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Changes Induced by Culture Solution Salinity to the Anatomy of Roots of Trifoliate Orange Grafted with Satsuma Mandarin

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Abstract

Anatomical changes in root tips of trifoliate orange grafted with satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsu Wase) at 0, 10, 25 and 50 mM NaCl in the culture solution were investigated after 3, 5, 8, and 10 days induction. At no-salt-control treatment, the root tips were always sharply pointed, apical initial cell layers were distinct and meristematic zone was wider. The rootcap and cortical cells apart from the root tip possessed only a few starch grains. The cortical cells were elongated and cell length gradually increased from the root tip towards the base. In contrast, under 10 mM NaCl, root tips gradually turned round and the meristematic zone finally shortened. Only a few starch grains accumulated up to 8 days of induction followed by degradation in the rootcap cells but cortical grains gradually increased. At 25 mM NaCl, severe sloughing of rootcap cells and the secretion of mucilaginous substances appeared on the 5th day. The epidermal and root tip injuries were found and the apical initial cell layers became indistinct from the 5th day onward. Lignification occurred in the central cylinder which gradually proceeded towards the root tip. Starch grain accumulation increased in the cortical cells but decreased in the rootcap cells along the exposure time to the saline solutions. The cortical cells tended to round and cell length increased near the root tip. At 50 mM NaCl solution, root tip and epidermal injuries and lignification in the central cylinder initiated on the 3rd day and became severe at longer exposure. Starch grains were not found in the rootcap cells but the cortical grains gradually increased. The cortical cell rounding and enlargement started from the very beginning of induction.

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). Compared to other agronomic and horticultural crops, citrus species are among the most sensitive to salinity (Maas and Hoffman, 1977; Walker and Douglas, 1983; Maas, 1990). NaCl causes foliar accumulation of Na (Behboudian *et al.*, 1986; Lloyd *et al.*, 1987) or Cl (Cooper and Gorton, 1952; Grieve 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) and has also other different effects on the growth of plants (Francois and Maas, 1978, 1985; Shalhevet and Levy, 1990). The various aspects of salinity effects on the physiological phenomenon of crop plants including citrus is also well established (Gale, 1975; Downton, 1977; Levitt, 1980; Walker *et al.*, 1981). To the best of our knowledge, the effects of salinity to the anatomy of citrus roots is not well studied and, especially the roots of trifoliate orange grafted with satsuma mandarin has not drawn the attention of scientists. Root is that part of the plant which has direct exposure to growing media salinity. This implies the immense importance of roots as a study material for increased understanding of the effects of NaCl on any plant species. The material should be studied up to the cellular levels for the distinct resolution of the effects. It has been recently shown that growing cells do not necessarily response to environmental stress in the same way as the mature tissue (Nonami and Boyer, 1989; Pritchard *et al.*, 1991), indicating that the presence of mature tissue may mask the response of growing cells. Thus determination of growth parameters in mature tissue does not accurately reflect controls of growth. Since the investigations on the growing tissue or cells are logical. Apparently the cell length patterns help the plant adapt to environmental stress (Silk, 1992). Therefore, cell length might be an useful parameter for the study of stress effects on plants.

This investigation was designed to elucidate the effects of different levels of culture solution salinity on the anatomical features of root tips emphasizing starch grain accumulation in the cortical and rootcap cells of trifoliate orange grafted with satsuma mandarin. The cell length patterns in the cortical layers close to the root apex were also taken into consideration.

Materials and Methods

The experiment was conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University 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 within a plastic house. Twenty four trees for uniformity of vigor were placed into twelve styrofoam boxes containing culture solution each 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).

The culture solution pH was adjusted everyday between 5.0-5.5 by using 1M H_2SO_4 or 3M NaOH as required. The solutions were continuously aerated and stirred by air pumps. After the formation of numerous new roots, they were marked with wax-coated paper tags and subjected to 0, 10, 25 and 50 mM of NaCl. Different levels of culture solution salinity were made by adding required amount of NaCl. Three boxes retaining six trees were considered as one treatment. To ensure an adequate supply of all essential elements, culture solutions were completely renewed at every 14 days interval. The house temperature was adjusted between 25-27°C during the experiment.

Roots of 3, 5, 8, and 10 days induction were sampled and washed gently several times with water to remove any adhering particles. The collected samples were fixed immediately in Karnovsky's solution (Karnovsky, 1965), dehydrated in a graded ethanol series, infiltrated and embedded in JB-4 resin. Median longitudinal sections of 3μm thickness were stained with iodine-potassium-iodide, viewed and photographed under light phase microscope. Data on the cortical cell length were obtained from prints with a total magnification of X1000 as was used by Betraud and Gandar (1985). For this purpose the continuously taken photographs of individual section of a root were arranged and pasted. The cell lengths at different distances from the root tip were measured. The same cell files were used for this purpose. Cell length at each point was taken as an average of the 25 to 35 cells. The three-year-observations were interpreted and average results were presented.

Results

Anatomical features of roots of trifoliate orange grafted with satsuma mandarin under different levels of culture solution salinity: Under no-salt-control treatment, three-day-induced roots had normal anatomical features. The root tips were sharply pointed, initial cell layers were distinct and meristematic zone was wider (Fig. 1A). Normal sloughing of older rootcap cells and the presence of well furnished column of cells were clearly observed. Five-day-exposed roots were also in normal anatomical state (Fig. 1B). Root tip was sharply pointed as 3-day-induced roots. Eight-day-induced roots remained in the similar anatomical state having sharply pointed tips (Fig. 1C). The apical initial cell had normal arrangement. The 10-day-induced roots had n differences in terms of anatomical features compared to th roots sampled after 3, 5 or 8 days induction (Fig. 1D). Th quiescent center was far apart from the root tip at a sampling dates. The cortical cells were always elongated. The roots under 10 mM NaCl solution also exhibited norm anatomical characteristics having distinct initial cell layer and wider meristematic zone on the 3rd day of inductio (Fig. 2A). The quiescent center was apart from the root tip The cortical cells were elongated at every distances fro the root tip. The columns of rootcap cells were distinc Five-day-induced roots also displayed overall norm anatomy having sharply pointed tips and elongated cortic cells (Fig. 2B). All other characteristics resembled the roo of 3 days induction. The adverse effects of this treatmen started on the 8th day with the narrowing of meristemati zone, proximity of the quiescent center to the root termin and enlargement of cortical cells little far from the root t (Fig. 2C). Ten-day-induced roots had shortening o meristematic zone and severe sloughing of rootcap cel (Fig. 2D). The cortical cells turned round and enlarged ne the root apex. The quiescent center became comparativel more proximal to the root tip. The lignification (arro heads) started in the central cylinder (Fig. 2D). Th intercellular spacing (IS) in the cortex was conspicuous (Fi 5H).

Three-day-induced roots at 25 mM NaCl solution ha pointed tips and wider meristematic zone (Fig. 3A). The 5 day-induced roots received meristematic zone shortenin and severe sloughing of rootcap cells (Fig. 3B). The cortic cells appeared larger and lignification (arrow heads) wa distinct in the central cylinder (Fig. 3B). Eight-day-induce root tips turned round, almost all the rootcap cells sloughe off and root tip (RI) and epidermal injuries (EI) wa observed (Fig. 3C). The meristematic zone became short and cortical cell enlargement occurred even near the ro tip. The lignification (arrow heads) in the central cylind became more prominent (Fig. 3C). The 10-day-induce roots received severe root tip injury (RI) (Fig. 3D). Th meristematic zone partially damaged and the cortical ce turned round even in the tip region. The epidermal injury (E was also distinct in few places (Fig. 3D). The lignificati (arrow heads) in the central cylinder continued and becam proximal to the root terminal (Fig. 3D). Intercellular spaci (IS) occurred in the cortex which increased with t exposure time (Figs. 5K,L).

Three-day-induced roots at 50 mM NaCl solution receiv root tip injuries (RI) with the secretion of mucilagino substances (Fig. 4A). The rootcap cells mostly sloughed o Cell rounding occurred in the cortex a little apart from t root tip. The lignification (arrow heads) appeared in t central cylinder (Fig. 4A). Five-day-exposed ro underwent severe root tip injuries (RI) (Fig. 4B). Compl root tip abolition occurred through the dissolution

rootcap cells. Lignification (arrow heads) was prominent in the central cylinder (Fig. 4B). Intercellular spacing (IS) was found even near the root terminal (Fig. 5N). After 8 days induction, root tip injury (RI) became more severe (Fig. 4C). The cell rounding was noticed near the root apex. The lignification (arrow heads) in the central cylinder also continued (Fig. 4C). Intercellular spacing (IS) continued (Fig. 5O). After 10 days induction, the entire root tip abolition through severe root tip injuries (RI) was found, lignification occurred in the central cylinder, and the meristematic region completely destroyed (Fig. 4D). Intercellular spacing (IS) increased even near the root tip (Fig. 5P).

Starch grain accumulation and degradation patterns in the rootcap and cortical cells under different levels of culture solution salinity: The rootcap cells accumulated many starch grains (SG), especially in the outer layers at no-salt control treatment (Figs. 1A-D). The cortical cells possessed no grains near the root tip (Figs. 1A-D) but the distal cells contained a few starch grains (SG) (Figs. 5A-D) and the accumulation increased towards the root base. However, the accumulation of grains in the cortical cells always maintained the same level. Under 10 mM NaCl solution, starch grain (SG) accumulation occurred in the rootcap cells on the 3rd and 5th days of induction which became less on the 8th day (Figs. 2A-C) and degraded completely on the 10th day (Fig. 2D). Starch grains were absent on the root and adjacent cortical cells (Figs. 2A-D) but the grain (SG) content increased in the distal cortical cells along the exposure time (Figs. 5E-H). Under 25 mM NaCl solution starch grains (SG) in the rootcap cells were found only on the 3rd day (Fig. 3A) which completely degraded during the following sampling dates (Figs. 3B-D). The root tip adjacent cortical cells still remained starch grain less (Figs. 3A-D) but the distal cells accumulated grains (SG) (Figs. 5I-L). The grains near the injured epidermis underwent degradation. At 50 mM NaCl solution starch grains were not found in the rootcap cells (Figs. 4A-D). Starch grain (SG) content increased in the cortical cells along the exposure time and in the root tip adjacent round cells possessed a few grains (Figs. 5M-P). The starch grain degradation was observed near the injured epidermis.

Cell length in the cortical layers close to the root apex under different levels of culture solution salinity: The cortical cell length profiles under different treatments varied considerably in the corresponding locations of root tips up to 5 mm studied. However, the cell lengths increased from the root apex towards the root base in all treatments. This increase followed a regular trend in the no-salt control treatment (Fig. 6) where the cell length up to 1 mm from the root apex remained within 20 μm. This length was unchanged in the following sampling dates. Under 10 mM treatment the occurrence of smaller cells (less than 20 μm) were restricted within 0.5 mm from the root apex in the initial stage (Fig. 7). Cell length increased at 0.5 mm back from the root apex and the cell enlargement gradually became proximal to the root tip. It became more proximal to the root tip at 25 mM NaCl solution (Fig. 8). Although smaller cells were found up to 0.5 mm from the root apex at the initial stage, became limited to the surroundings of quiescent center on the 5th day. The cell enlargement extended up to the root tip from the initial stage of exposure under 50 mM solution (Fig. 9).

Discussion

The proportional reduction of growth in increased concentration of culture solution salinity was reported in citrus (Zekri *et al.*, 1992). The uptake and transport of Cl by plants are associated with the salinity and some times physiological disturbances can occur before the appearance of visible symptoms caused by salinity (Downton, 1977; Walker *et al.*, 1981). This was postulated to be resulted from the inability of plants to withstand osmotic stress caused by the presence of salt in the soil solution (Gale, 1975; Levitt, 1980). Our present observation revealed the anatomical effects, especially the rounding of cells in the meristematic cortex under different levels of salinity. Severe intercellular spacing also appeared at higher levels of salinity indicating these treatments as adverse for normal plant growth. Physiological disturbances and growth reductions even in a low to moderate levels of salinity have also been similarly reported in other citrus rootstocks (Cooper and Shull, 1953; Kirkpatrick and Bitters, 1969; Walker *et al.*, 1982). The epidermal injuries in the present study at higher doses of salinity, especially at longer exposure was consistent with the report of Storey and Walker (1987). Disorganization and injuries in the rootcap and initial cells of salt treated roots obviously showed growing media salinity as an influential limiting factor for root growth in citrus. The rootcap cells provides the protective mechanisms to the initial cells and, in adverse condition, these are sloughed off. The initial cell is comparatively persistent to adverse conditions and mature tissue usually mask the response of growing cells under stress conditions but could not endure the salinity effects in the present study. This kind of disturbance is presumed to be resulted in the cessation of growth of roots. Similar root tip injuries were reported under high temperature and low soil moisture stress condition (Mohammad *et al.*, 1996).

The presence of only a few starch grains in the control tree roots was probably due to the instant utilization of the synthesized products by the trees for their normal growth and development. While the starch grain accumulation under higher salinity in this study could be presumed as the result of disturbance in the normal physiological activities of plants causing hindrance in the utilization of stored grains. Mohammad and Shiraishi (1998) has reported that starch grain accumulation occurred under cold stress condition in satsuma mandarin which was utilized readily with the resumption of favorable temperatures. The degradation of grains near the injured epidermis in this experiment might be

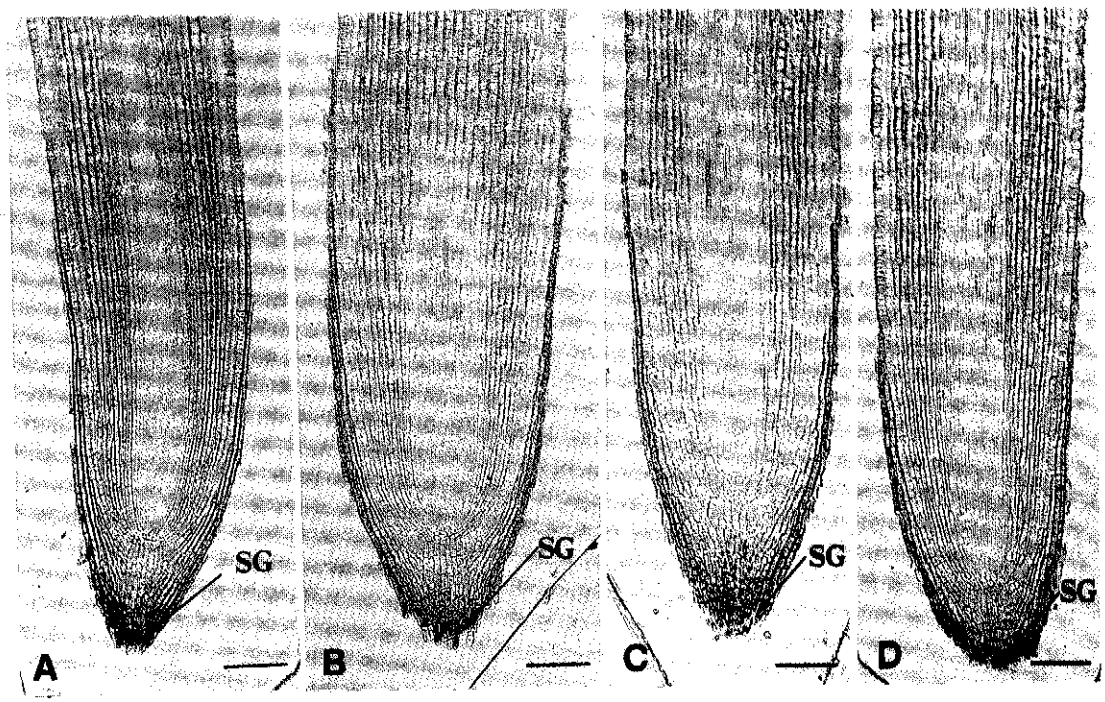

Fig. 1: Light micrographs of median longitudinal sections of roots in trifoliate orange grafted with satsuma mandarin under no-salt control treatment. A: Three-day-induced. B: Five-day-induced. C: Eight-day-induced. D: Ten-day-induced. Note: Normal anatomy with pointed tips, wider meristematic zone and a few cortical starch grains apart from the root apex were found irrespective of exposure time. SG, Starch grain. Bar = 150μm.

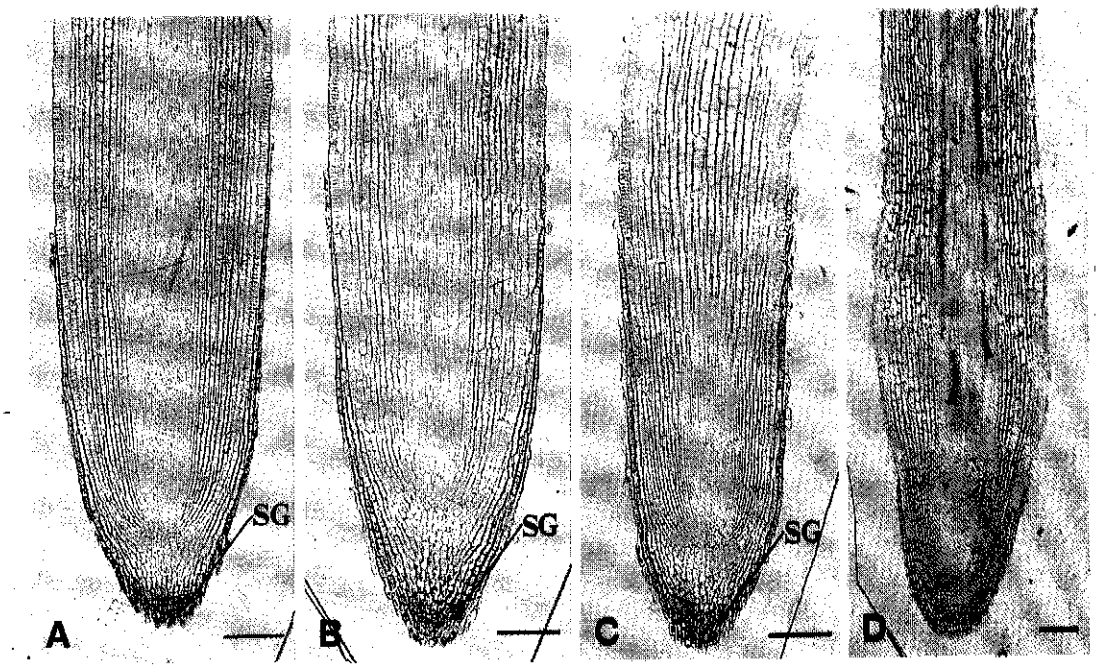

Fig. 2: Light micrographs of median longitudinal sections of roots in trifoliate orange grafted with satsuma mandarin unde 10 mM NaCl solution. A: Three-day-induced. B: Five-day-induced. C: Eight-day-induced. D: Ten-day-induced. Not Adverse effects of salinity became evident on the 8th and 10th days of induction having larger cells near the ro tip and abnormal sloughing of rootcap cells. SG, Starch grain. Arrow heads indicate lignification. Bar = 150μm

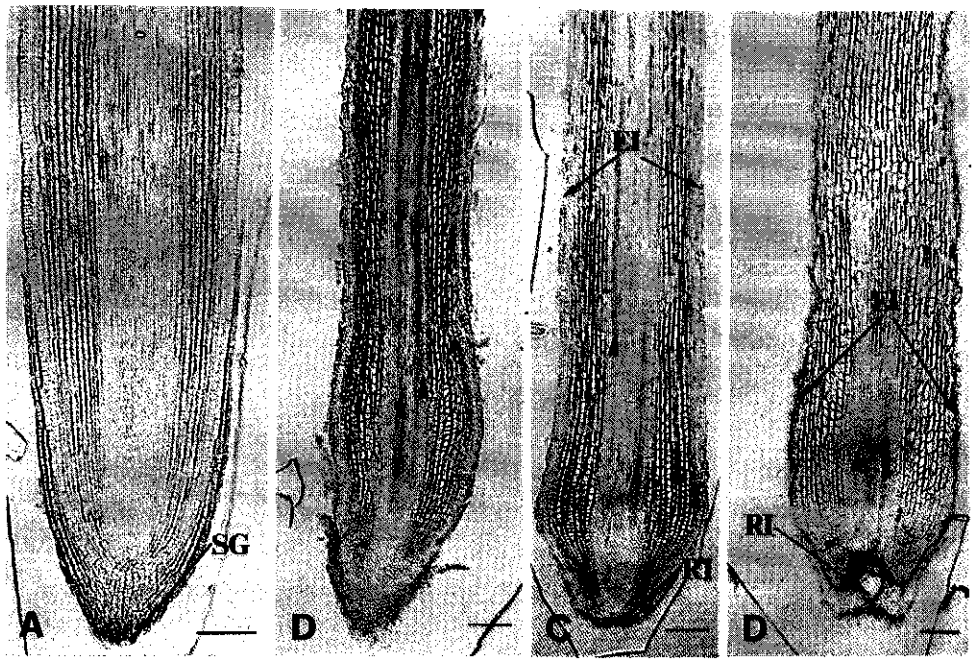

Fig. 3: Light micrographs of median longitudinal sections of roots in trifoliate orange grafted with satsuma mandarin under 25 mM NaCl solution. A: Three-day-induced. B: Five-day-induced. C: Eight-day-induced. D: Ten-day-induced. Note: Severe injuries in the root tip and epidermis appeared along the exposure time. SG, Starch grain; EI, Epidermal injury; RI, Root tip injury. Arrow heads indicate lignification. Bar = 150μm.

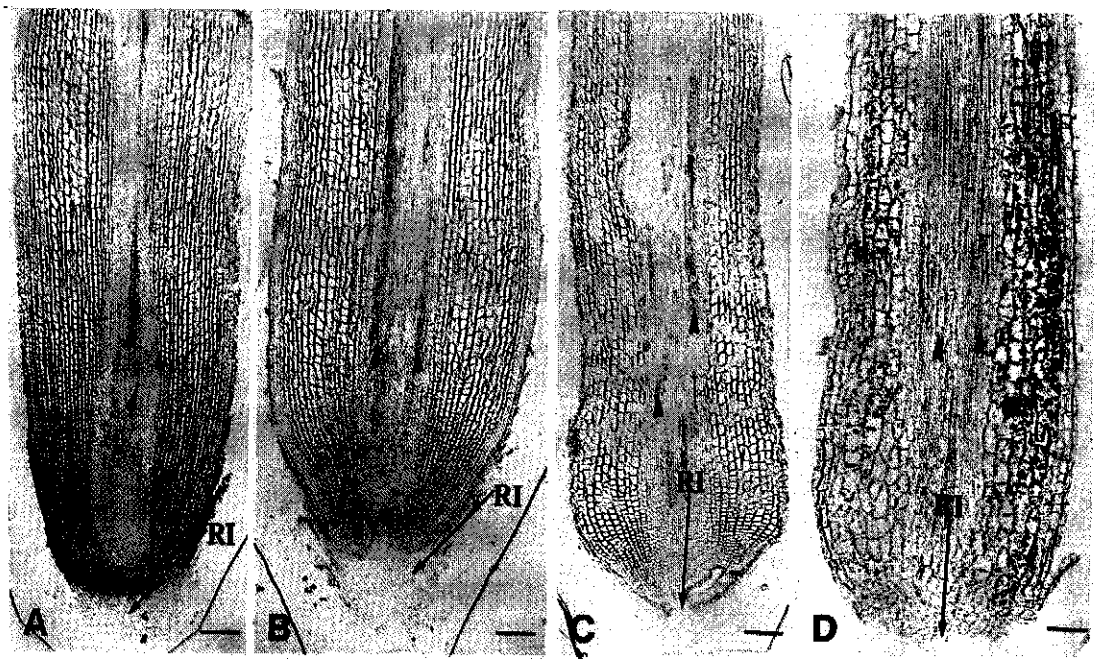

Fig. 4: Light micrographs of median longitudinal sections of roots in trifoliate orange grafted with satsuma mandarin under 50 mM NaCl solution. A: Three-day-induced. B: Five-day-induced. C: Eight-day-induced. D: Ten-day-induced. Note: Severe root tip and epidermal injuries appeared even at short exposure. RI, Root tip injury. Arrow heads indicate lignification. Bar = 150μm.

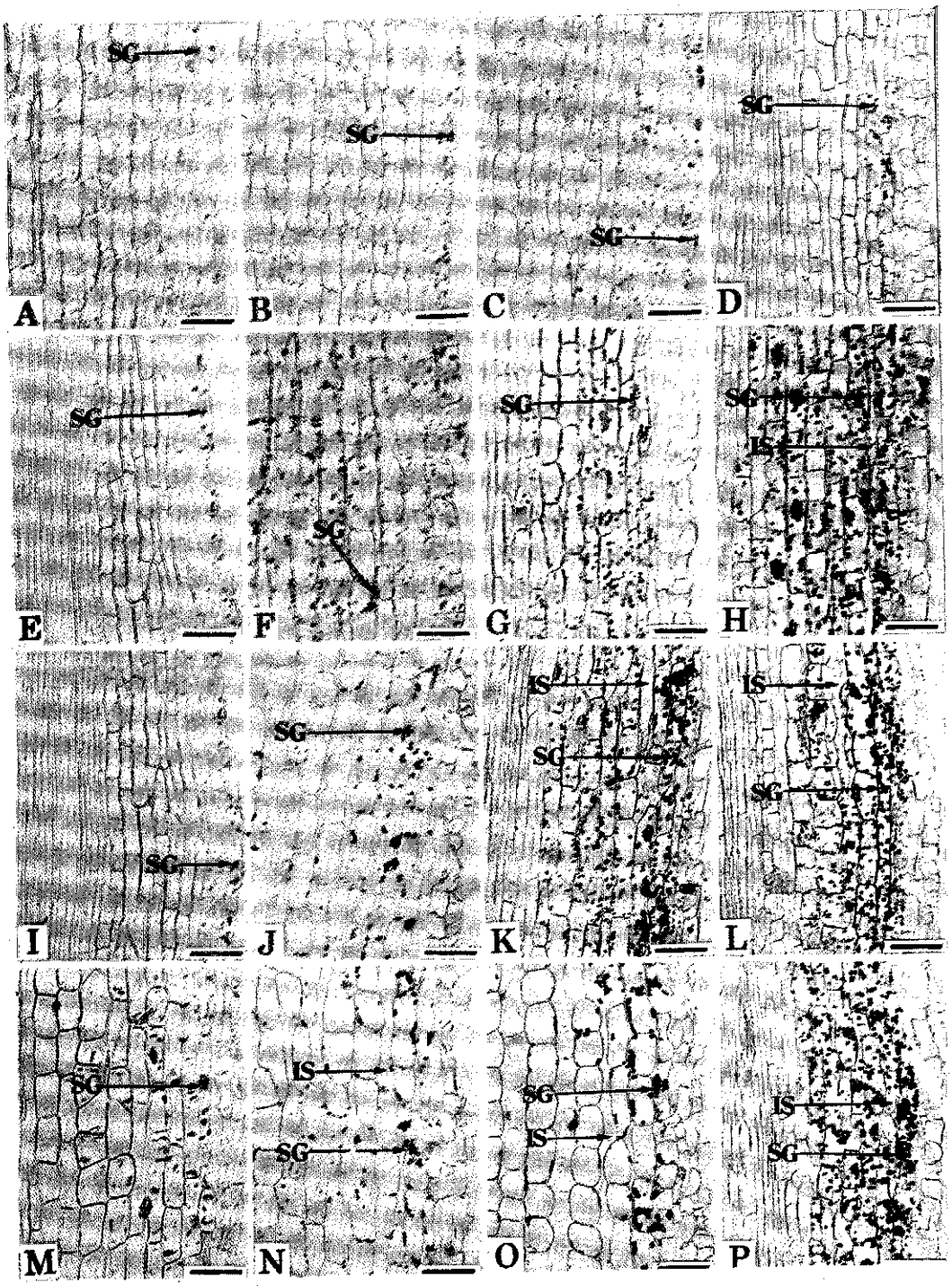

Fig. 5: Localized views of longitudinal sections of roots in trifoliate orange grafted with satsuma mandarin. (A-D dend no-salt-control, E-H represent 10 mM, I-L show 25 mM and M-P present 50 mM NaCl solution. A, E, I, M: Thr day-induced. B, F, J, N: Five-day-induced. C, G, K, O: Eight-day-induced. D, H, L, P: Ten-day-induced. Note: O a few starch grains deposited on the cortical cells apart from the root tip under control. Starch accumulat increased along the increased concentration of salinity with longer exposure. The cell rounding proceeded towa the root tip to response the increased salinity along the exposure time. SG, Starch grain; IS, Intercellular spa Bar = 100μm.

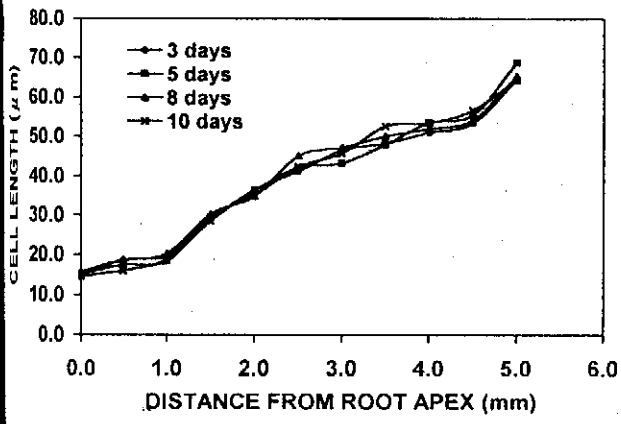

Fig. 6: Cortical cell lengths at different distances from the apex of roots in trifoliate orange grafted with satsuma mandarin under no-salt control treatment.

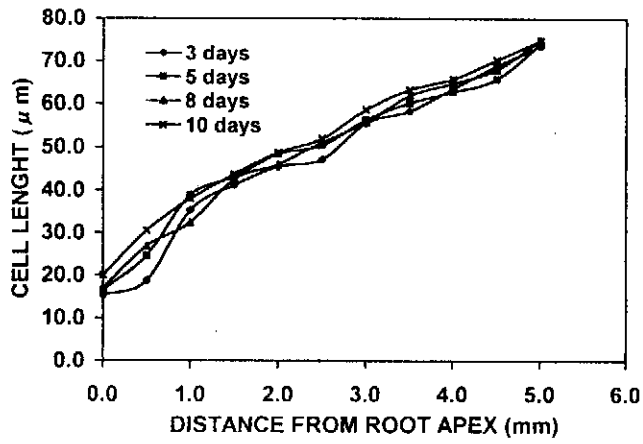

Fig. 8: Cortical cell lengths at different distances from the apex of roots in trifoliate orange grafted with satsuma mandarin under 25 mM NaCl solution.

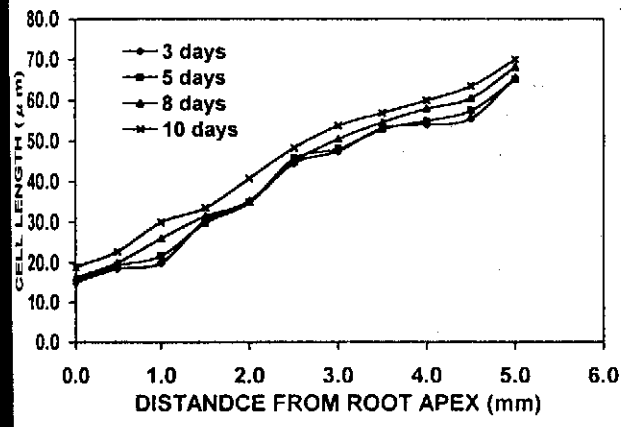

Fig. 7: Cortical cell lengths at different distances from the apex of roots in trifoliate orange grafted with satsuma mandarin under 10 mM NaCl solution.

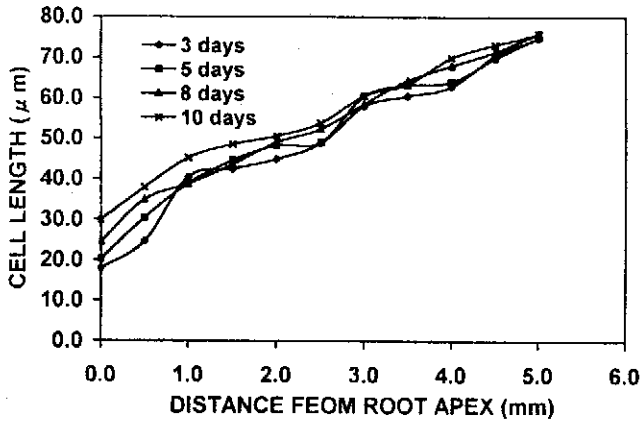

Fig. 9: Cortical cell lengths at different distances from the apex of roots in trifoliate orange grafted with satsuma mandarin under 50 mM NaCl solution.

an adaptive mechanism of plants under adverse condition. This means that the plants tried to cope with the situation by the expense of stored starch grains. Lignification usually occurs when tissues are under injury. Therefore, the present lignification in the central cylinder might be due to the injuries
in the root structure from the adverse effects of salinity. As the lignification became gradually proximal to the root terminal along the exposure time under higher salinity indicating these conditions as the gradual impedance to root health.

To understand the functional significance of the cell size profile, contrasting demands of intercellular and intracellular transport should be considered. Intracellular transport is greatly facilitated by small cell size, while intercellular transport is impeded by the presence of cell wall. Developing cells are metabolically very active, so that in developing tissue a small cell size is necessary for the elevated rates of protein production. In contrast, intercellular transport is facilitated by longer cells. The meristem must import sucrose and other metabolites from the phloem, and phloem is functional many cell lengths from the meristem. Although plasmodesmatal frequency and structure may be modified to facilitate symplasmic transport, imports of metabolites is impeded to some extent by the cell wall. In stressed tissue, movements of metabolites from the phloem into the meristem is facilitated by two anatomical adaptations. Slow growth is usually associated with phloem differentiation close to the meristem (Rost and Baum, 1988). The increase in cell length at the base of the meristem also promotes intercellular transport. The comparatively larger cells near the root apex under saline condition therefore indicated the condition as adverse and the elongated but smaller cells near the root apex and enlargement of cells towards the root base under control stood in favor to opine the condition as ideal from our study.

In conclusion, it can be stated that trifoliate orange rootstock might undergo different kinds of anatomical changes and root tip injuries if this is exposed to saline conditions and even a very low concentration of NaCl (10 mM) may affect the roots if they are left for longer periods. The adverse effects of salinity increase with the increased concentration of salt and exposure time.

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