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Varietal Responses of Pollen Development to Salt Stress in Barley

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ABSTARCT : **Thirty-nine out of eighty-five barley varieties/strains survived until heading stage in the saline experimental field (0.03 - 0.05 % salt) and they were used for pollen study. Light and scanning electron microscopic observations revealed two distinctive types of barley pollens: one transparent and small in size and the other dark and larger. In addition, both types of pollens were stained with Alexander's stain and it was found that the smaller and transparent pollen was cytoplasm-devoid (CD) while the larger pollen was cytoplasm-rich (CR). Sixteen out of 39 barley varieties/lines grown in the saline soil had CR pollens, which were rarely observed in the barley plants grown in the non-saline soil. Moreover, it was observed that salt stress severely reduced seed setting in the varieties having degenerated pollens. These results suggest that salt stress affects the fertility of barley pollen. The sterile pollen was undersized and lack of cytoplasm probably due to abortion. Furthermore, a varietal difference existed in the response of pollen development to salt stress.**

Keywords: barley, genotype, pollen development, salinity

Salinization of soils is not new and is one of the oldest and the most serious environmental problems. It is also a problem, which is increasing rapidly (Karen, 1994; Szabolcs, 1994). Large areas of land are suffering from serious degradation of one kind or another. A typical degradation of soil results from salinization, sodicity, water logging or a combination of these factors. Salinity is one of the major constraints among environmental stresses that consistently reduce crop production in all agricultural areas. Effects of salinity on plant vegetative growth are well documented (Cramer *et al.*, 1990; Munns, 1993; Noble & Rogers, 1992) but the salinity effect on plant during the reproductive stage has received far less attention, despite it is one of the most sensitive stage to environmental stresses, which could cause yield reduction as much as 50% in cereals (Dorion *et al.*, 1996; Khan & Abdullah, 2003; Namuco & OToole, 1986). The aim of this study was to investigate the effect of salinity on the pollen fertility of barley (*Hordeum vulgare* L) and to screen salt tolerant barley varieties at the reproductive stage.

MATERIALS AND METHODS

Eighty-five barley (*Hordeum vulgare* L.) varieties/lines were grown in the saline experimental field of Kyehwado sub-station, Honam Agricultural Research Institute, NICS, with the salt concentration ranging from 0.03-0.05% and in the non-saline field (as a control) in Chonbuk National University, Korea in 2002-2004. Thirty-nine varieties/strains survived until heading stage and they were used for the investigation of pollen fertility (Table 1).

Pollens were collected from the mature central open spikelets having visible anthers and were used for morphological observations. Light and scanning electron microscopes (SEM) (JEOL JSM-6400) were used to conduct the morphological studies of freshly collected pollen from barley varieties grown in saline and non-saline soil. A thin layer of platinum was coated on pollen surface before SEM study Alexanders staining (Alexander, 1980) was applied in the current experiment to observe the existence of cytoplasm (Biasi *et al.*, 2001) in barley pollen grains grown in saline and non-saline soils.

RESULTS AND DISCUSSION

Scanning electron microscopic (SEM) observations revealed two distinctive types of barley pollen grains (Fig. 1). One was comparatively larger in size (±35 μm) and regular shaped while the other smaller (±20 μm) and irregular shaped. Similarly, two types of pollen grains, a dark looking, larger in size and regular shaped and the other transparent, smaller and irregular shaped, could be observed before and after hydration at light microscopic level (Fig 2 & 3). The

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Table 1. List of barley varieties/lines grown in saline field, showing different responses of pollen development to salt stress.

Pollen cytoplasm	Seed set	Genotype
CR	Y	0010-304, 0010-36, 0010-511, 0019-10, 9231-263, 9321-260, Chal-bori, Chapssal-bori, Chunpayukgakmak, Daebaek-bori, Daejin-bori, Gang-bori, IT-017038, IT-017547, IT-108312, IT-110533, IT-140594, Jinkwang-bori, Jinyang-bori, Koanghalssal-bori, Saegang-bori, Saeol-bori
CD	L	Hyeunchalssal-bori, IT-108301, Naehanssal-bori, Ol-bori
	N	9321-262, Alchan-bori, IT-019683, Jaikang-bori, Keunal-bori, Nakyeong-bori, Namhyang-bori, Owol-bori, Sacheon-6, Saidun-bori, Sinho-bori, Tapgol-bori

CD= Aborted/cytoplasm-devoid pollen, CR= Normal/Cytoplasm-rich pollen, Y= Seed set in over 30% spikelets of a spike, L= Seed set in less than 30% spikelets of a spike, N= No seed set.

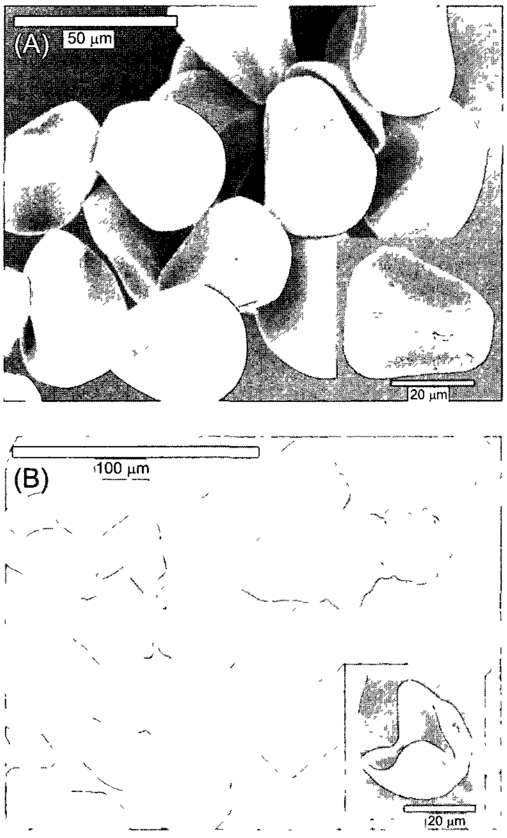

Fig. 1. Scanning electron micrographs of normal / cytoplasm-rich (A) and aborted / cytoplasm-devoid pollens (B)

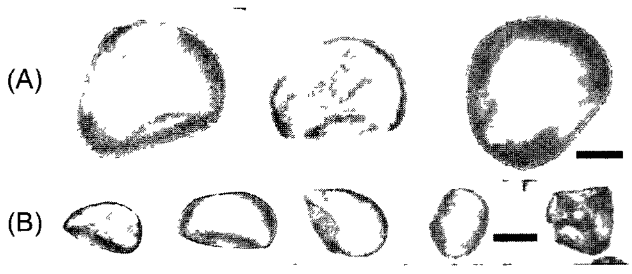

Fig. 2. Light micrographs of normal/cytoplasm-rich (A) and aborted/ cytoplasm-devoid pollens (B) before hydration. Bar 10 µm

pollen grains of former type were normal while the pollens of later type were abnormal/aborted compared to pollen grains from the plants grown in non-saline soil. Furthermore, Alexanders stain test showed that large, dark looking and regular shaped pollen grains stained positively, that is, pollen grains were cytoplasm-rich (CR) while the others were negatively stained, implying pollen grains were cytoplasm-devoid (CD) (Fig. 4). Seed setting was poor in CD type as compared to the CR type at harvest (Table 1).

Sixteen out of 39 barley varieties/lines (Table 1) grown in saline field grouped into two classes, aborted or degenerated and cytoplasm-devoid (CD) pollen grains compared to the CR pollen from the plants grown in non-saline soils. However, the proportion of CD pollens varied from 30 to 100%. It was also observed that some of the pollens were completely devoid of cytoplasm while others were filled with various levels of cytoplasm, reflecting that pollen development was stopped at the various stages. These results indicate that a large number of undersized pollen grains of salt-sensitive varieties may be due to the failure of pollen development and abortion caused by salt stress.

The results also indicate that some of the barley varieties with degenerated/CD pollen grains were salt sensitive at reproductive stage. Pollen development period is considered as one of the most sensitive stages to the environmental stresses, e.g. drought, temperature, salinity etc The pollen sterility induced by the environmental stresses could result in the yield reduction as much as 50% in most of the cereals (Dorion *et al.*, 1996; Khan & Abdullah, 2003; Namuco & O'Toole, 1986). Therefore, it is important to evaluate the pollen sterility and to select plant genotype tolerant to environmental stress at reproductive stage. These observations also signify the importance of male gametophyte in salt tolerance because gametophytes contribute significantly in the inheritance of salt tolerance in barley (Koval1, 2000). Furthermore, poor seed set demonstrates the loss of pollen fertility under the saline condition. Although, stigma surfaces of flowers were not investigated in present experiment but it

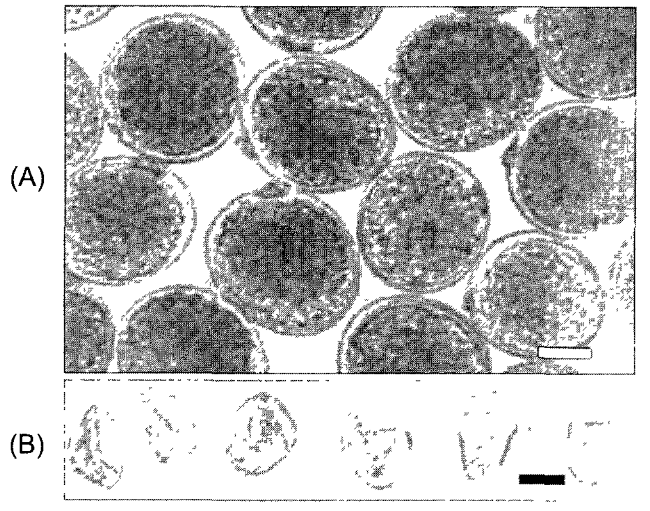

Fig. 3. Light micrographs of normal / cytoplasm-rich (A) and aborted / cytoplasm-devoid (B) pollens after hydration Bar 10 μm.

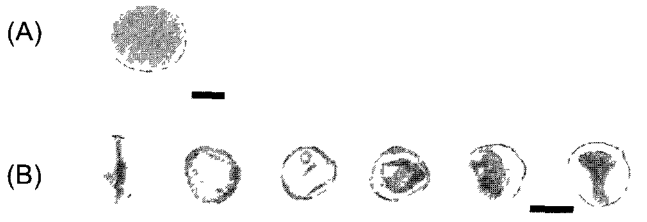

Fig. 4. Pollen grains treated with Alexanders stain Normal / ytoplasm-rich pollens (A) stained positively while aborted/ cytoplasm-devoid pollens (B) stained negatively, confirming the presence of cytoplasm in the former case vice versa Bar: 10 μm

may be possible that salt stress also caused dysfunction of the female reproductive parts. Despite, the pollen sterility could be a major cause of poor seed set under salt stress condition.

In conclusion, the results suggest that pollens of the salt-sensitive barley genotypes were smaller in size and lack of cytoplasm, probably due to abortion by salt stress. Pollen morphology could be a useful indicator for the performance of the male organ under salt stress, and could provide additional information to aid selection for salinity tolerance at the reproductive stage.

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