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Structural Changes in Root and Shoot of *Bacopa monniera* in Response to Salt Stress

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Various concentrations of salt (NaCl) were shown to have an influence on the differentiation of tissues in the root and stem of *Bacopa monniera* (L.) Wettst. Higher concentrations induced drastic changes in roots grown on salt-supplemented media; epidermal and cortical cells experienced changes in shape, size, and orientation and/or were got disintegrated. A low concentration of salt induced a profuse development of root hairs which gradually disappeared at higher concentrations. Air spaces in the stem cortex were enlarged and xylem cell walls in the vascular ring were thickened.

Keywords: aerenchyma, anatomy, *Bacopa monniera*, root, salt stress, shoot

Soil salinity has long been a major factor in limiting crop production in more than 50% of arable/cultivable land (Subhashini and Reddy, 1990). Salinity of soil or water poses a stress condition for crop plants, primarily in coastal, arid, and semi-arid regions of the world, which comprise over 25% of the earths surface (Carter, 1975). A halophyte/glycophyte expresses three basic responses when exposed to a saline environment; it maintains favourable water relation, copes with potentially toxic ions, and obtains required nutrient ions from the external media (Rains et al., 1980). Supraoptimal concentrations of NaCl inhibit various metabolic processes in plants, resulting in reduced plant growth and development. However, information on the differentiation of plant tissues in response to NaCl is meagre (Yermanos et al., 1967; Kurth et al., 1986; Benzioni et al., 1992; Chretien et al., 1992).

Bacopa monniera (L.) Wettst., known as a memory vitalizer, is a small creeping herb of the family Scrophulariaceae. High frequency of regeneration makes it a model system for in vitro studies (Ali et al., 1996). Salt and heavy metal tolerant regenerants (Ali et al., 1997,1998 a,b; Ali et al., 1999) of this species have previously been produced. However, no systematic study of the effect of salt on its anatomy has yet been carried out. The present investigation examines the effects of salt stress on the root and stem structures of *B. monniera* grown in vitro.

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MATERIAL AND METHODS

Establishment of Cultures

Regenerants were established from the nodal segments of *B. monniera* (Ali et al., 1996) on Murashige and Skoog's (1962) media supplemented with various levels of NAA (0.1-0.5 mg · L^{-1}), BAP (0.5-5.0 mg · L^{-1}), and casein hydrolyzate (500 mg · L^{-1}). The cultures were maintained on the maintenance medium (MM), ie., MS (sucrose 2%) added with NAA (0.2 mg · L^{-1}), BAP (0.5 mg · L^{-1}), and glutamine (50 mg · L^{-1}), which was also supplied with NaCl (5,10, and 15 g · L^{-1}). All cultures were maintained at 25 ± 2°C with 55 ± 5% relative humidity in a culture room provided with a cool white fluorescent bulb light at a total intensity of 40 μ mol m^{-2} s^{-1} for 15 h.

Sectioning of Root and Stem

The regenerants were harvested after four weeks of growth on salt-supplemented media. For anatomical studies, root and shoot segments were taken from sites 1 cm away from the root-shoot junction. The segments, fixed in FAA and dehydrated in graded ethanol series, were embedded in paraffin wax. Transverse sections of the root were cut quite a distance behind the root tip. The sections obtained by a rotary microtome, stained with safranin O-fast green, and mounted in Canada balsam, were observed under a light microscope (Olympus Vanox-S, Japan).

RESULTS AND DISCUSSION

The regenerants grown on various concentrations (5-15 g · L^{-1}) of NaCl showed suppressed growth (Fig.1). The plantlets maintained at low concentrations were less affected than those at higher concentrations (Ali et al., 1997). The shoots and roots exhibited various anatomical changes as compared to the control (Figs. 2 and 3).

In the roots of control plants without salt treatment, the epidermal cells were relatively thick-walled and compactly arranged, the multilayered hypodermis was well organized and the cortex was composed of radial stretches of parenchymatous cells separated by air cavities. The vascular cylinder comprised of a centripetal xylem and a peripheral phloem as usual (Fig. 2I).

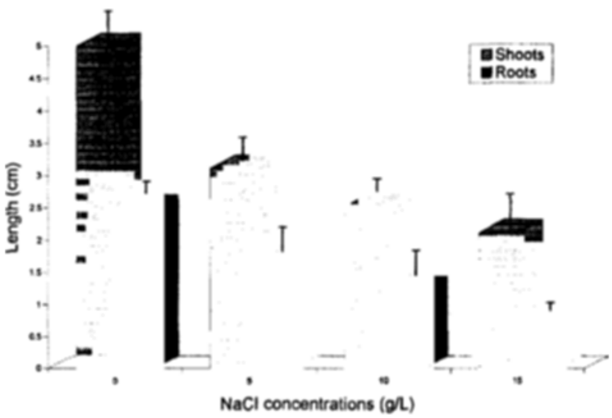

Figure 1. Root and shoot length of *B. monniera*, four-week-old regenerants grown under various concentrations of NaCl.

Plants grown on 5 g · L^{-1} NaCl, showed no specific changes in the root structure except for the appearance of enormous multicellular root hairs (Fig. 2II). The root hairs were abundant in number at a low concentration such as 5 g · L^{-1}. At 10 and 15 g · L^{-1} NaCl, epidermal cells became uneven in appearance. The cortical cells became radially elongated, the multilayered radial strips became thinner, and the air spaces got enlarged (Fig. 2III). The root hairs disappeared gradually with the increase in the concentrations of NaCl (Fig. 2III).

Stem anatomy showed wide variation under salt stress. In the control plants, epidermal cells were well arranged. The cortical tissue was composed of parenchymatous cells with evenly distributed air cavities. The endodermis, followed by the pericycle, was distinct. The vascular bundles were normal surrounding a well-developed pith consisting of thick-walled parenchyma (Fig. 3I). Plants grown on 5-15 g · L^{-1} NaCl, did not show any major change in the epidermal cells (Fig. 3, II and III). However, the aerenchymatous cells formed prominent radial strips giving rise to larger air cavities. The vasculature looked relatively more compact, the xylem cells being particularly thicker and heavily lignified. In addition, the pith was

Figure 2. Transverse sections of roots of *B. monniera*, four-week-old regenerants grown under various concentrations of NaCl (I. 0 g · L^{-1}, II. 5 g · L^{-1}, III. 10 g · L^{-1}). Ae = aerenchyma, Ep = epidermis, RH = root hairs, VB = vascular bundle. All figures at × 100.

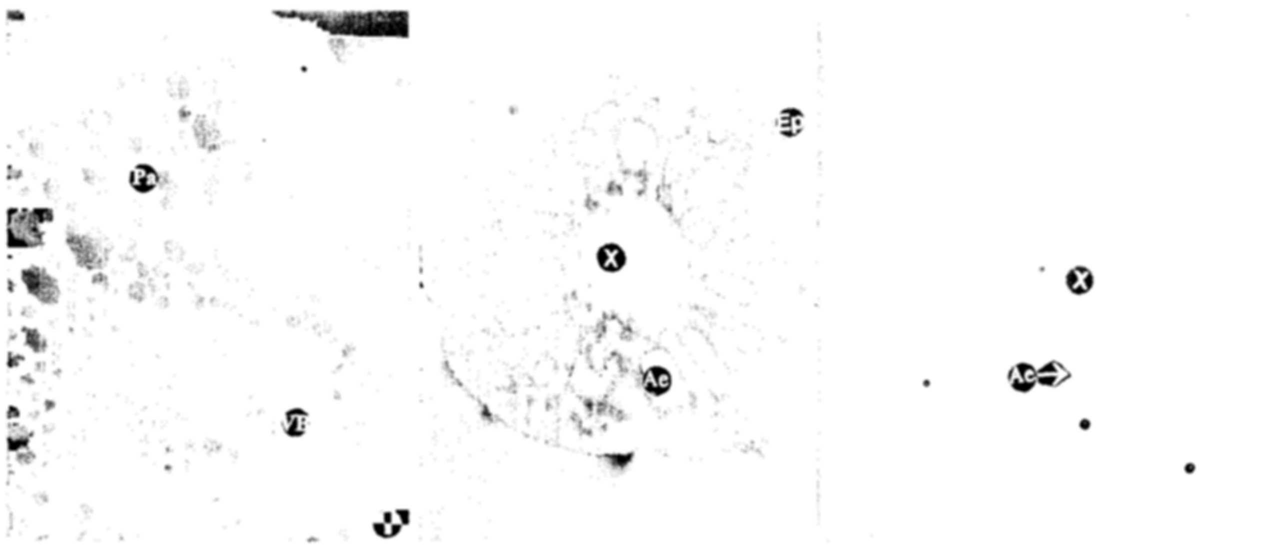

Figure 3. Transverse sections of shoots of *B. monniera,* four-week-old regenerants grown under various concentrations of NaCl (I. 0 g · L^{-1}, II. 5 g · L^{-1}, III. 10 g · L^{-1}). Ae = aerenchyma, Ep = epidermis, Pa = parenchyma, VB = vascular bundle, X = xylem I at × 200, II & III at × 100.

extremely reduced (Fig. 3, II and III).

Plants that accumulate and store salt, also manage to exclude it to some extent, possibly through the endodermal layer of the root (Salisbury and Ross, 1974). The cells that regulate osmotic potential swell by storing more water and organic compounds such as proline in the cytoplasm. They are also capable of exuding the salt through the leaves (Salisbury and Ross, 1974). At the plant cell level, one of the strategies for survival in a saline environment is pumping out of the cells most of the salt that diffuses in. Osmotic stress can thus be relieved by accumulation of organic solutes to maintain turgor. Cells adapted to salt have mechanisms for pumping the mineral ions from the cytosol outwards to the free space and inwards to the vacuole. Na^+ could be driven out of the cytoplasm by H^+/Na^+ antiport at the plasmalemma and at the tonoplast, using the H^+ gradient as an energy source (Kavikishore, 1988).

Salt stress, causing reduction in plant growth, is usually attributed to osmotic stress due to lowering of external water potential (Mass and Nieman, 1978) or to specific effects of ions on the metabolic process in the cell (Greenway and Munns, 1980). The Na^+ exclusion and K^+ uptake in the stressed roots are less efficient than in the roots of the normal genotype (Ben-Hayyim and Kochba, 1983). When the callus cultures of potato were exposed to NaCl, accumulation of the Na^+ ion was lower, and level of K^+ was more stable in osmotically-adapted cells (Sabbah and Tal, 1990). At higher external salt concentrations, accumulation of Cl^- and Na^+ was more maximized in the salt-adapted than in the non-adapted suspension cultures (Sabbah and Tal, 1990).

The present study indicates that salt (NaCl) induces differential anatomical changes in the root and stem of *B. monniera*. The response to salt elicited by the roots was disorganization and disintegration of cells/tissues. Disintegration of epidermal and cortical cells appears to be the outcome of salt accumulation in toxic concentrations in these tissues. The salt ions seem to attack various cellular components, including the cell wall and membranes, leading to various alterations which ultimately cause disorganization. Accumulation of metals in toxic amounts in the root and shoot tissues has been reported in many species (Woolhouse, 1983; Setia and Bala, 1994). Chretien et al. (1992) observed cytological changes in two-month old salt adapted calli of the Jojoba plant, especially an increase in cell diameter and cell wall thickness. Similarly, Yermanos et al. (1967) noted that high soil salinity caused a thickening of leaf lamina in Jojoba, primarily due to an increase in parenchyma cell size. The presence of profuse root hairs at a low salt concentration, as noted in the present study also, indicates that the plants need more and more water to protect themselves from salinity. At higher concentrations, these hairs fail to survive because of the high amounts of salt absorbed. Thinning of aerenchymatous cells and formation of large cavities must facilitate osmosis in view of the higher concentrations of salt outside the cells. When Ca^{2+} was provided, cell enlargement also occurred in cotton roots exposed to salt stress (Kurth et al., 1986). Correlating with the cell enlargement, the thickening of the cell wall may improve its rigidity and ability to resist high turgor pressure, possibly in conjunction with modifications in the assembly of wall sub-units (Roland and Vain, 1979). The anatomical changes in shoot and root of *Bacopa* might be correlative to the increased osmotic pressure due to high accumulation of ions, as was observed in leaves of Jojoba plants grown under high soil salinity (Yermanos et al., 1967; Benzioni et al., 1992; Chretien et al., 1992), and could be interpreted as a process of adaptation to salinity. This study suggests that apparent inhibition of growth in *Bacopa* involves and could be an outcome of histological disturbance and interference with cell division and cell elongation.

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