http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (4): 1118-1124, 1999 — Research Article

Morphological Deformations of Root Hasirs in Trifoliate Orange Grafted with Satsuma Mandarin under High Summer Temperature and Limited Soil Moisture Conditions

Pear Mohammad and Masaya Shiraishi*
The United Graduate School of Agricultural Sciences
*Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan

Abstract
The morphological deformations of root hairs in trifoliate orange grafted with satsuma mandarin *(Citrus unshiu* Marc. cv. Okitsu Wase) under high summer temperature (40-45°C) and limited soil moisture (pF 2.7-2.9) conditions were observed under scanning electron microscope. The hair zone positioned apart from the root tip before treatments which gradually proceeded nearer the root tip under only high temperature stress. However, the speed of this effect was so slow that even after 25 days of exposure the hair zone was not found to be completely proximal to the root tip. The elongation of hairs was noticed within 5 days which gradually deformed and after 25 days of exposure almost all the hairs were degraded. In contrast, at high temperature accompanied low soil moisture condition, hair zone appeared extremely nearer the root tip within 5 days followed by the random occurrence of hairs from root tip up to a certain distance towards the base. Although slightly elongation of hairs was found at the initial stage, severe degradation ensued at the following sampling dates. As the consequences, after 25 days of treatment the complete destruction of hairs occurred where only the traces of abolished hairs were left. In general, only the high temperature stress primarily resulted the formation of dense hairs but this dense hair formation was instantly inhibited as the interactive effects of high temperature and low soil moisture stress.

Introduction
Most of the citrus grown in Japan are stressed to some extent by low soil moisture during the high temperature period of summer. Rootstocks are the first lines to response this adverse soil condition. However, citrus rootstocks have been reported to confer different drought tolerance to the scion (Wutscher,1979).
Although the effects of temperature as a whole on the growth of citrus roots is poorly understood (Reuther, 1973), it has been described as a significant production restraint (Reuther *et al.*, 1979) and an influential factor for tree growth (Bevington and Castle,1985). Root growth is restricted below 15°C (Inoue and Harada, 1988) but optimum and maximum temperatures have been reported to be 26 and 37°C, respectively (Hodgson and Cameron,1943; Poerwanto *et al.*, 1989). Other complementary studies suggested that considerable high temperature within the range of 20-30°C was favorable for its intense root growth (Bevington and Castle, 1985; Inoue and Harada, 1988). Moreover, under high temperature condition, trees are usually exposed not only to heat stress but also to water stress (Li *et al.,* 1996). Recently we reported that the summer prevailing high temperature (40-45°C) and limited soil moisture (pF 2.7-2.9) condition changed greatly the color, shape and anatomy of roots in trifoliate orange grafted with satsuma mandarin (Mohammad *et al.,* 1996, 1999).
Root hairs are thought to increase the absorptive capacity of the root by increasing the surface area (Clarkson, 1985). Although they generally constitute a very small portion of the root surface and form a unicellular layer, their functions are closely tied to those of the root of which they are an integral part. These functions vary widely with the environmental factors (Bristow, 1975; Kramer, 1983). However, the effects of high temperature and low soil moisture condition on root hairs are not yet known. As the morphology of roots was modified by this condition (Mohammad *et al.,* 1996, 1999), we anticipated that root hairs might also be similarly affected.
This investigation was intended to elucidate the morphological characteristics of root hairs in trifoliate orange grafted with satsuma mandarin Linder only high summer temperature (40-45°C) and that temperature accompanied limited soil moisture (pF 2.7-2.9) conditions under scanning electron microscope.

Materials and Methods
The experiment was conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University, Japan from April 1 to August 28 of 1996-1998 using three-year-old satsuma mandarin trees on trifoliate orange rootstocks. Twenty four trees were selected for uniformity of vigor and grown in eartheri pots filled with a mixture of sandy loam soil and granite having a pH mean 6.5 in April 1 of each year. Sufficient amount of manure was added with the pot mixture. A basic fertilization was applied consisting of 120, 50 and 80 mg/dm^3 of N, P, and K, respectively. The pots were primarily maintained under the natural environment. At 60 days from planting, N was added at 50 mg/dm^3 followed by application of irrigation water. The trees were also daily irrigated with sufficient amount of water up to the water stress treatment. The potted trees were transferred to the plastic house on the 3rd August for imposing water stress

treatment. For sampling, the soil pF was adjusted between 2.7-2.9 everyday by constant reading and adding required amount of water. Samples were collected 6 times with 5 days intervals including one sampling before water stress treatment on the 3rd August as control. Other samplings were done on the 8th, 13th, 18th, 23rd and 28th August every year. For each sampling, two trees were uprooted and the available new roots were collected. The average daily soil and air minima/maxima were recorded during the sampling period which ranges between 24-27/32-42 and 21-24/40-45°C, respectively.

The collected roots were washed gently several times with water to remove any adhering particles. Segments of five millimeter length were excised from the root apices and fixed immediately in 4% glutaraldehyde buffered to pH 7.4 in sodium cacodylate, post-fixed in osmium tetroxide, dehydrated in an ethanol series and freeze dried. The dried samples were coated with gold, observed and photographed only from the middle portions crf root hair zones under Hitachi S-2250N scanning electron microscope at 20 kV. About ten roots were used for photography at every sampling dates. The three-year-observations were summarized and interpreted.

Results

Morphological deformations of root hairs in trifoliate orange grafted with satsuma mandarin under high summer temperature: The hair zone positioned far apart from the root tip and limited within a definite zone in the samples collected on the 3rd August immediately before imposing treatment (Fig. 1A). These hairs were mostly shorter with only a few- elongated (Fig. 2A). They were sparsely positioned, smoothly surfaced, mostly possessed dome-shaped tips and there was no symptom of morphological abnormality. The hair zones were also accompanied by many elevated epidermal cells as the initial of hairs. After five days i.e., on the 8th August also, hair zone positioned apart from the root terminal (Fig. 1B). The hairs were elongated and remained highly dense but their tips were mostly injured (Fig. 2B). However, only the short-stature initial hairs appeared normal. Except a few hair tips, mostly malformed. The overall hair surface also became different irregular in few cases. On the 13th August, the roc possessed numerous hairs apart from the tip (Fig. 1C). The hairs were elongated but their tips were swollen, variously malformed or in few cases partially destroyed (Fig. 2C). Along with the elongated hairs, a considerable number of

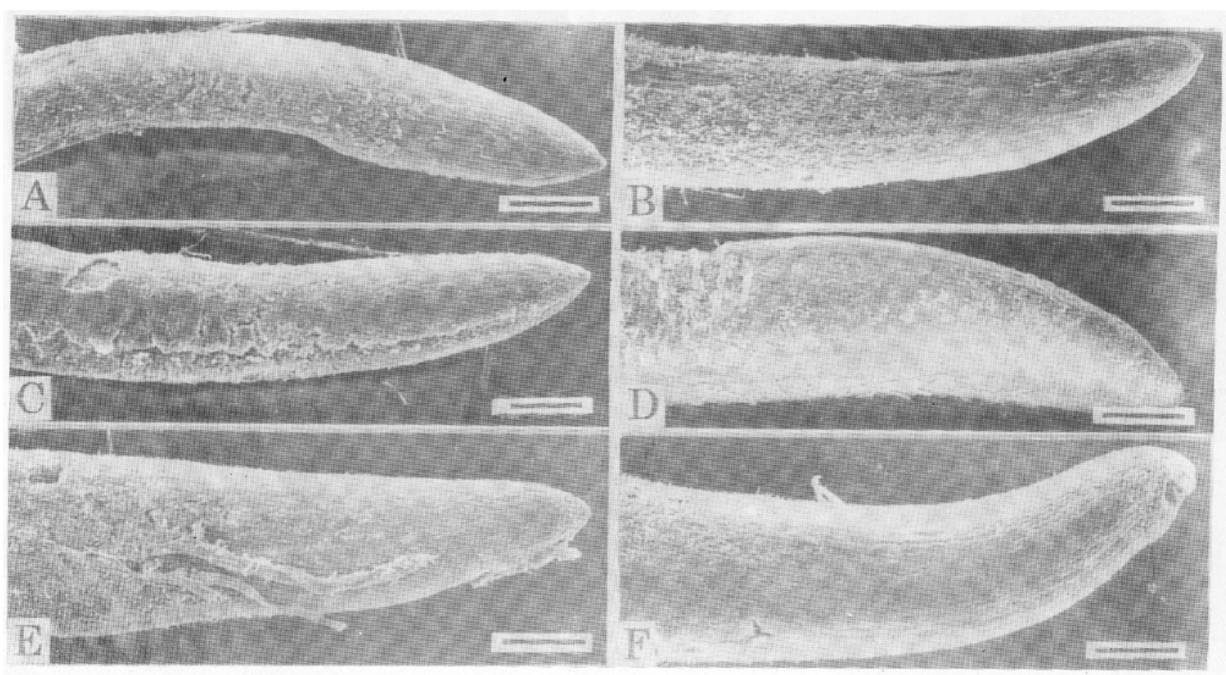

Fig. 1: Low magnified views of roots of trifoliate orange grafted with satsuma mandarin under high temperature of summer. (A) Samples collected on the 3rd August. (B) Samples of 8th August. (C) Sampled on the 13th August. (D) Samples taken on the 18th August. (E) Sampled on the 23rd August. (F) Samples collected on the 28th August. Note: Root hair zone positioned gradually nearer the root tip along with the exposure time. Bar = 500 µm

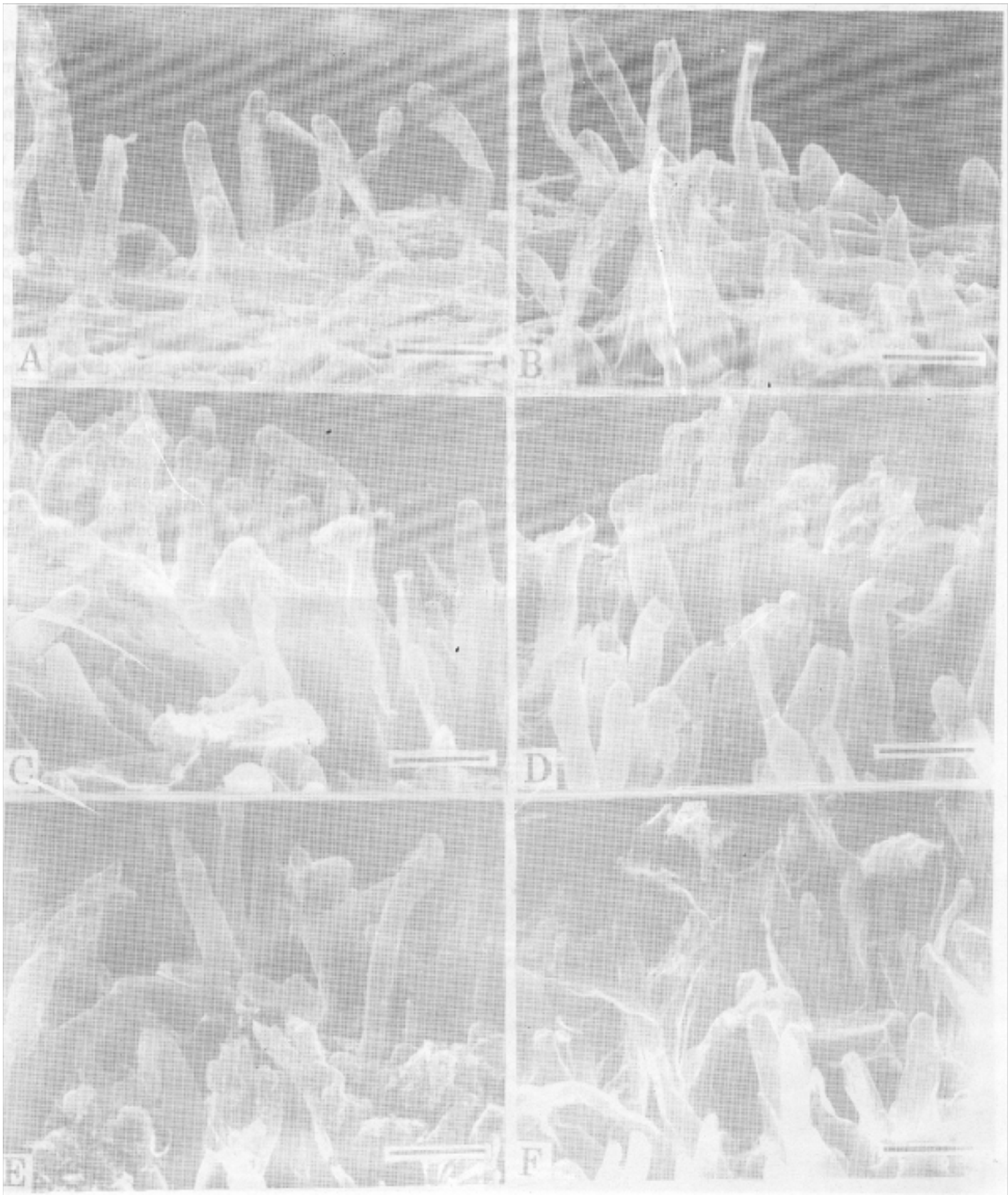

Fig. 2: Localized views of root lkrirs of trifoliate orange grafted with satsuma mandarin under high temperature of summer. (A) Roots collected on the 3rd August. (B) Samples collected on the 8th August. (C) Sampled on the 13th August. (D) Roots taken on the 18th August. (E) Roots collected on the 23rd August. (F) Roots sampled on the 28th August. Note: Slight modification of root hairs was found along the exposure time and finally root hairs degenerated forming ribbon-shape. Bar = 25 μm

hairs were composed of smaller or variously malformed ones or the epidermal cells contained only the traces of destroyed hairs. On the 18th August, the actively growing zone of root slightly shortened and the position of hair zone became comparatively more nearer the root tip (Fig. 1D). Although the hairs remained elongated, they were under different destruction processes (Fig. 2D). The tips of most hairs damaged and few other hairs were completely destroyed. On the 23rd August, the hair zone positioned similarly nearer the root tip (Fig. 1E). Although the hairs were elongated, they were mostly damaged (Fig. 2E). The hair tips were severely injured. A considerable portion of root tip epidermis was devoid of hairs or possessed the traces of abolished hairs. On the 28th August, the actively growing zone of root was highly shortened and the hair zone positioned very near but not extremely proximal to the root tip (Fig. 1F). The elongated hairs took ribbon shape and underwent random damage (Fig. 2F). Even rarely a few hairs showed normal or nearly normal morphological features.

Morphological deformations of root hairs in trifoliate orange grafted with satsuma mandarin under low soil moisture and high summer temperature: The roots collected on the 3rd August exhibited normal tips and the hair zones positioned far apart from their tips beyond the actively growing zone (Fig. 3A). The root hairs were composed of shorter and moderately elongated ones. They had dome-shaped tips and smooth or regular surface (Fig. 4A). The root hairless epidermal areas also possessed the initial cells for hair formation. The hair tips were similarly dome-shaped and the hair zone positioned nearer the root tip due to the reduction in actively growing zone of root after five days of treatment (Fig. 3B). The hairs were moderately elongated but their tips were under slightly injury induction process (Fig. 4B). In few cases, hair surface was also slightly irregular and their population was sparse as normal. After 10 days of induction, hair zone positioned both in near and apart areas from the root tips (Fig. 3C). The root hairs were sporadically destroyed but their overall population sligh" increased. The surfaces of other existing hairs were also

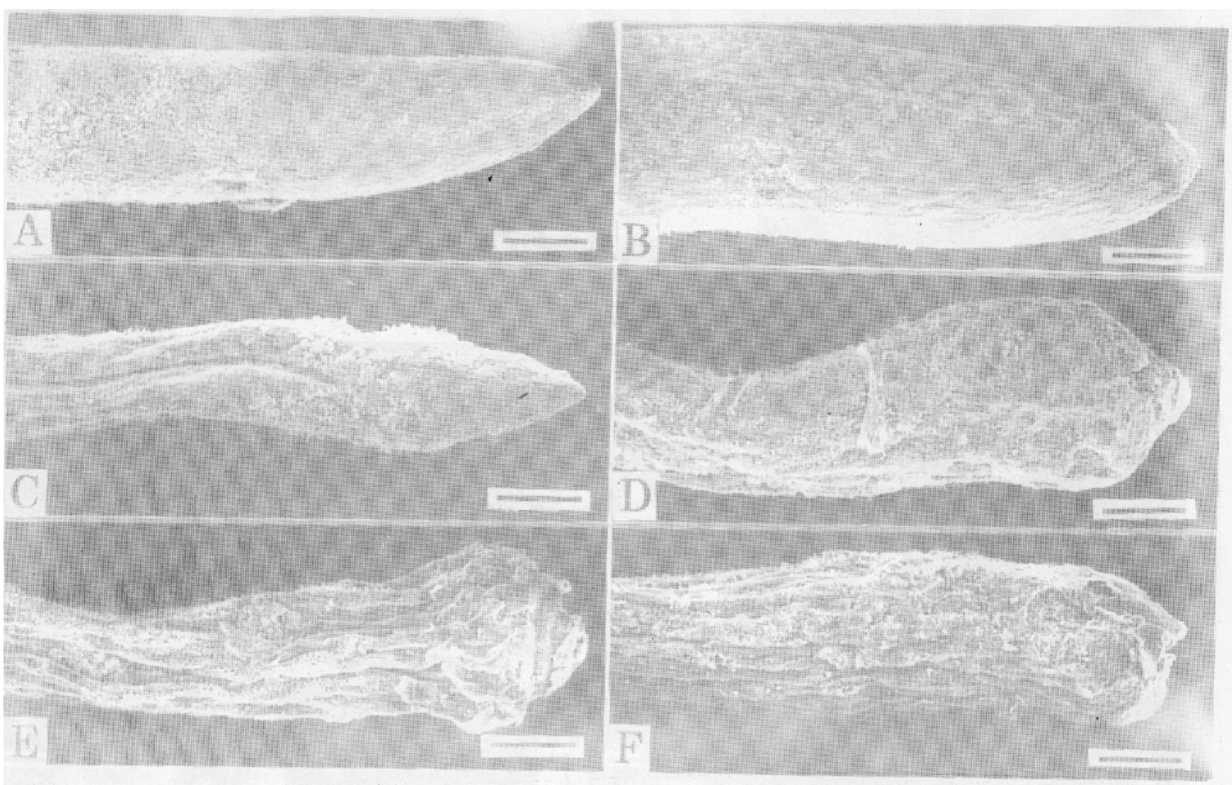

Fig. 3: Low magnified views of roots of trifoliate orange grafted with satsuma mandarin under limited soil moisture and high summer temperature condition. (A) Samples collected on the 3rd August just prior to impose water stress treatment. (B) Samples taken after 5 days of treatment. (C) Sampled after 10 days of treatment. (D) Sampling was done after 15 days of treatment. (F) Samples taken after 20 days of treatment. (F) Samples collected after 25 days of treatment. Note: The position of root hair zone shortly became proximal to the root tip. Bar = 500 μm

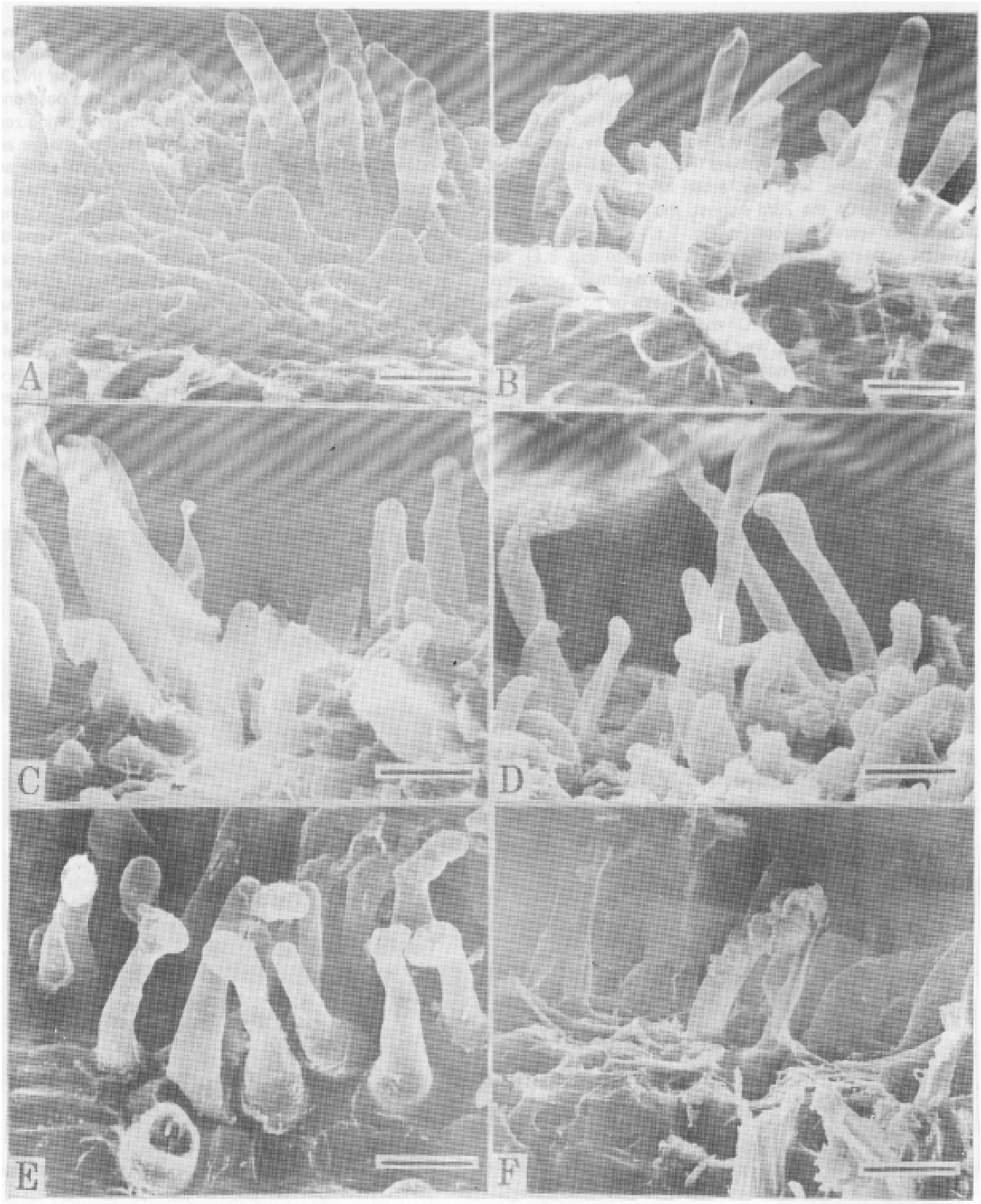

Fig. 4: Localized views of root hairs of trifoliate orange grafted with satsuma mandrain under limited soil moisture and high summer temperature condition. (A) Roots collected on the 3rd August just peior to impose water stress treatment. (B) Samples taken after 5 days of treatment. (C) Roots sampled after 10 days of treatment. (D) Sampling was done after 158 days of treatment. (F) Samples taken after 20 days treatment. (F) Samples collected after 25 days of treatment. Note: Root hairs initially became elongated followed by their abnormality and degradation. Bar = 25 µm

irregular. In few cases, the hair tips were injured (Fig. 4C). After 15 days, the position of hairs similarly remained random throughout the root tip (Fig. 3D). The hairs were highly elongated and thin but their tips modified to different irregular shapes as a result of injuries (Fig. 4D). Few tips were also destroyed partially or completely. Few other hair tips took bulbous shape even at their primary elongating stage. After 20 days, the epidermal cells were also destroyed (Fig. 3E). The existing hairs were under serious tip malformation or destruction process and their surface was severely irregular (Fig. 4E). Few hairs were completely destroyed leaving only the traces of their origin on the epidermis. After 25 days of treatment, the root surface was severely destroyed (Fig. 3F). The root hairs were seriously malformed and degenerated (Fig. 4F). The subjected hair surface was serrated and randomly damaged. The rest portions of epidermis had no hairs or possessed only the traces of abolished hairs. In general, at all sampling dates, the root hair density appeared lower under this treatment compared to that of only high temperature stress.

Discussion

The morphological studies of root hairs have led to successive updates, from Uphof *et al*. (1962) and Aeschbacher *et al*. (1994), which give a fairly clear picture of the variability propensity existing in the structure of root hairs. In our study, the morphology of root hairs were studied under high temperature and low soil moisture conditions. Under these treatments, the root hair zone gradually became proximal to the root tip but the occurrence of this event was more rapid in high temperature accompanied low soil moisture condition than that of only high temperature stress. The former situation was reported as more adverse for root elongation compared to that of later one (Mohammad *et al.,* 1999). The hair zone of most roots is situated just behind the zone of active root elongation (Jaunin and Hofer, 1988). The formation of root hairs may extend nearer the tip if root growth is slowed down (Johnson-Flanagan and Owens, 1985; Jaunin and Hofer, 1988). The proximity of the hair zone to the root tip in this study was therefore presumed to be resulted from the cessation of elongation growth of roots. As high temperature accompanied low soil moisture condition is more rapid to cause growth cessation, the root hair zone proximity was also more advanced compared to that of only high temperature condition. The elongation of root hairs at the initial stage of stress effects in both conditions was observed in our study. Root hair size was reported to vary with several environmental factors (Ewens and Leigh, 1985; Jaunin and Hofer, 1988; Marschner, 1995). Uptake efficiency is correlated with root hair length (Caradus, 1979; Itoh and Barber, 1983). The elongation of hairs in the moderately modified roots was therefore presumed to be the adaptation strategy of this plant species under the present 'stress condition by extending their absorption areas. However, although the root hairs elongated in this condition, they were under surface depression followed by injuries which indicated the distinct effects of these adverse conditions on their morphology. The roots ultimately produced the ribbovshaped hairs and finally their complete destruction ensued. The population density of hair was higher at high temperature compared to that of high temperature accompanied low soil moisture stress. This phenomenon probably indicated that the trees tried to accommodate themselves With the moderately adverse condition like high summer temperature by increasing their hair density. While the severely adverse condition like high temperature accompanied low soil moisture appeared destructive to that strategy.

The first effect of stress condition was distinct in the hair tips forming their different shapes. Meekes (1986) also showed that in *Ceratopteris thalictroides* L. swollen root hair tips could be obtained by the addition of Congo Red, a cellulose crystalization inhibitor. The growth inhibiting effects of the stress conditions in the present study therefore presumed to be resulted from this malformed hair tips. Probably for that reason, the trees were as exposed to the stress conditions for longer periods, the modification of hair tips also became more prominent.

As the extent of modification in roots increased in our study the hair tips changed differently. Schnall and Quatrano (1992) also reported that the swelling of root hair base were the characteristics of changes in root hair development due to water stress. Along with these changes, the degradation of hair tips and finally. their complete abolition appeared in the present study. This kind of extreme situation probably appeared due to the interactive effects of high temperature and low 'soil moisture conditions as acute stress for the normal functioning of root hairs. However, although both stress conditions under study showed root hair degradation, process was different. High temperature alone modified the hairs and finally destroyed them through forming ribbon-shape but the high temperature accompanied low soil moisture condition attacked the hair tips and gradually caused their destruction leaving only the traces of hairs on the epidermis.

In conclusion, although both high temperature and high temperature accompanied low soil moisture conditions cause the proximity of hair zone to the root tip, the cumulative effects of the later situation is severe. Both treatments initially increase the length of hairs followed by their modification as ribbon-shape or irregular injury and complete destruction. High temperature increases root hair population but if water stress is also accompanied, hair population can not increase due to the instant and severe adverse activities.

References

Aeschbacher, R.A., J.W. Schiefelbein and P.N. Benfey, 1994. The genetic and molecular basis of root development. Annu. Rev. Plant Physiol. Plant Mol. Biol., 45: 25-45.

Bevington, K.B. and W.S. Castle, 1985. Annual root growth pattern of young citrus trees in relation to shoot growth, soil temperature and soil water content. J. Am. Soc. Hort. Sci., 110: 840-845.

Bristow, J.M., 1975. The Structure and Function of Roots in Aquatic Vascular Plants. In: The Development and Function of Roots, Torrey, J.G. and D.T. Clarkson (Eds.). Academic Press, London, pp: 221-236.

Caradus, J.R., 1979. Selection for root hair length in white clover (*Trifolium repens* L.). Euphytica, 28: 489-494.

Clarkson, D.T., 1985. Factors affecting mineral nutrient acquisition by plants. Ann. Rev. Plant Physiol., 36: 77-115.

Ewens, M. and R.A. Leigh, 1985. The effect of nutrient solution composition on the length of root hairs of wheat (*Triticum aestivum* L.). J. Exp. Bot., 36: 713-724.

Hodgson, R.W. and S.H. Cameron, 1943. Some instances of scion dominance in citrus. Proc. Am. Soc. Hort. Sci., 43: 131-138.

Inoue, H. and Y. Harada, 1988. Tree growth and nutrient absorption of young satsuma mandarins under different temperature conditions. J. Japanese Soc. Hort. Sci., 57: 1-7.

Itoh, S. and S.A. Barber, 1983. A numerical solution of whole plant nutrient uptake for soil-root systems with root hairs. Plant Soil, 70: 403-413.

Jaunin, F. and R.M. Hofer, 1988. Calcium and rhizodermal differentiation in primary maize roots. J. Exp. Bot., 39: 587-593.

Johnson-Flanagan, A.M. and J.N. Owens, 1985. Development of white spruce (*Picea glauca*) seedling roots. Can. J. Bot., 63: 456-462.

Kramer, D., 1983. Root Ecology and its Practical Application. Bundesanstalt fur Alpenlandische Landwirtschaft, Irdning, Austria, pp: 153-156.

Li, Z., M. Oda, K. Okada and H. Sasaki, 1996. Changes in thermotolerance of photosynthetic apparatus in cucumber leaves in response to water stress and exogenous ABA treatments. J. Japanese Soc. Hort. Sci., 65: 587-594.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edn., Gulf Professional Publishing, London, ISBN: 9780124735439, Pages: 889.

Meekes, H.T.H.M., 1986. Inhibition and recovery of cell wall formation in root hairs of *Ceratopteris thalictroides*. J. Exp. Bot., 37: 1201-1210.

Mohammad, P., M. Shiraishi and S. Koike, 1996. Morphological deformations of satsuma mandarin roots under high temperatures and limited soil moisture conditions during the summer season. Proceedings of the 8th International Citrus Congress, Volume 2, May 12-17, 1996, South Africa, pp: 1033-1037.

Mohammad, P., M. Shiraishi, T. Kashiwazaki and F. Khan, 1999. Effect of high summer temperatures and low soil moisture conditions on the morphology and anatomy of roots of trifoliate orange budded with Satsuma Mandarin. Sarhad J. Agric., 15: 181-191.

Poerwanto, R., H. Inoue and I. Kataoka, 1989. Effects of temperature on the morphology and physiology of the roots of trifoliate orange budded with Satsuma mandarin. J. Japanese Soc. Hort. Sci., 58: 267-274.

Reuther, W., 1973. Climate and Citrus Behaviour. In: The Citrus Industry, Vol. IV. Crop Protection, Reuther, W., E.C. Calavan and G.E. Carman (Eds.). University of California, Berkeley, pp: 280-337.

Reuther, W., E.M. Nauer and C.N. Roistacher, 1979. Some high-temperature effects on citrus growth. J. Am. Soc. Hort. Sci., 104: 353-356.

Schnall, J.A. and R.S. Quatrano, 1992. Abscisic acid elicits the water-stress response in root hairs of *Arabidopsis thaliana*. Plant Physiol., 100: 216-218.

Uphof, J.C. and K. Hummeh, 1962. Plant Hairs. In: Encyclopedia of Plant Anatomy, Zimmerman, W. and P.G. Ozenda (Eds.). Vol. 4, Gebruden Borntraeger, Berlin-Nikolassee.

Wutscher, H.K., 1979. Citrus Rootstocks. In: Horticultural Reviews, Volume 1, Janick, J. (Ed.). John Wiley and Sons, Hoboken, NJ, USA., pp: 237-269.