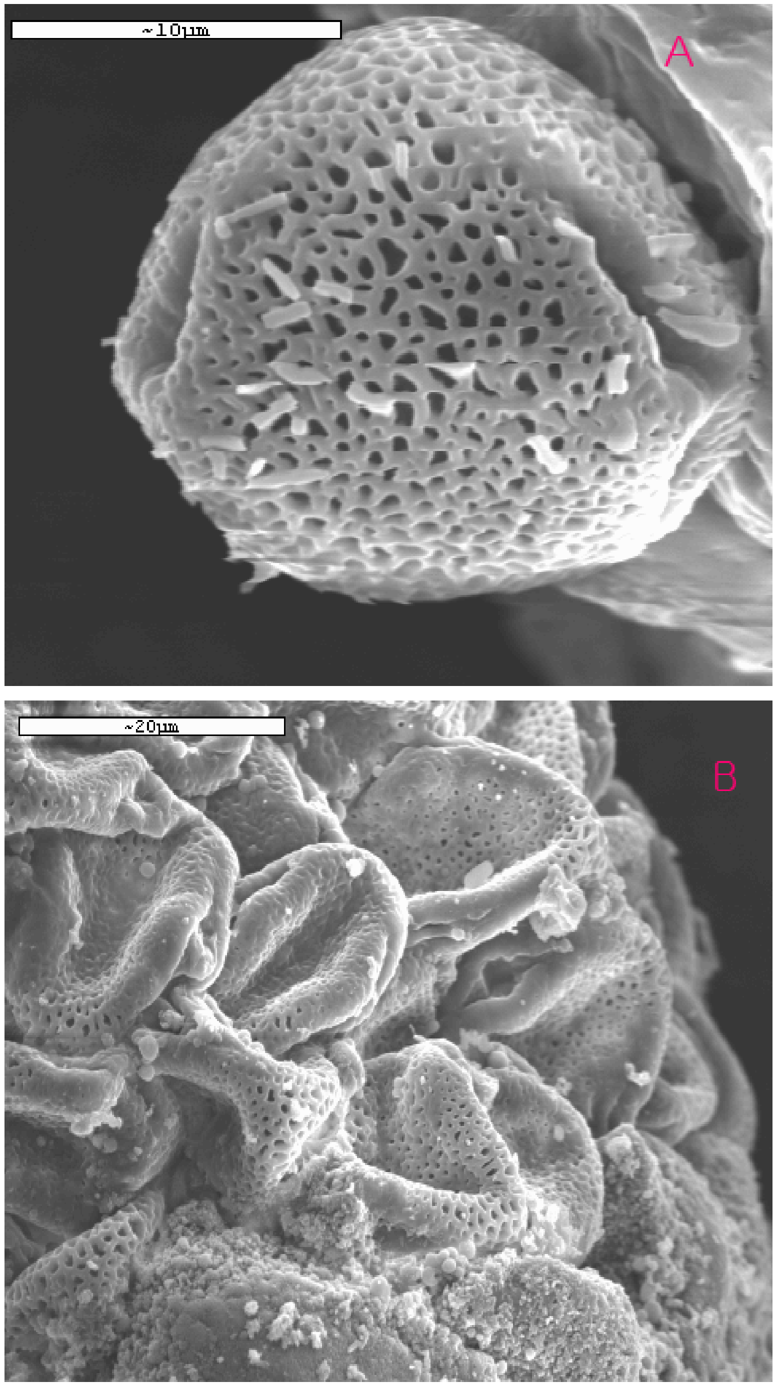

Fig. 1. Scanning electron micrographs of fertile (normal/cytoplasm-rich) (A) and sterile (cytoplasm-devoid) pollens (B) of Japanese angelica *(Aralia elata* L.).

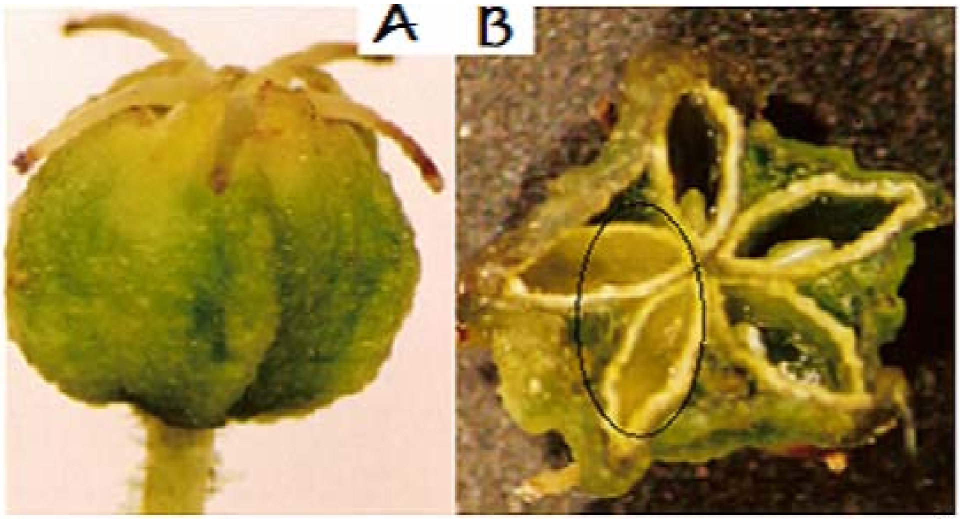

Fig. 2. Light electron micrographs of immature pod (A) seedless pod (B) of Japanese angelica *(Aralia elata* L.).

Energy dispersive X-ray analysis (EDX) (Fig. 4) verified the intensity of K peaks at aperture area of pollen. The appearance of high intensity of K at the aperture area of pollen, does suggest that it is most probably responsible for the rapid uptake of water, resulting in rapid swelling. This result is consistent with previous report (Fan *et al*., 2001) that K influx may play in the regulation of pollen turgor pressure. The consistent appearance of K at the aperture area of pollen in sesame and barley was previously reported by Rehman *et al*., (2002, 2004). The presence of potassium (K) at the aperture area of pollen was considered responsible for the rapid hydration of pollen (Rehman *et al.*, 2004). EDX techniques have shown that K was highly concentrated at the aperture area of pollen regardless of number of apertures as in case of barley with a single aperture (Rehman *et al.*, 2004) and sesame with 12 apertures (Rehman *et al*., 2002).

Potassium is widely known for its rapid action as an osmotic regulator (Heslop-Harrison & Heslop-Harrison, 1996). Our results suggest that the role of K in swelling of pollen could be due to osmotic effect. However, K in pollen may have accumulated from pollen space (Zhang *et al*., 1996) during maturation. The consistent appearance of K at the aperture area of pollen was considered one of the factors that regulate the quick uptake of water and rapid swelling of the pollen and the rapid imbibition may be a prerequisite for rapid pollen tube emergence because in most cases the emergence of pollen tube takes a few seconds to a few hours after being placed in favourable germination conditions (Rehman *et al*., 2005).

These results support our hypothesis that if K drives rapid imbibition of water then it should be located in the aperture area of pollen. In conclusion, these results demonstrate that a likely relationship exists between K located at the aperture areas and swelling of pollen.

Acknowledgment

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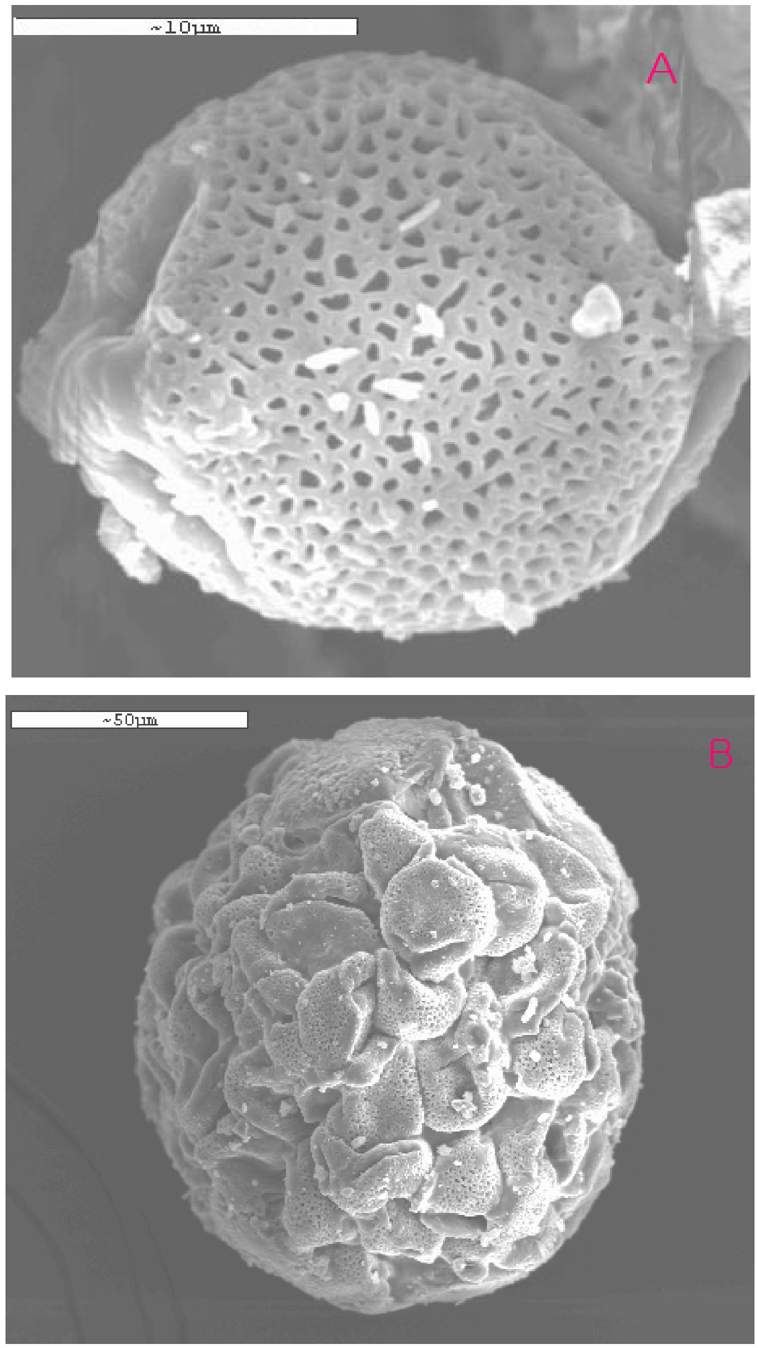

Fig. 3. Scanning electron micrographs of Pollen having four clearly visible apertures (A) and bunch of pollen stick with each other (placed in different angles) just like a ball (B) of Japanese angelica *(Aralia elata* L.).

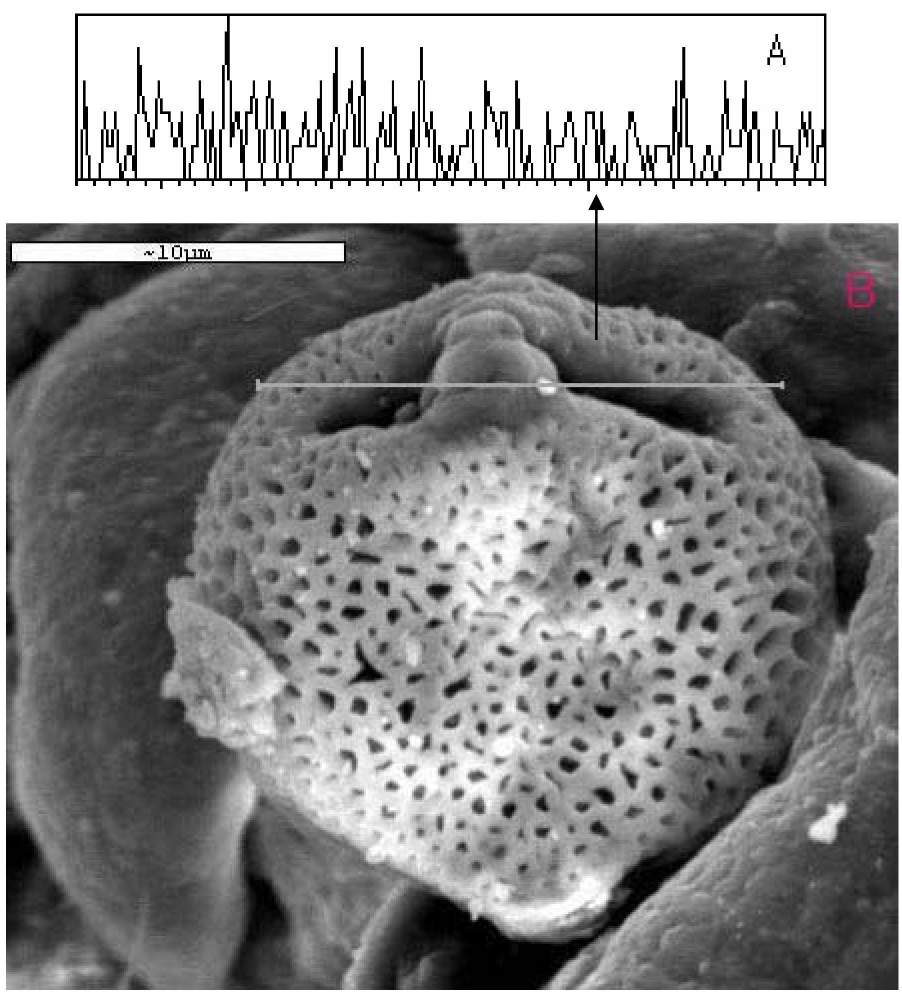

Fig. 4. Energy dispersive X-ray analysis (EDX) spectrum with scanning electron microscope (SEM) micrograph aperture areas of Japanese angelica (*Aralia elata* L.) shows the (A) EDX-profile for K after X-ray beam was passed across the aperture (B) SEM micrograph of pollen aperture indicating the corresponding K peak area.

References

Brewbaker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *J. Exp. Bot.*, 50: 859-865.

Fan, L.M, Y.F. Wang, H. Wang and W.H. Wu. 2001. *In vitro Arabidopsis* pollen germination and characterization of the inward potassium currents in *Arabidopsis* pollen grain protoplasts. *J. Exp. Bot*., 52: 1603-1614.

Feijo, J.A., R. Malho and G. Obermeyer. 1995. Ion dynamics and its possible role during in vitro pollen germination and tube growth. *Protoplasma*, 187: 155-167.

Heslop-Harrison, Y. and J.S. Heslop-Harrison. 1996. Lodicule function and filament extension in the grasses: potassium ion movement and tissue specialization. *Ann. Bot*., 77: 573-82.

Marschner, H. 1995. *Mineral nutrition of higher plants*. Harcourt Brace and company, Academic Press Publishers, London.

Obermeyer, G. and M.R. Blatt. 1995. Electrical properties of intact pollen grains of *Lilium longiflorum*: characteristics of non germinating pollen grains. *J. Exp. Bot*., 46: 803-813.

Rehman, S., K.J. Lee, E.S. Rha, S.J. Yun and J.K. Kim. 2002. Mechanisms involved in rapid swelling of sesame (*Sesamus indicum* L.) pollen. *New Zealand J. Crop Hort. Sci.,* 30: 203-213.

Rehman, S., E.S. Rha, M. Ashraf, K.J. Lee, S.J. Yun, Y.G. Kwak, N.M. Yoo and J.K. Kim. 2004. Does barley (*Hordeum vulgare* L.) pollen swell in fractions of a second? *Plant Sci.*, 167: 137-142.

Rehman, S., N.H. Yoo, M.R. Park and S.J. Yun. 2005. Confocal potassium imaging: Giving new insight into potassium concentrated at the aperture area of barley (*Hordeum vulgare* L.) pollen. *Plant Sci*., 169: 457-459.

Shukla, A.K., M.R. Vijayraghavan and B. Chaudhry. 1998. *Biology of pollen*. Aph publishing corporation, New Delhi, India.

Tayler, L.P., and P.K. Hepler. 1997. Pollen germination and tube growth. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48: 461-491.

Zhang, W.H. Z. Rengel and J. Kuo. 1998. Determination of intracellular Ca in cells of intact wheat roots: loading of acetoxymethyl ester of Fluo-3 low temperature. *Plant J.*, 15: 147-151.

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