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The Effects of Culture Solution pH on Root Formation, Root Growth and Root Morphology in Trifoliate Orange Budded with Satsuma Mandarin

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Abstract
The formation rate, elongation and periclinal growths, and the morphology of roots in trifoliate orange budded with satsuma mandarin under culture solution pH 4.5-5.6, 6.0-6.3 and 7.5-7.6 were studied to know the favorable range of culture media acidity for this plant. Root formation was earlier and maximum and the elongation growth was highest at pH 4.5-5.6 followed by pH 6.0-6.3 and 7.5-7.6. The periclinal growth also increased in terms of stelar and overall diameters along with elongation under pH 4.5-5.6 but periclinal development became slower or remained constant at pH 6.0-6.3 and 7.5-7.6. Normal sloughing of older rootcap cells leaving sharply pointed root tips and the presence of distinctly visible intact epidermal cells were observed under pH 4.5-5.6 at all exposures. While at pH 6.0-6.3, moderately severe removal of rootcap cells was evident at short exposure followed by root tip injuries and root surface ridging on prolonged induction. The severe removal of rootcap cells under pH 7.5-7.6 became apparent in a short period and root tip injuries occurred subsequently on prolonged exposure. Epidermal peel off and root surface ridging were prominent due to the destruction of epidermal cells and gradual root death with the increase of exposure time. This study suggests that the favorable range of growing media pH for trifoliate orange budded with satsuma mandarin lies between 4.5-5.6.

Introduction
Growing media pH is an important factor which has considerable effects on the overall growth of plants. Often these effects are complex and it is very difficult to separate them from other indirect phenomenon associated with the changes in solubility of various biologically important mineral elements (Islam *et al*., 1980). Until to date enormous reports appeared on the influence of media pH on nutrient solubility and physiological activities in different crop plants (Medappa and Dana, 1970; Zieslin and Snir, 1989; Rosen *et al*., 1990; Brunet, 1994; Sasada *et al*., 1994; Zieslin and Abolitz, 1994; Koyama *et al*., 1995). Majority of these reports were made on the influence of growing media pH on the above ground parts of plants but these influences on the underground parts were unheeded. Fortunately, although the effects of growing media pH on both above and underground parts of citrus have been studied, the available findings are contradictory.

Smith (1957, 1971) reported that root elongation ceased in citrus below pH 4.0 and that vigorous tree growth occurred at. pH 6.0. On the other hand, Yokomizo and Ishihara (1973) found vigorous tree growth in satsuma mandarin under pH 3.8 and 4.5 in solution culture which depressed with decaying of roots in rising pH above 5.2. Recently, Soprano and Koller (1996) have also suggested pH 5.0-5.5 as the best range and pH 6.0 as the worst one for cleopetra mandarin. Moreover, Chapman (1968) reviewed that a wide range of growing media pH between 3.8-9.7 was tolerated by citrus plants. The ultimate influence of growing media pH in citrus is thereby until now in serious contention which suggested us not to embrace any range of pH hastily as favorable. Therefore, it was implicative to note the necessity of increased understanding of the appropriate range of growing media pH for citrus. Roots are directly in contact with media pH and are potentially the first line to response to the media environment. The morphology of growing roots was reported to be changed along with growth cessation in satsuma mandarin plants under high temperature and water-stress conditions (Takao *et al*., 1996). This means that the morphological states of roots might be the indicators of growing states of plants. We were therefore inspired to study roots as the best logical representative organ for our studies on satsuma mandarin plants.

Experiments were designed to elucidate the effects of different levels of growing media pH on roots of trifoliate orange budded with satsuma mandarin in terms of formation rate, elongation and periclinal growths and external morphology emphasizing surface characteristics and tip statures. Based on these characteristics, the favorable range of pH for this plant was also concluded.

Materials and Methods
Two experiments were conducted under this program in the Citriculture Laboratory, Faculty of Agriculture, Ehime University, Japan from April 14 to June 30 for three consecutive years (1996-1998). Three-year-old satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsu Wase) trees grafted on trifoliate orange were used. Eighteen trees for uniformity of size were selected for each experiment and were placed into styrofoam boxes retaining nutrient solution each year. Two plants were allocated for one box and the boxes were buried into the soil. The nutrient solution contained N, Ca, K, S, Mg, P, Na, Cl, Mn, Fe, B, Mo, Zn,

and Cu at approximately the following 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 used as one treatment. The treatments were pH 4.5, 6.0 and 7.5 which were adjusted by titrating nutrient solution with 1 M H_2SO_4 or 3 M NaOH as were required. The nutrient solutions had a tendency for the pH to drift up to 5.6, 6.3 and 7.6, respectively in every 24 hours. Therefore, although the pH of the nutrient solutions were adjusted daily to keep them near the desired levels, the treatments turned to pH 4.5-5.6, 6.0-6.3 and 7.5-7.6, respectively. Nutrient solutions were completely renewed at two weekly intervals to ensure an adequate supply of all essential elements and were continuously aerated and stirred by air pumps throughout the experimental period. Regular observations were made on all trees under different treatments to trace out the formation of new roots. New roots were tagged soon after detection with wax-coated paper tags and cotton threads. The total number of new roots formed during the experimental period was thereby obtained. The solution temperatures during rooting were recorded as 22-26°C.

In experiment II, similarly eighteen trees were placed in the nutrient solution under pH 5.0. The cultural practices were same as were in experiment 1. On the formation of numerous new roots, the apparently similar but minor aged roots were marked with individual numbers in the wax-coated paper tags. The trees were subjected to the treatments of pH 4.5-5.6, 6.0-6.3 and 7.5-7.6 after recording the initial length of roots. Roots were collected after 5, 10, 15 and 20 days after placement in the solutions and their lengths were measured. At least 10 roots for each treatment were used. The elongation growth of each root was obtained subtracting their initial lengths from the subsequent ones.

The elongated roots were fixed immediately in 4 percent glutaraldehyde, buffered to pH 7.4 in sodium cacodylate, dehydrated in a graded ethanol series, infiltrated and embedded in JB-4 resin. Cross sections of three-micrometer thickness taken at 2, 4, 6, 8 and 10 mm apart from root tips were stained with iodine-potassium-iodide. The sections were observed and photographed under light microscope. Five roots of each treatment were examined each year. Data on stelar and overall root diameters at different distances from the root apex were obtained from the photo prints.

For scanning electron microscopy, roots for every sampling dates were fixed immediately in 4 percent glutaraldehyde buffered to pH 7.4 in sodium cacodylate buffer, post-fixed in 2 percent osmium tetroxide, dehydrated in a graded ethanol series, dried in a critical point drier and coated with gold in an ion sputter. The samples were viewed and photographed under HITACHI 5-2250N scanning electron microscope at 20kV. At least five roots of 5, 10, 15 and 20 days after induction from each treatment were used for these observations every year. The overall results of three year experiments were summarized and presented.

Results

Formation rates of roots under different levels of culture solution pH: The trees under pH 4.5-5.6 produced the highest number of roots among the treatments which was followed by pH 6.0-6.3 and 7.5-7.6 during the experimental period each year (Fig. 1). Root formation varied in different dates even within the same treatment during the experimental period but higher number of roots under pH 4.5-5.6 was decisive. Root formation was also earlier in pH 4.5-5.6 where formation began 33-37 days (May 16-20) after placement of trees to the culture solution. While pH 6.0-6.3 treated trees formed new roots 38-42 days after placement (May 21-25), i.e., rooting was late by few days under this treatment compared to pH 4.5-5.6. The root formation under pH 7.5-7.6 had an identical trend as pH 6.0-6.3 and that was about 38-42 days after placement (May 21-25). The rooting intensity and the period of root formation in different treatments followed a similar trend with respect to experimental years.

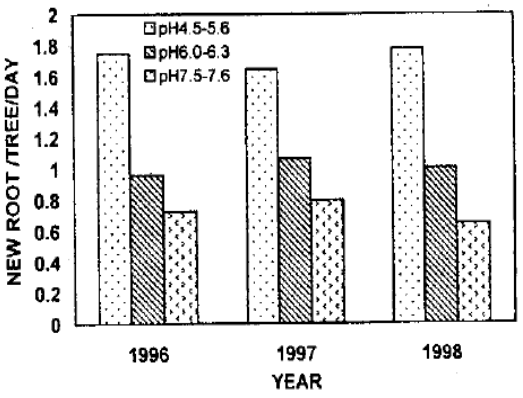

Fig. 1: Number of new roots/tree/day under different treatments during the experiments from 1996-998. The trees under pH 4.5-5.6 produced the highest number of roots followed by pH 6.0-6.3 and 7.5-7.6

Elongation growth of roots under different levels of culture solution pH: Elongation growth varied considerably in different treatments along with comparable exposure time (Fig. 2). This growth was highest at pH 4.5-5.6 treatment and root elongation continued even up to 20 days of their placement to the treatment as observed. On the other hand, although elongation was slightly different, pH 6.0-6.3 and 7.5-7.6 at the preliminary stage were comparable to that of pH 4.5-5.6 with only marginal variation. Successively, gradual decrease and finally complete growth cessation

occurred under pH 6.0-6.3 and 7.5-7.6 along with exposure times. However, this growth cessation was observed on the 15th and 10th days after placement at pH 6.0-6.3 and 7.5-7.6, respectively (Fig. 2). Although the pH of culture solutions were adjusted everyday to the desired levels, fluctuations in different rates within 24 hours were recorded. The pH 4.5 treatment drifted up to 1.1, pH 6.0 up to 0.3 and pH 7.5 up to 0.1 (Fig. 3). The decreasing trends of pH in each treatment, however, followed similar patterns, where the dripping was higher just after renewal of solutions and gradually became lower.

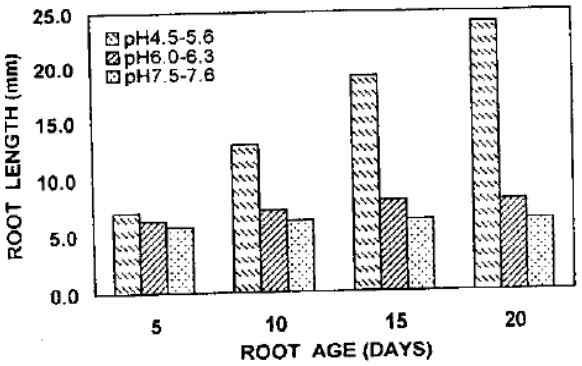

Fig. 2: Elongation growth of roots along with their exposure times under different pH levels. Elongation growth decreased with the increase of pH levels of culture solution from 4.5-6.6 up to 7.5-7.6

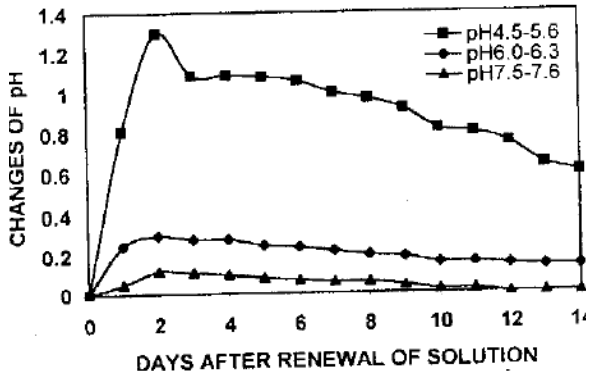

Fig. 3: Gradual changes of pH in different treatments after renewal of culture solution. The pH drift was up to 1.1, 0.3 and 0.1 for pH 4.5, 6.0 and 7.5, respectively

Periclinal growth of roots inferred from anatomical records: Periclinal growth of comparable distances from the root apex in terms of stelar and overall diameters varied considerably in different treatments each year (Fig. 4, 5). However, both stelar and overall root diameters toward the base of roots up to 10 mm studied followed a gradual increasing trend under pH 4.5-5.6. With the increase of root diameter, the stelar diameter also increased. Under pH 6.0-6.3, the stelar and overall root diameters increased marginally or remained constant from root tip toward root base. Similar results were found under pH 7.5-7.6. However, the diameters were always higher at pH 4.5-5.6 than those of pH 6.0-6.3 arid 7.5-7.6 treatments. The ratio of stelar to overall diameter also varied among the treatments. This ratio showed that pH 4.5-5.6 had lower values than those of other two treatments in the root apex but gradually became higher towards the root tip (Fig. 6). In contrast, under higher pH (pH 6.0-6.3 and 7.5-7.6), the ratio remained constant.

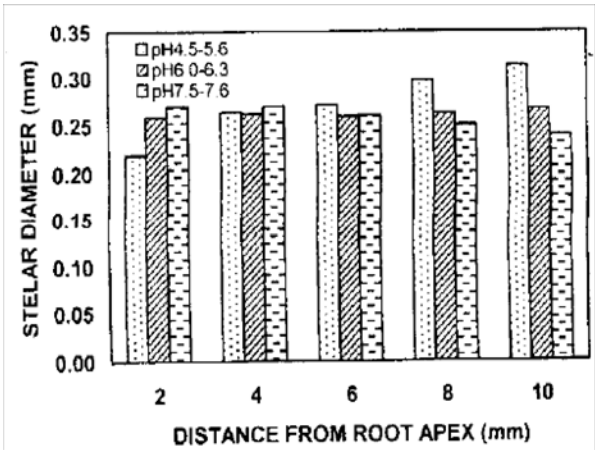

Fig. 4: Stelar diameters of roots of trifoliate orange budded with satsuma mandarin at different pH levels

Morphology of roots at different levels of culture solution pH under scanning electron microscope: The removal of older rootcap cells were distinct in 5-day-induced roots under pH 4.5-5.6 (Fig. 7A). The intact epidermal cells near the root tip was also clearly observed (Fig. 8A). Even at 10-day-induction, the removal of older rootcap and epidermal cells were apparent and root tips were sharply pointed (Fig. 7B). The intact epidermal cells under the peeled off sites were conspicuously evident (Fig. 8D). The 15-day-exposed roots showed clear epidermal cells and root tip became slightly blunt but received no injuries or abnormalities (Fig. 7C, 8G). The 20-day-induced roots also exhibited similar characteristics (Fig. 7D, 8J).

The 5-day exposed roots under pH 6.0-6.3 were slender.

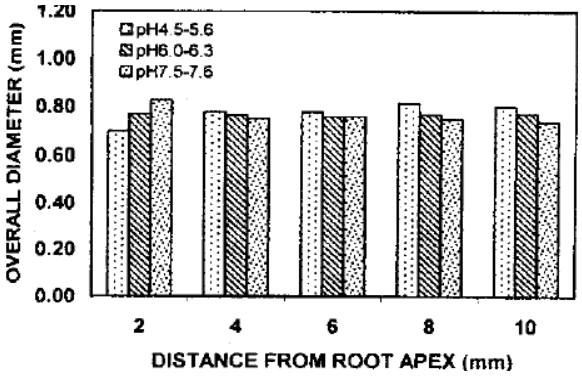

Fig. 5: Overall diameters of roots of trifoliate orange budded with satsuma mandarin at different pH levels

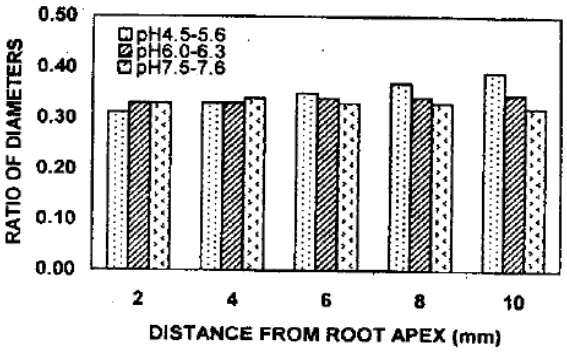

Fig. 6: Ratio of stelar to overall diameters of roots of trifoliate orange budded with satsuma mandarin at different pH levels

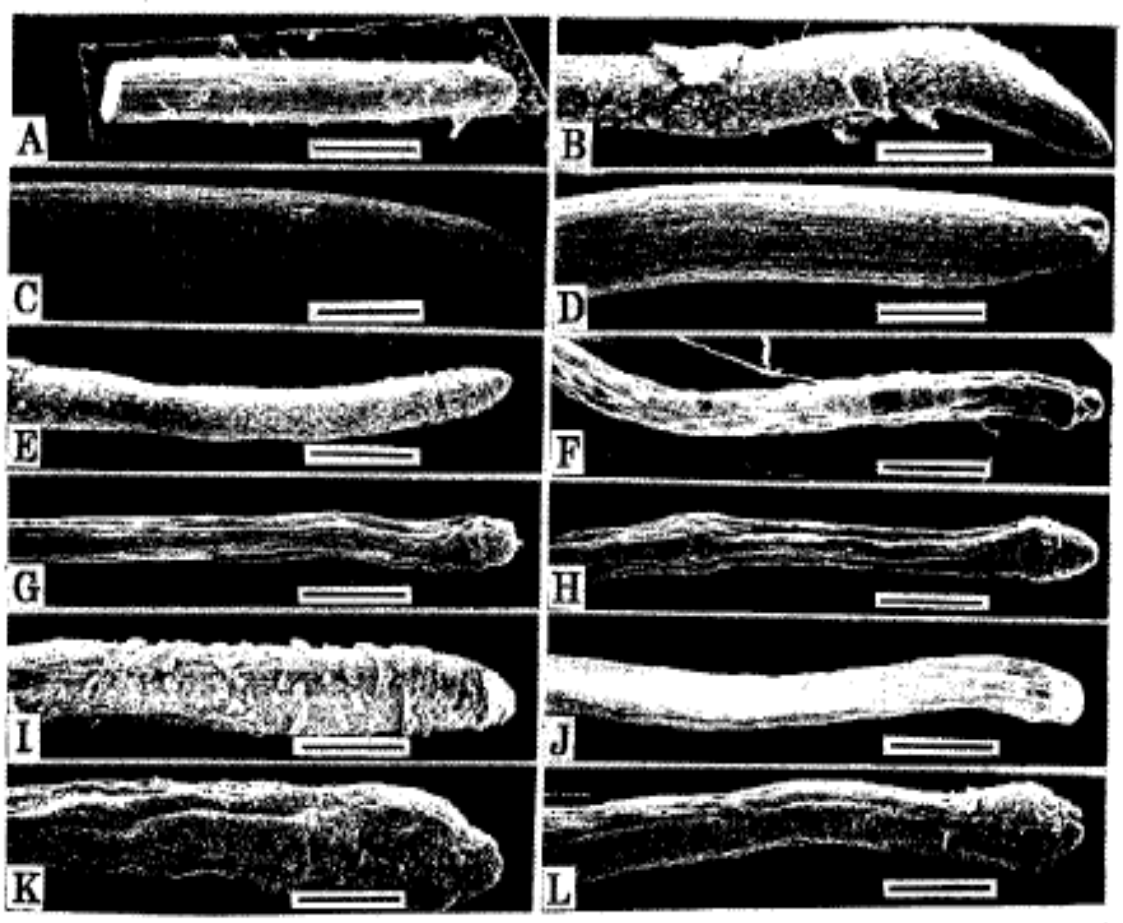

Fig. 7: Morphology of roots of trifoliate orange budded with satsuma mandarin under different levels of culture solution pH. Photographs were taken under SEM at 20kV. A, B, C, 5-,10-,15- and 20-day-induced roots, respectively at pH 4.5-5.6. Continued elongation growth was observed having the normal morphology of roots under this treatment. E,F, G,H: 5-,10-,15- and 20-day-exposed roots, respectively at pH 6.0-6.3. Gradual injuries of roots and surface ridging were observed under this condition. I,J, K,L: 5-,10-, 15- and 20-day-suffered roots at pH 7.5-7.6. Severe abnormal-lities were observed in this treatment. Bar = 1 mm

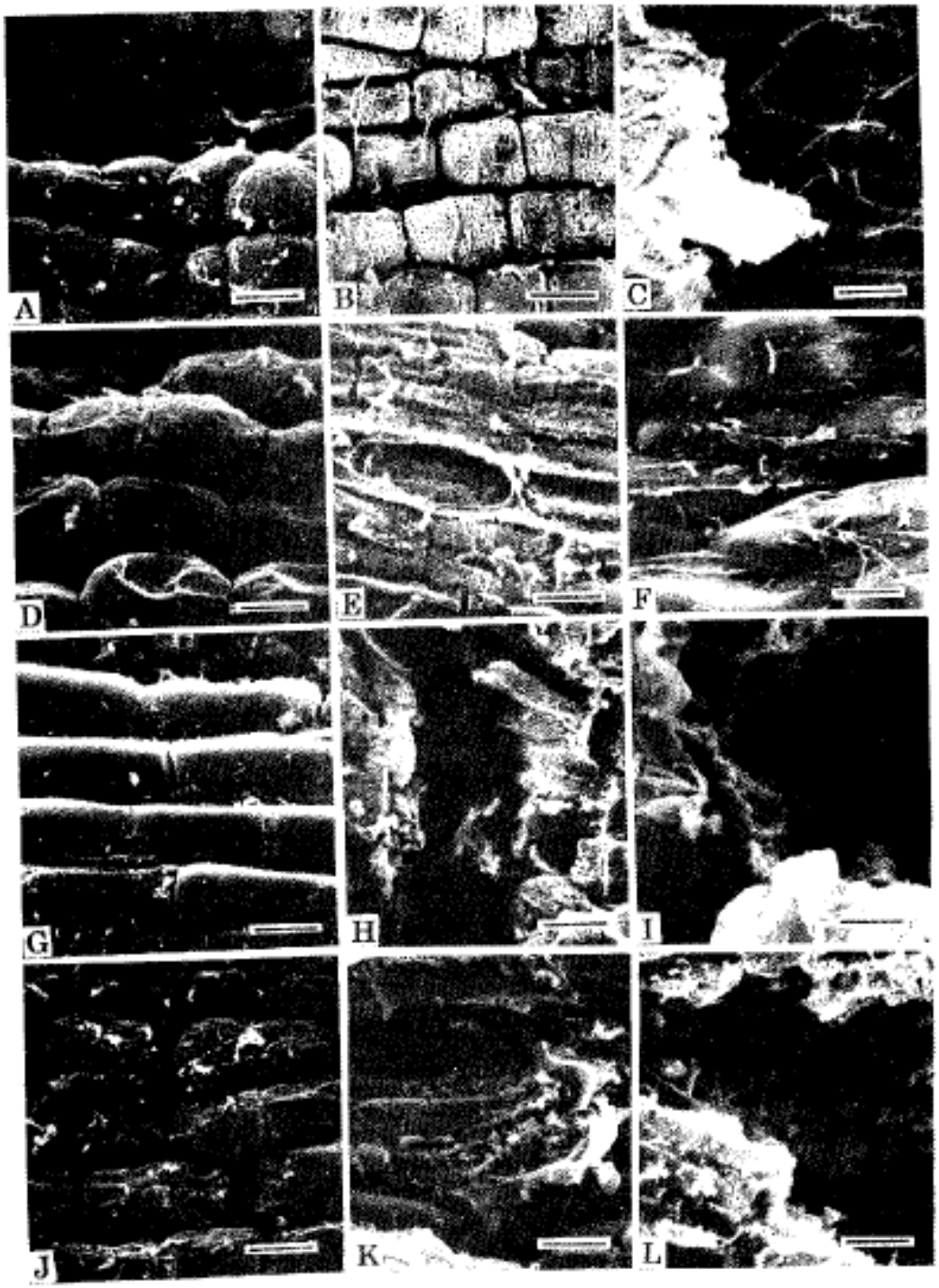

Fig. 8: Focalized vin vs ol sonic eilects on roots under different levels of culture solution pH under SEM at 20 kV. A, B, C: Five clay induced roots. Note Normal epidermal cells in pH 4.5 5.6 iA), cell iniunes under pH 6.0-6.3 (B). and pH 7.5-7.6 (C); D, E, F: Ten day-exposed roots. Note: normal epidermal cell in pH .4.5-5.6 (D). More injuries under pH 6.0-6.3 (E) and 7.5 /.6 (F); G. H, 1: Fifteen-day.induced roots. Note: normal development of epidermal cells under pH 4.5-5.6 (G), surface ridging and injuries at pH 6.0 6.3 (H) and 7.5-7.6 (I) J, K, L: Twenty day-exposed roots. Note: normal cellular development under pH 4.5-5.6 (J), severe injuries at pH 6.0-6.3 (K) and 7.5-7.6 (L) Bar 5 m

Root tip was sharp, where moderately severe removal of rootcap cells were observed (Fig. 7E). The destruction of epidermal cells was noticed in few places (Fig. 8B). After 10 days, root tip injuries or bulbous malformation in the root tip shape were distinct (Fig. 7F). Root surface was also ridged and roughed and the roots, as a whole, were comparatively slender even after longer exposures. The epidermal cells were found to be variously malformed (Fig. 8E). The 15-day-exposed roots were under more adverse effects. All the root tips became severely deformed with [njuries or other abnormalities (Fig. 7G). The epidermal cell death in these roots was distinct which resulted serious surface ridging and clear cellular injuries (Fig. 8H). The 20day-exposed roots also showed similar root tip injuries and root surface ridging along with cellular injuries (Fig. 7H, 8K).

The tips of 5-day-exposed roots at pH 7.5-7.6 became Borne-shaped (Fig. 7I). Abnormal epidermal peeling off was noticed (Fig. 8C). The roots were comparatively coarser than those of other two treatments. The whole root surface became rough due to the abnormal and complete or incomplete removal of the rootcap and epidermal cells. The 10-day-induced roots were characterized by tip injuries (Fig. 7J). The epidermis became ridged and rough-surfaced, and the epidermal cell death was conspicuously observed (Fig. 8F). The 15-day-exposed roots were under more adverse effects. The root tip became severely deformed with injuries and other abnormalities (Fig. 7K). The stern injuries on the root surface produced holes (Fig. 8J). Roots severely exhibited an overall abnormal morphology. The 20-dayexposed roots had also identical abnormalities (Fig. 7L, 8L).

Discussion

The effects of pH on the formation, growth, and morphology of roots in trifoliate orange budded with satsuma mandarin were investigated in the present experiment. However, the earlier and maximum rooting with higher elongation and periclinal growths of roots at pH 4.5-5.6 suggested this range to be better in this studied material. Smith (1971) reported that citrus roots under pH 5.0 were coarser than those of pH 6.0 which produced slender roots which were more than 11 inches in length after first month. On the other hand, Yokomizo and Ishihara (1973) found vigorous tree growth at pH. 3.8 and 4.5 in solution culture, but in rising pH above 5.2, tree growth depressed, and root rotting markedly increased. The present study indicated that although the roots were slender in the early growth stage at pH 4.5-5.6 and 6.0-6.3, they gradually became coarser along with their rapid elongation growth up to 20-day-exposure as observed under pH 4.5-5.6 and remained slender throughout the period at pH 6.0-6.3. The elongation growth was also higher at pH 4.5-5.6 than those of 6.0-6.3 and 7.5-7.6 treatments. Similarly Soprano and Koller (1996) noticed best response of cleopetra mandarin trees at pH 5.0-5.5 i.e., lower than the recommended range (6.0) for citrus. Therefore, our observations slightly differed from the report of Smith (1971) but stood in accord with the findings of Soprano and Koller (1996) and Yokomizo and Ishihara (1973) regarding the elongation growth in roots. The gradual increase of stelar diameter in the roots under pH 4.5-5.6 apart from the root apex toward base and the increase of the overall root diameter might have resulted from the normal growth of roots under this treatment. On the contrary, the slow increase or static condition of stelar diameter under pH 6.0-6.3 and 7.5-7.6 indicated an inhibition in secondary growth of roots under these high pH ranges. Root thickening occurs through the increase of stelar diameter (Fraser *et al*., 1990). Therefore, the slow increase or static condition of stelar diameter under higher ranges of pH also suggested these conditions as adverse for root growth. Production of slender roots under pH 6.0-6.3 was probably due to the shrinkage of cells as was observed under SEM with ridged surfaces and injured tips. The decrease in plant growth under higher pH was ascribed to be caused by the low availability of trace elements such as boron (Soprano and Koller, 1996). The present study, also suggests that root injuries resulting from root rotting under higher pH might also play an adverse role to the plant growth.

Although the pH levels of culture solutions were adjusted everyday, it changed in various rates at different treatments indicating a trend as the higher the initial pH, the lesser the pH drift. Similar perturbation of pH ranges were reported by Rosen *et al*. (1990) in solution culture of blueberry and cane berry plants. On the other hand, Soprano and Koller (1996) found decreasing trend of pH in the soil culture. However, we concurred with Rosen *et al*. (1990) but differed with that of Soprano and Koller (1996). This controversy might have resulted from the differences of rooting media used in our experiments. In another study, Smith (1971) found that there was a tendency for the pH to drift up to 2 weeks and beyond this, all solutions tended to become more acidic. This phenomenon may also explain for the differences of our findings with that of Soprano and Koller (1996). In our study, we renewed the culture solutions and recorded the pH up to 14 days but Soprano and Koller (1996) recorded at the end of long term experiment.

The normal removal of older rootcap cells resulting sharply pointed root tips followed by the formation of distinct epidermal cells at pH 4.5-5.6, could probably be due to the rapid growth of roots in its early stage at this treatment. The continued prevalence of this characteristic feature along with incessant elongation growth of roots therefore led us to ascribe this condition as favorable. On the other hand, the abnormal peeling of the epidermal cells at pH 6.0-6.3 suggested that those roots were already under injury induction process. The slight injury in the epidermal cells observed under SEM also supported this assumption. The abnormal configuration of root tips and the ridged surface of roots under this treatment on prolonged exposures

exhibited a severe adverse effects on the growing roots. Soprano and Koller (1996) reported that plant growth in cleopetra mandarin decreased linearly in higher pH (6.0-7.0). The present study noted root injury at high pH levels indicating it as the physical mechanism of growth inhibition under that condition. Although plant growth was not studied in the present experiment, it can be logically presumed that if root injuries occur, it is not irrelevant that plant growth will also be hampered as a consequence. It is believed that roots are mediator capable of monitoring the changing conditions in the growing media and transmitting the information to the shoot.

The dome-shaped tips, epidermal and tip injuries of 5-dayexposed roots under pH 7.5-7.6 indicated the severe effects of higher pH (7.5-7.6). The complete peeling of epidermal cells in 10-day-exposed roots leaving the cortex bare may have resulted from the continued adverse effects due to the longer exposure of roots in this treatment. The overall root tip abnormalities is indicative of the stern effects of higher levels of growing media pH on the growth and morphology of roots of trifoliate orange budded with satsuma mandarin. The present investigation was made only on the growth and external morphology of roots under different pH levels. However, this study added substantial knowledge on the responses of roots in trifoliate orange budded with satsuma mandarin to different levels of media pH. For the complete resolution of controversies regarding culture media acidity and plant growth in citrus, the molecular level studies on the matter were also warranted. In conclusion, the state of root growth in the field conditions and the morphology of roots under SEM suggested that pH 6.0 may not be the best for satsuma mandarin onto trifoliate orange as was known earlier. The best range lies between pH 4.5-5.6. Above this range favorable root growth could not be found but rather injury, abnormality and root death were distinct in the present study. Concurrently, studies on different ultrastructural effects of culture solution pH in the same material are in progress. These studies are expected to provide substantial information on the same problem in the cellular level.

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References

Brunet, J., 1994. Interacting effects of pH, aluminium and base cations on growth and mineral composition of the woodland grasses *Bromus benekenii* and *Hordelymus europaeus*. Plant Soil, 161: 157-166.

Chapman, H.D., 1968. The Mineral Nutrition of Citrus. In: The Citrus Industry, Volume 2, Reuther, W., L.D. Batchelor and H.J. Webber (Eds.). University of California, Berkeley, pp: 266-268.

Fraser, T.E., W.K. Silk and T.L. Rost, 1990. Effects of low water potential on cortical cell length in growing regions of maize roots. Plant Physiol., 93: 648-651.

Islam, A.K.M.S., D.G. Edwards and C.J. Asher, 1980. pH optima for crop growth: Results of a flowing solution culture experiment with six species. Plant Soil, 54: 339-357.

Koyama, H., T. Toda, S. Yokota, Z. Deqair and T. Hara, 1995. Effects of aluminum and pH on root growth and cell viability in *Arabidopsis thaliana* strain Landsberg in hydroponic culture. Plant Cell Physiol., 36: 201-205.

Medappa, K.C. and M.N. Dana, 1970. The influence of pH, CA, P and FE on the growth and composition of the cranberry plant. Soil Sci., 109: 250-253.

Rosen, C.J., D.L. Allan and J.J. Luby, 1990. Nitrogen form and solution pH influence growth and nutrition of two *Vaccinium* clones. J. Am. Soc. Hortic. Sci., 115: 83-89.

Sasada, N., Y. Yamamoto and H. Matsumoto, 1994. Responses to acid stress in suspension-cultured tobacco cells. Plant Cell Physiol., 35(Suppl.): 133-141.

Smith, P.F., 1957. Studies on the growth of citrus seedlings with different forms of nitrogen in solution cultures. Plant Physiol., 32: 11-15.

Smith, P.F., 1971. Hydrogen ion toxicity on citrus. J. Am. Soc. Hortic. Sci., 96: 462-463.

Soprano, E. and O.L. Koller, 1996. Effect of lime incorporation depth and lime levels on the development of two citrus rootstocks under greenhouse conditions. Proceedings of the 8th International Citrus Congress, Sun City, South Africa, Volume 2, (ICC'96), International Society of Citriculture, Nelspruit, pp: 832-835.

Takao, K., S. Masaya and P. Mohammad, 1996. Effects of high temperature and dry condition of summer season on the changes of root tip morphology in satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsu Wase). Proceedings of the Symposium and Spring Meeting of Japanese Society for Horticultural Science, Volume 65, (JSHS'96), Japan, pp: 60-61.

Yokomizo, H. and M. Ishihara, 1973. Studies on the mineral nutrition of fruit trees in sand and water culture. I. Effects of nutrient solution composition on the growth of satsuma trees. Bull. Hortic. Res. Station Series A, 12: 29-77.

Zieslin, N. and M. Abolitz, 1994. Leakage of phenolic compounds from plant roots: Effects of pH, Ca^{2+} and NaCl. Scient. Hortic., 58: 303-314.

Zieslin, N. and P. Snir, 1989. Responses of rose plants cultivar Sonia and *Rosa indica* major to changes in pH and aeration of the root environment in hydroponic culture. Scient. Hortic., 37: 339-349.