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The Effects of Salinity on Formation, Growth and External Morphology of Roots in Trifoliate Orange Grafted with Satsuma Mandarin

Pear Mohammad, Masaya Shiraishi* and Touru Manabe*

The United Graduate School of Agricultural Sciences

*Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan

Abstract

The formation, elongation and periclinal growths, and morphology of roots in trifoliate orange grafted with satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsu Wase) were studied under different levels of culture solution salinity. Root formation and root elongation were highest in no-salt-control treatment which decreased as the culture solution salinity was increased up to 50 mM. Both stelar and overall diameters gradually increased towards the root base under control. While these diameters became considerably higher near the root tip and increased slightly towards the root base under saline solutions. This increasing trend was drastically slowed by higher doses of salinity. The ratio of stelar to overall diameters fairly increased towards the root base under control but tended to be similar throughout the root tip in saline solutions. The continued growth of roots under control was accompanied by the distinct removal of older rootcap cells and the observation of intact epidermal cells. In contrast, epidermal cell dehydration initiated under 10 mM NaCl solution. The roots subjected to 25 and 50 mM of NaCl attributed root tip injuries along with severe epidermal cell dehydration and cell death even at short exposure. The formation of holes on the epidermis and distinct malformation of root tip increased considerably with respect to both increased salinity from 25 to 50 mM and the exposure time of roots to these saline solutions.

Introduction

All soils and irrigation waters contain soluble salts, many of which are required for normal growth and development of plants. While many soils and waters contain excessive amount of salts that are harmful. According to the estimates of the Food and Agriculture Organization (FAO) and United Nations Educational, Scientific and Cultural Organization (UNESCO), as much as half of all the existing irrigation systems of the world are, more or less, under the influence of secondary salinization (Szabolcs, 1994). Every year about 10 million ha of irrigated land is abandoned because of the adverse effects of salinity as a result of irrigation, mainly secondary salinization and alkalization and at present no continent is free of the occurrence of this very serious phenomenon as was cited by Szabolcs (1994). Salinity often induces growth inhibition, although most plants are capable of tolerating a certain range. This range varies in different species, varieties, and ecosystems. In some plants, the range is rather narrower; in others it is wide (Poljakoff-Meyber and Lerner, 1994). A study on the relationships between root form, structure, and conditions of habitat revealed that salinity may induce morphological changes to the root in a range of species (Simsburg, 1969). In *Pisum sativum* such changes have included thickening (Solomon *et al.*, 1986) and reduction in the cross-sectional diameter of roots (Setia and Narang, 1985). Compared to other agronomic and horticultural crops, citrus is among the most sensitive to salinity (Maas and Hoffman, 1977; Walker *et al.*, 1983; Maas, 1990). NaCl results in foliar accumulation of Na (Behboudian *et al.*, 1986; Lloyd *et al.*, 1987) or Cl (Cooper and Gorton, 1952; Heve and Walker, 1983; Walker and Douglas, 1983;

Storey and Walker, 1987) in citrus. It inhibits chlorophyll synthesis by decreasing photosynthesis and increasing respiration through the dehydration of mesophyll cells (Kramer, 1984). Besides these, different aspects of salinity on the physiological phenomena in citrus plants is also well established (Gale, 1975; Downton, 1977; Levitt, 1980; Walker *et al.*, 1981). However, majority of these findings were obtained studying the aboveground parts of citrus plants. To the best of our knowledge, literature regarding the effects of salinity on citrus roots is hardly found. Root is that part which has direct exposure to growing media salinity and can be considered as the sensor of plants in the media. It is through the root that the whole plant is affected by changing growing media conditions. This implies the immense importance of roots as study material for increased understanding of the effects of different growing media conditions.

This investigation was intended to elucidate the effects of different levels of salinity on the roots of trifoliate orange grafted with satsuma mandarin with respect to their formation, elongation and periclinal growths, and external morphology. Suitable level of salinity for this plant species was suggested.

Materials and Methods

Two experiments were conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University, Japan from August to October of three consecutive years (1996-1998) using three-year-old satsuma mandarin trees grafted on trifoliate orange rootstocks. The experiment was set inside a plastic house. Twenty four trees were selected for uniformity of size and placed into twelve styrofoam boxes containing nutrient solution for each experiment every year.

The nutrient solution contained N, Ca, K, S, Mg, P, Na, Cl, Mn, Fe, B, Mo, Zn, and Cu at the following concentration in ppm, respectively: 198, 160, 80, 38, 23, 15, 11, 1, 0.5, 0.4, 0.25, 0.1, 0.05 and 0.02 as was used by Smith (1971).

In experiment I, three boxes retaining six trees were considered as one treatment. Different treatments were 0, 10, 25 and 50 mM of NaCl which were adjusted by adding required amount of NaCl to the respective culture solutions. The pH of the culture solutions were maintained between 5.0-5.5 daily by using 1M H₂SO₄ or 3M NaOH as required. The culture solutions were continuously aerated and stirred by air pumps. Solutions were completely renewed at every 14 days interval to ensure an adequate supply of all essential elements to the plant. The house temperature was controlled between 25-27°C. Regular observations were made on the formation of new roots in each tree under different treatments. The new roots as found were tagged with wax-coated paper tags bearing individual numbers. The total number of roots under different treatments were thereby noted during the whole period of the experiment and utilizing this information, number of roots/tree/day was calculated for each treatment.

In experiment II, all trees were primarily placed in the culture solution where NaCl was not added. After the formation of numerous new roots, they were marked with wax-coated paper tags bearing individual numbers. Roots were measured individually and their lengths were recorded. The trees were then brought under 0, 10, 25 and 50 mM NaCl solutions as different treatments. Six trees in three boxes were considered as one treatment. After 3, 5, 8 and 10 days of induction, the root lengths were measured to calculate their elongation growth. For light microscopy, 3, 5, 8 and 10 days induced roots were sampled and washed gently several times with water to remove any adhering particles. The samples were fixed in Karnovsky's solution (Karnovsky, 1965), dehydrated in a graded ethanol series, infiltrated and embedded in JB-4 resin. Transverse sections of 3 µm thickness, taken at different distances from the root tips, were stained with iodine-potassium-iodide and observed and photographed under light phase microscope. Data on the stelar and overall diameters at different distances from the root apex were obtained from the prints. Five roots of each treatment were used every year.

For scanning electron microscopy, roots were immediately fixed in Karnovsky's solution (Karnovsky, 1965), post-fixed in osmium tetroxide, dehydrated in a graded ethanol series and dried in a critical point drier. The dried samples were coated with gold, observed and photographed under Hitachi S2250N scanning electron microscope at 20kV. Three-year-observations were summarized in all cases and reported.

Results

Formation of roots under different levels of culture solution salinity: Number of roots/tree/day was maximum under no-salt-control treatment which gradually decreased as the

culture solution salinity was increased up to 50 mM (Fig. 1). As a result, although rooting decreased at 10 mM NaCl solution compared to control, was not severe as 25 and 50 mM solutions. The decreasing trend of rooting also had a harmony with the increased concentration of salinity. Finally rooting reduced to about 50 percent at 50 mM NaCl solution compared to control. Although the number of new roots under different treatments varied slightly among the years, the formation trends corresponding to the concentration of culture solution salinity were always identical.

Elongation growth of roots under different treatments

Root elongation was higher in control compared to different levels of culture solution salinity (Fig. 2). This elongation also continued under control showing a regular trend of growth. Under 10 mM NaCl solution also, the elongation growth was in increasing trend but the increasing rate was lesser compared to that of control. Up to 5th day of exposure, at this treatment, the elongation growth was comparable to that of control. On the 8th day although there was a slight increase of root length, ranked lower than control. On the 10th day, root length remained same as 8 days induced roots. In contrast, root elongation was minimum from the onset of induction to 25 mM solution and at 5-day-exposure, elongation growth nearly ceased which had the same level up to 10th day. Under 50 mM root elongation was very negligible at the early stage of induction that completely ceased at 5-day-exposure. Compared to control, this treatment attributed less than half root length within 3 days which remained constant and became very negligible with respect to control during the following exposure times.

Periclinal growth of roots inferred from anatomical records

Both stelar and overall diameters maintained a gradual increasing trend towards the root base up to 10 mm studied under control (Figs. 3, 4). As the overall diameter increased the stelar diameter also increased and vice-versa but the increasing rate of stelar diameter was higher than that of overall diameter. Under 10 mM NaCl, both diameters similarly increased but these increasing rates, especially overall diameter decreased. The stelar and overall diameters tended to be constant having only a slight increase in diameters towards the root base under 25 mM. However, the increasing rate of overall diameter was higher than that of stelar diameter at this treatment. Under 50 mM, stelar and overall diameters remained completely constant from the root tip towards the root base. However, both stelar and overall diameters differed considerably among treatments at comparable distances from the root apex. The increasing rates of diameters towards the root tip were always higher in control than those of other treatments. The diameters under saline solution were comparatively higher near the root tip compared to control.

As a result, the ratio of stelar to overall diameter was lesser under control near the root apex that increased gradually towards the root base (Fig. 5). On the contrary, this ratio tended to be similar throughout the root tip as the levels of culture solution salinity were increased.

Morphology of roots at different levels of culture solution salinity under scanning electron microscope: Three-day-induced roots under control showed normal removal of older rootcap cells and possessed sharply pointed tips (Fig. 6A), and intact epidermal cells (Fig. 7A). On the 5th day, the removal of older rootcap cells continued, root tip remained sharp and root surface was smooth (Fig. 6B). The epidermal surface remained bare and the intact epidermal cells were conspicuously observed (Fig. 7B). On the 8th day, removal of older rootcap cells similarly continued and root tips were sharply pointed (Fig. 6C). The epidermal cells also remained intact as before (Fig. 7C). The 10-day-induced roots did not differ in terms of overall morphology than the roots of 3rd, 5th or 8th days induction (Fig. 6D) and the epidermal cells remained unchanged (Fig. 7D).

Three-day-induced roots under 10 mM NaCl were characterized by the removal of older rootcap cells and holding of intact epidermis (Figs. 6E, 8A). On the 5th day, the complete removal of older rootcap cells and the slightly surface depression of the epidermal cells were noticed (Figs. 6F, 8B). These depressed areas were blackish compared to their surroundings. On the 8th day, root tip started turning to round from sharply pointed one and dehydration of epidermal cells continued (Figs. 6G, 8C). On the 10th day, root tip rounding and dehydration of epidermal cells became more prominent (Figs. 6H, 8D).

Three-day-induced roots under 25 mM NaCl solution differed from others by severe and abnormal removal of rootcap cells and slight epidermal cell depression (Figs. 6I, 9A). On the 5th day, root surface became ridged and root tip tended to be swollen (Fig. 6J). All epidermal cells were under dehydration and injury which produced distinct holes in few places of the epidermis (Fig. 9B). On the 8th day, root tip turned to bulbous shape completely (Fig. 6K). The dehydration and injuries of epidermal cells resulted distinct holes on the entire surface of roots (Fig. 9C). On the 10th day, root tip injuries and epidermal cell dehydration became more prominent (Figs. 6L, 9D).

Three-day-induced roots at 50 mM concentrated solution were characterized by the abnormal shape and conspicuous dehydration of the epidermal cells (Figs. 6M, 10A). On the 5th day, root tip injuries and root tip swelling were observed. The distinct dehydration of the epidermal cells resulted numerous holes on the epidermis (Figs. 6N, 10B). On the 8th day, root surface was irregular with ridged and undulating surface with terracing on the root tip (Fig. 6O). The dehydration of epidermal cells became severe and caused serious malformation of roots (Fig. 10C). On the

10th day, complete terracing of root tips occurred and the epidermal injuries were distinctly found (Figs. 6P, 10D). In severe cases, the dehydrated and injured epidermal cells also exhibited the destruction of cellular contents.

Discussion

Present study revealed that the formation of roots and their elongation growths were affected by culture solution salinity and the severity of these effects increased at higher concentration of NaCl. Root growth has been reported previously to be limited under saline condition in citrus (Zekri and Parsons, 1992; Zekri, 1993) and in tomato (Snapp and Shennan, 1994). Therefore, the present decrease in formation and elongation growth of roots were consistent with these reports, but surprisingly differed from the finding of Alva and Syvertsen (1991) where root density was reported to be increased with high doses of salinity in carrizo citrange and sour orange rootstocks. In our study, the used satsuma mandarin trees were grafted on trifoliate orange rootstocks which has been reported to be severely affected by NaCl (Zekri and Parsons, 1992). Therefore, the differences in the used materials and comparatively higher salinity tolerance of carrizo citrange and sour orange rootstocks used by Alva and Syvertsen (1991) might be the reason of inconsistency. In favor of this opinion it can also be mentioned that root growth was reported to be accelerated by higher salinity in the salt tolerant asparagus, table beet and sea aster plants (Uno *et al.*, 1996).

The increase of stelar diameter under control treatment from the root apex towards the base and similar increase of overall diameter might be resulted from the normal growth of roots under this treatment. On the other hand, increase of root diameters near the tip followed by slower increase or the static condition of stelar diameter towards the root base under saline solutions indicated the inhibition of growth of roots by salinity. Root thickening occurs through the increase of stelar diameter (Fraser *et al.*, 1990). Therefore, the slower increase or static condition of stelar diameter under saline solution was consistent with the inhibition of root growth under this condition. Root thickening (Solomon *et al.*, 1986) and reduction in the cross sectional diameter of pea roots (Setia and Narang, 1985) were reported earlier. Our study made it clear that under saline solution root thickening extends up to the root tip. The cross sectional diameter although decreased in general, the root tip adjacent diameter increased. This was probably due to the induction of earlier aging of tissue near the root tip as was found in pea roots by Hasson and Poljakoff-Mayber (1981).

The continuous removal of older rootcap cells showing distinct epidermal cells along with sharply pointed tips indicated the normal growing condition of roots under control. Ridged and, in extreme cases, undulating surfaces under different levels of culture solution salinity can be

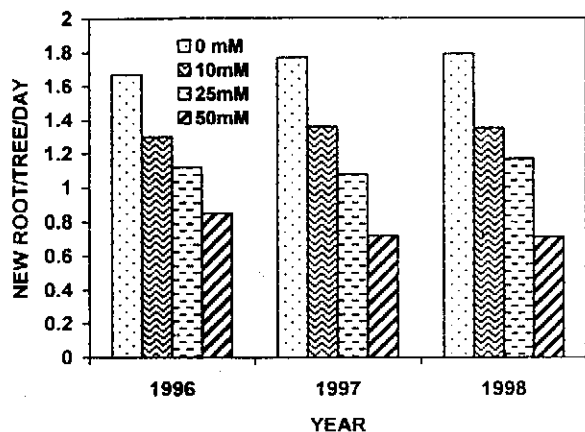


Fig. 1: Formation of roots in trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity during the experimental period. Note: Root formation decreased with the increased concentration of NaCl.

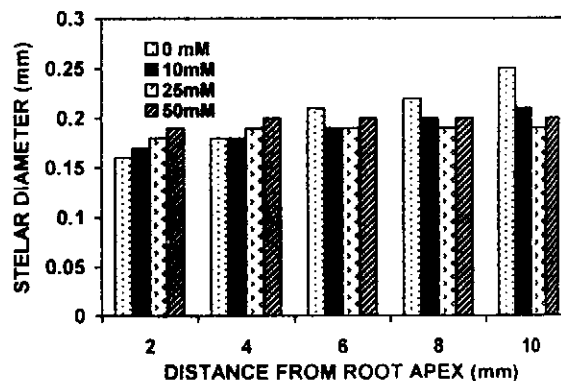


Fig. 3: Stelar diameters of roots in trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity. Note: Stelar diameter increased along the root base but culture solution salinity slowed that increasing trend.

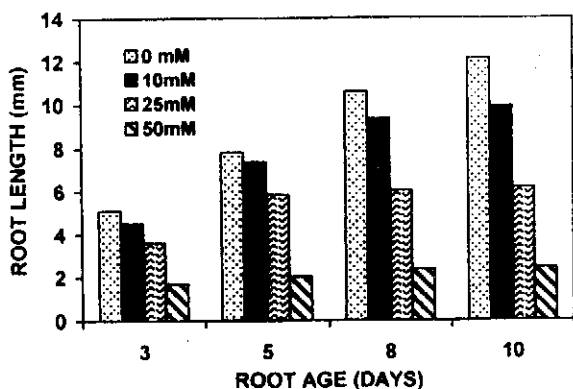


Fig. 2: Elongation growth of roots in trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity. Note: Elongation growth decreased with the increased concentration of NaCl.

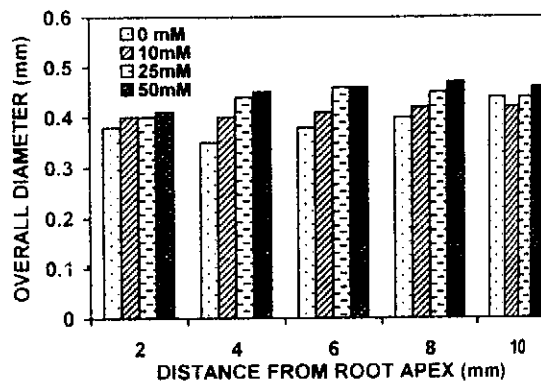


Fig. 4: Overall diameters of roots in trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity. Note: Overall diameter slightly increased towards the root base.

presumed as the shrinkage of epidermal cells which resulted these abnormalities. Root morphology determines the total surface area of root system that is exposed to salinity (Barber and Silberbush, 1984). The present study indicated

that root morphology in trifoliate orange grafted with satsuma mandarin was considerably modified by growth media salinity indicating the immense effects of this factor to the growing roots.

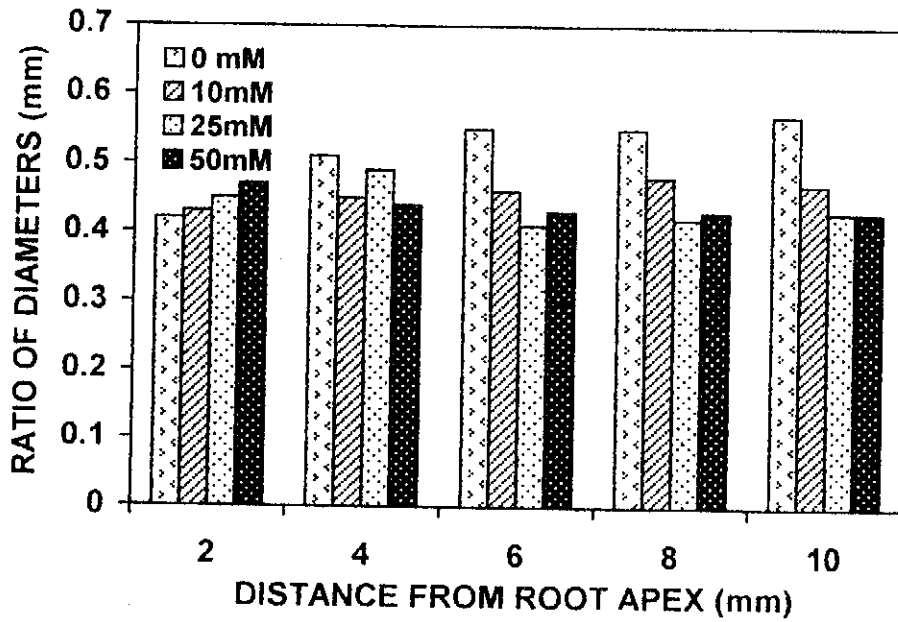


Fig. 5: The ratio of stelar to overall diameters of roots in trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity. Note: The ratio under control gradually increased which tended to be constant under saline solutions.

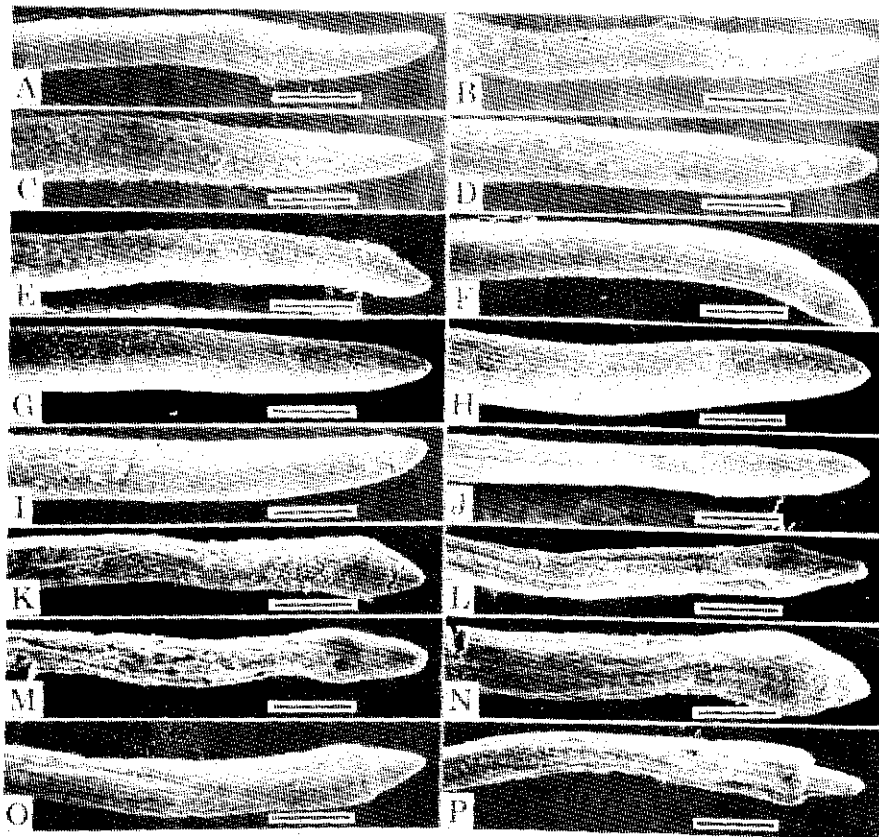


Fig. 6: Low magnified views of roots in trifoliate orange grafted with satsuma mandarin at different levels of culture solution salinity under SEM. (A-D represent no-salt-control, E-H denote 10 mM NaCl solution, I-L indicate 25 mM NaCl and M-P express 50 mM NaCl solution). A, E, I, M: Three-day-induced. B, F, J, N: Five-day-induced. C, G, K, O: Eight-day-induced. D, H, L, P: Ten-day-induced. Note: Root tip swelling, surface ridging and root tip terracing gradually appeared in saline solutions. Bar = 500 μ m.

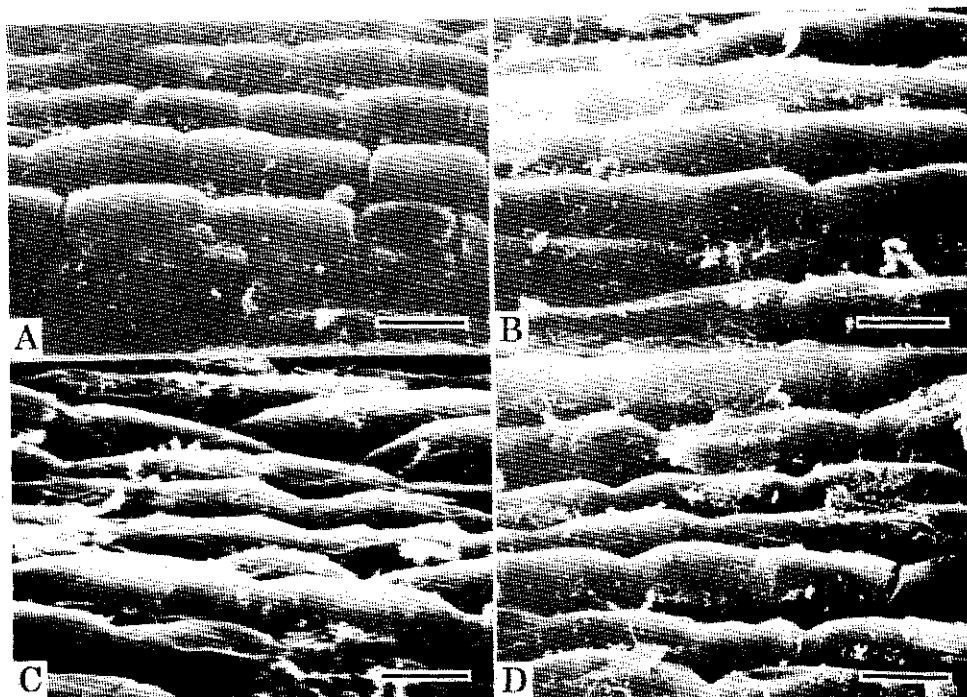


Fig. 7: Epidermal cells of roots in trifoliate orange grafted with satsuma mandarin under no-salt-control treatment. A: Three-day-induced, B: Five-day-induced, C: Eight-day-induced, D: Ten-day-induced. Note: The epidermal cells were intact irrespective of exposure time. Bar = 10 μ m.

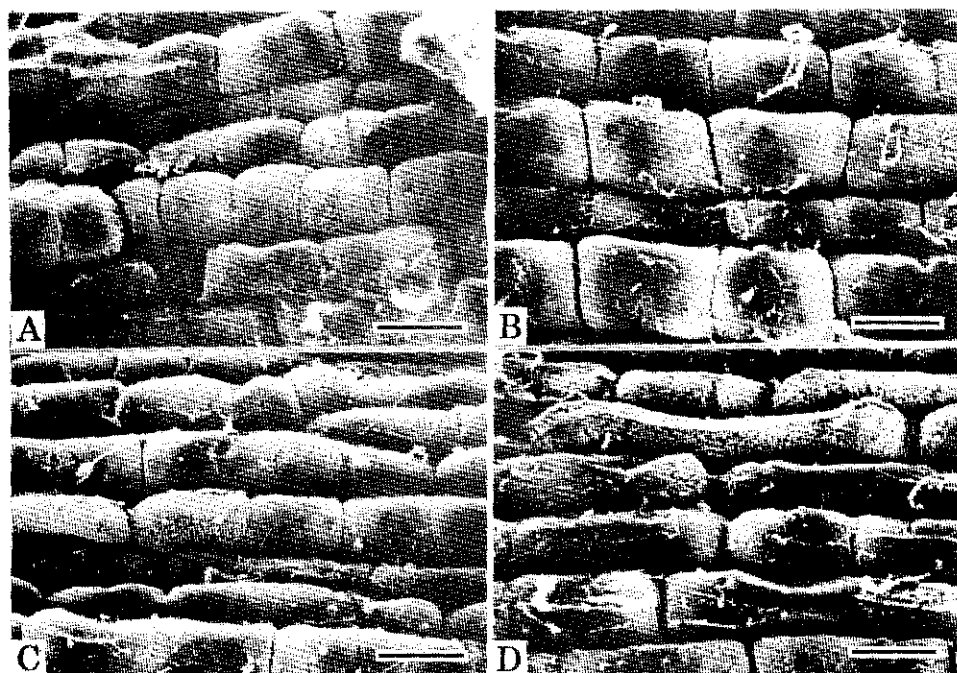


Fig. 8: Epidermal cells of roots in trifoliate orange grafted with satsuma mandarin under 10 mM NaCl solution. A: Three-day-induced, B: Five-day-induced, C: Eight-day-induced, D: Ten-day-induced. Note: Cell dehydration initiated longer exposure. Bar = 10 μ m.

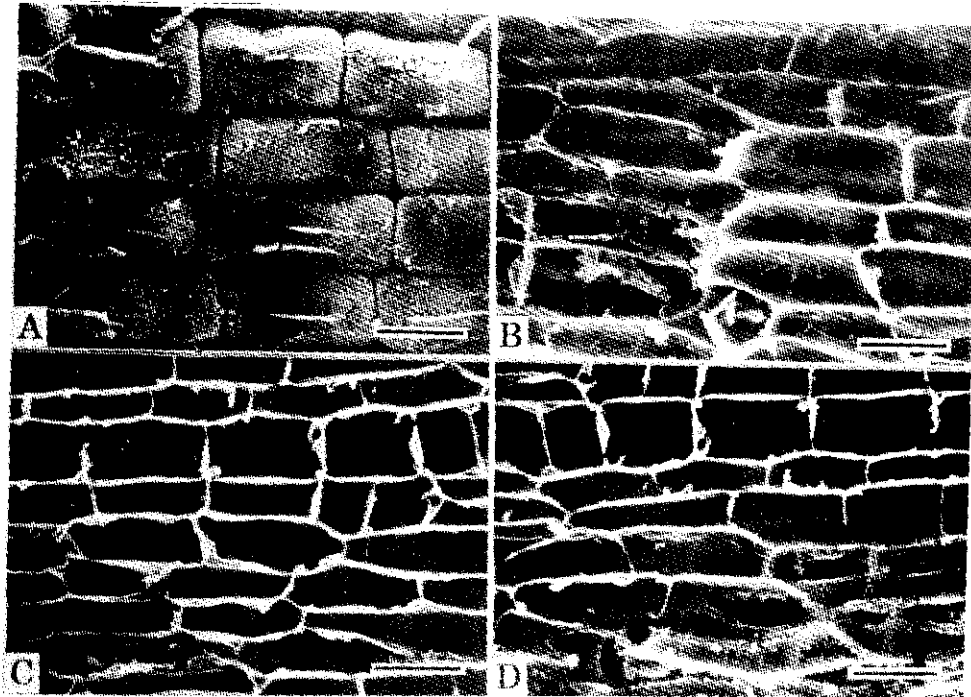


Fig. 9: Epidermal cells of roots in trifoliolate orange grafted with satsuma mandarin under 25 mM NaCl solution. A: Three-day-induced, B: Five-day-induced, C: Eight-day-induced, D: Ten-day-induced. Note: Cell dehydration and injuries appeared gradually along the exposure time. Bar = 10 μ m.

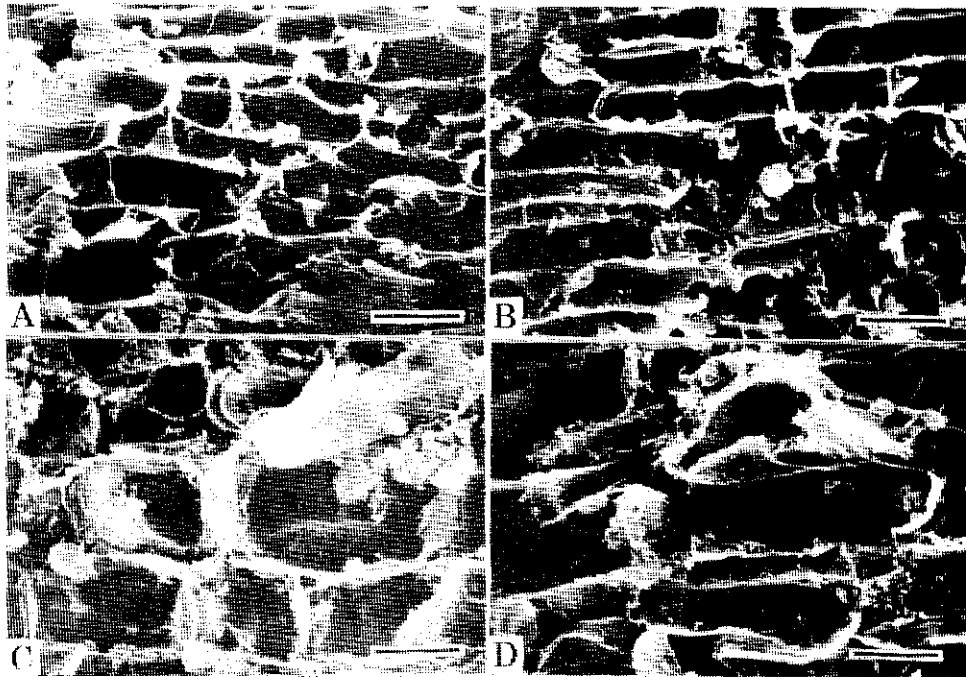


Fig. 10: Epidermal cells of roots in trifoliolate orange grafted with satsuma mandarin under 50 mM NaCl solution. A: Three-day-induced, B: Five-day-induced, C: Eight-day-induced, D: Ten-day-induced. Note: Distinct formation of holes were observed on the root surface. Bar = 10 μ m.

The epidermal cell dehydration of 3-day-induced roots under saline solutions was consistent with the finding of Solomon *et al.* (1986) who also found salinity-induced morphological and anatomical changes in pea roots, even within 24 hours of placement in the culture solution. This first noticeable changes of epidermal cell dehydration in our study was consistent with Perez-Alfocea *et al.* (1996) who suggested that the degree of salt stress on a plant depends on cell dehydration. Similarly, Kramer (1984) has described mesophyll cell dehydration under salt stress condition. The complete dehydration of epidermal cells and production of holes on the epidermis at longer exposure of roots to the salt stress condition might be the consequences of continuous adverse effects.

Root tip injuries and, in extreme cases, root tip terracing were observed under saline condition in our experiment. Maas (1993) suggested that the obvious effects of salinity on plants were growth suppression and various symptoms of leaf injuries. On the other hand, studying excised pea roots, Hodson and Mayer (1987) reported that pea root tip was severely affected after 24 hours exposure to salinity. Therefore, we concurred with the findings of Maas (1993) and Hodson and Mayer (1987). Studying salt-treated plants of Rangpur lime, accumulation of higher concentration of Cl^- shape from sharp to a dome or round in the salt treated tree roots in our study was probably due to the inhibition in growth under stress condition by the accumulation of Cl^- as reported by Walker *et al.* (1984). In our another study this near the root tip compared with the proximal part of root was reported (Walker *et al.*, 1984). The changing in root tip kind of root growth cessation was observed under temperature-stress condition (Mohammad *et al.*, 1996).

In conclusion, the trifoliolate orange rootstock is very much sensitive to culture solution salinity in terms of root formation and root growth. The roots may undergo different kinds of morphological deformations and tip injuries if this is exposed to saline condition, even a very low concentration (10 mM) of NaCl may affect roots to a great extent. As the salt concentration and exposure time increase, the degree of adverse effects also increases. The growth inhibiting strategies might be root tip injuries and epidermal cell dehydration. No-salt-control treatment can be suggested as the favorable range for trifoliolate orange grafted with satsuma mandarin. For clear understanding of the mode of adverse activities of culture solution salinity, the ultrastructural observations on the cellular and sub cellular levels are warranted.

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