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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 3 (2): 219-222, 2000

SEM Observations on the Root Hairs of Trifoliate Orange Grafted with Satsuma Mandarin under Low Soil Moisture and High Summer Temperature Condition

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Abstract: The root hairs of trifoliate orange grafted with satsuma mandarin *(Citrus unshiu* Marc. cv. Okitsu Wase) at low soil moisture (-0.046 to -0.059 MPa) and high summer temperature (40-45°C) condition was studied under scanning electron microscope. The hair zone in actively growing roots positioned far apart from the root terminal and the hairs were mostly smaller with domft-shaped tips. Along with the severity of modification in roots under the present treatment hair zone positioned gradually nearer the root tip and the hairs gradually degraded. As a result the moderately modified roots possessed a few intact hairs but the extremely modified ones had only a few degenerated hairs or they were completely devoid of that. In that case, only the traces of abolished hairs were left with the root epidermis. At the initial stage of modification in roots, hairs elongated and this elongation was also found in the moderately modified roots but they had slightly irregular shapes. As the modification of root hairs progressed, their tips variously malformed having bending or swollen structure or they were severely injured. The malformed or injured root hairs subsequently destroyed completely.

Key words: High temperature, low soil moisture, root hair, scanning electron microscope, trifoliate orange.

Introduction

Citrus trees are sensitive in many ways to their physical environment. They react adversely to excessive heat and dry soil conditions (Marsh, 1973). High soil temperature basically causes slowing of transpiration which results to less water consumption (Smith *et al*., 1931) and considerable growth reduction in plants (Fretz, 1971; Ingram, 1981; Ingram and Buchanan, 1981; Wong *et al*., 1971). A large proportion of the total citrus roots exists in the upper soil layers which have also been reported to be subjected to high temperature-induced growth retardation process (Bevington and Castle, 1985). Moreover, under high temperature condition, trees are usually exposed not only to heat stress but also to water stress (Li *et al.,* 1996).

In Japan, satsuma mandarin orchards are also frequently enforced to water stress prior to fruit maturing, for increasing sugar in juice during late summer to autumn. Soil drying has also been suggested to force citrus trees to induce flowering (Inoue, 1989; Southwick and Davenport, 1987), Recently, the roots of trifoliate orange grafted with satsuma mandarin have been reported to be subjected to morphological and color changing process and considering these changes the roots were classified into eight morphological groups (Mohammad *et al*., 1999). Detailed studies on these stress effects have not yet been done. However, the previous studies revealed only the morphological changes in roots. Their functional characteristics were not considered.

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 (Von Guttenberg, 1968; Bristow, 1975; Kramer, 1983). However, the effects of high temperature and low soil moisture condition on the morphology of root hairs are not yet known. As the morphology of roots was affected by this condition in previous studies, it can also be presumed that the root hairs may also undergo structural changes.

This study was designed to observe the morphological changes in the hairs of recently reported eight morphologic kinds of roots in trifoliate orange grafted with satsuma mandarin under low soil moisture condition during the peak temperature period of summer using 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 to 1998 using three-year-old satsuma mandarin trees grafted on trifoliate orange rootstocks. Twenty four trees were selected for uniformity of vigor and planted in earthen pots, filled with sandy loam soil and granite mixture with a pH mean 6.5 in April 1 every year. Sufficient amount of manure was added with the pot mixture. A basic fertilization treatment was applied consisting of 120, 50 and 80 mg/dm^3 of N, P and K, respectively. The potted trees were maintained under a plastic house. 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. Long-standing establishment of the trees gave them sufficient development and the root samplings were done during the peak summer temperatures of August 3 to 28 in every session. Soil moisture stress treatment was first imposed by withholding the application of water from 3rd August and the soil mixture metric potential was adjusted daily between -0.046 to -0.059 MPa after reaching this level by everyday reading with tensiometer up to 20 cm depth and adding a little amount of water if necessary during the sampling period. The air and soil mixture minima/maxima were recorded everyday which ranged between 23-27/33-43 and 21-24/42-45°C, respectively. Four trees were uprooted in each sampling date and the available young roots were collected. First sampling was done immediately prior to impose water-stress treatment on the 3rd August and another five samples were taken with consecutive five days interval i.e., on the 8th, 13th, 18th, 23rd and 28th August of every session. On samplings, roots were separated into different morphologic forms on the basis of root tip modification under stereo microscope and their forms were denoted as A to H as was done by Mohammad *et al.* (1999).

The collected roots were washed gently several times with water to remove any adhering particles. Segments of 5 mm length from the root apices of each form were excised, fixed immediately in 4 percent glutaraldehyde buffered to pH 7.4 in sodium cacodylate buffer, post-fixed in osmium tetroxide, dehydrated in a graded ethanol series and freeze dried. The dried samples were coated with gold in an ion sputter and viewed under Hitachi S-2250N scanning electron microscope at 20kV. About ten roots from each type were investigated and the root hairs were photographed from the middle portions of hair zones. Three-year-observations were summarized and presented.

Results

Type A roots which were collected just before imposing water stress treatment had an elongated growing zone and hairs positioned a far apart from their tips towards the base (Fig. 1 A). Hair zone was limited only to a specific region immediately back to the actively growing zone of roots. The hairs were mostly smaller but accompanied by only a few elongated ones (Fig. 2 A). Their tips were mostly dome-shaped and bases were thicker. The overall hair surface was smooth. Although there existed a few partially degenerated hairs, where only their tips were abolished and other portions were smooth and regular.

Type B roots resembled type A in many characters. The actively growing zone here little shortened as a result the root hair zone became comparatively proximal to the root tip (Fig. 1 B). The most root hairs slightly elongated and their tips differently malformed (Fig. 2 B). In few cases the hair tips were swollen and others were pointed or dome-shaped. The hairs as a whole were irregular-shaped where a few were shrank. In other cases, their bases were narrower than the tips and vice-versa. Near the numerous root hairs, hairless areas of epidermis were also found.

Type C roots showed comparatively more proximity of the hair zone to the root tip (Fig. 1 C). The hairs were elongated but few were damaged or took irregular shape (Fig. 2 C). Even a considerable number of hairs had normal morphology. The affected hairs possessed damaged tips, which broke down fully or partially. Other hairs, especially the elongated ones were ridged and their tips formed dome shape or underwent swelling. The root hair zone was also accompanied by few degenerated hairs.

Type D roots were characterized by the extreme proximity of the hair zone to their terminal (Fig. 1 D). A few hairs elongated and became narrower but among them some were injured (Fig. 2 D). The thicker hairs mostly underwent injury induction process, especially their tips were destroyed. The comparatively unchanged hairs had mostly dome-shaped tips.

Type E roots had extremely elongated hairs and positioned up to the end of root tips (Fig. 1 E). The elongated hairs had tip injuries or different kinds of modifications (Fig. 2 E). The appearance of root hairs, as a whole, was irregular. A few hairs were partially or completely destroyed. Other hairs were tore down in few places and rarely their tips were completely destroyed.

Type F roots were also similarly accompanied with hairs up to their tips and spread throughout the tip region (Fig. 1 F). Root hairs were elongated but mostly their tips were modified into different shapes (Fig. 2 F). However, most tips formed bulbous shape and the rests were variously injured. They were accompanied by the partially or completely destroyed hairs. The modification also turned the hairs to be bend or irregularly shaped throughout.

Type G roots possessed short hairs throughout their tips (Fig. 1 G). The hairs were damaged totally from their tips towards the bases. Not a single hair was found to be free of injury (Fig. 2 G). The hair tips were irregularly injured and only a few tips formed dome shape. The sites having completely damaged root hairs were also found on the epidermis leaving only the injured bases of hairs.

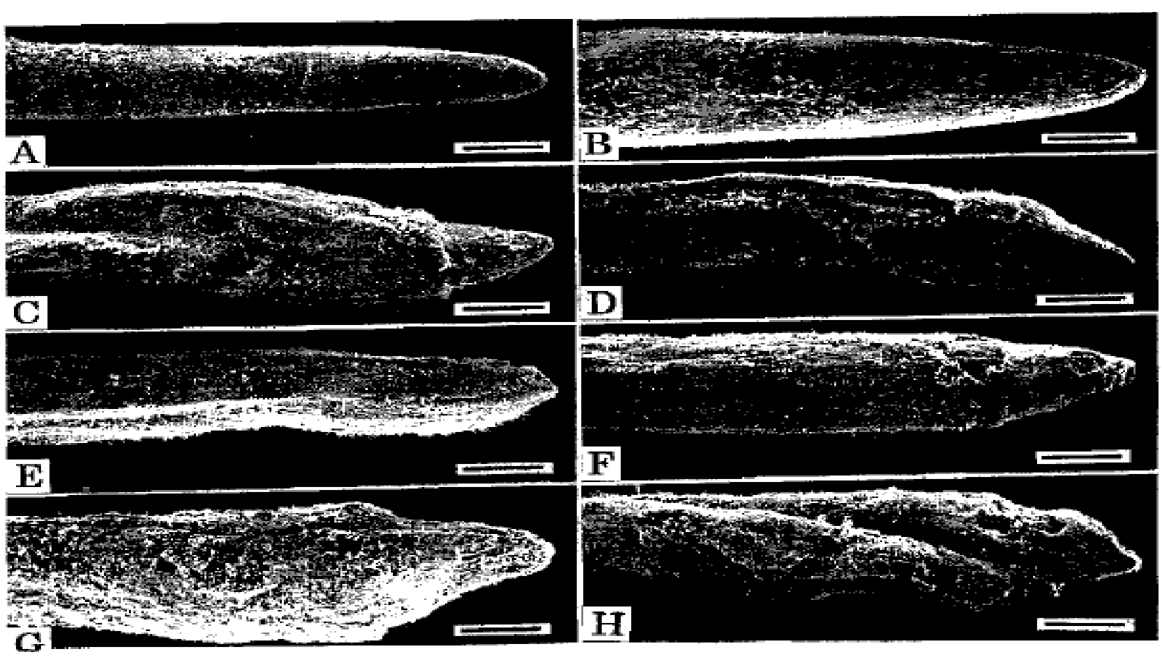

Fig. 1: Low magnified views of roots of trifoliate orange grafted with satsuma mandarin showing the position of hair zones under limited soil moisture condition during the high temperature period of summer, A: Type A. B: Type B. C: Type C. I): Type D. E: Type E. F: Type F. G: Type G. H: Type H. Note: Along with the modification of roots, the hair zones became gradually proximal to the root tip. Bar = 500 µm

Fig. 2: Localized views of root hairs of trifoliate orange grafted with satsuma mandarin under low soil moisture condition during the high temperature period of summer. A: Type A. B: Type B. C: Type C. 0: Type D. E: Type E. F: Type F. G: Type G. H: Type H. Note: Along with the modification of roots, hairs became elongated followed by their gradual degradation

Type H roots showed the extreme degradation of hairs (Fig. 1 H). Mostly the root tip surface was devoid of hairs and in few cases the epidermal cells possessed the injured bases of hairs (Fig. 2 H). The rest hairs were severely malformed and were under distinct destruction. This situation showed that the degeneration of hairs occurred from the tips towards their bases. The comparatively elongated hairs also had rough surface. The epidermal cells mostly dried.

Discussion

Research on the morphology of root hairs has led to successive updates, from Hesse (1904) to Uphof (1962), Von Guttenberg (1968) and Aeschbacher *et al*. (1994), which give a fairly clear picture of the variability propensity existing in the structure of root hairs. The hair zone became gradually proximal to the root tip as the modification in root tip proceeded in our study. The hair zone of most roots is situated just behind the zone of active root elongation (Jaunin and Hofer, 1988). However, the formation of root hairs may extend nearer the tip if growth is slowed down (Johnson-Flanagan and Owens, 1985; Jaunin and Hofer, 1988). The presently imposed high temperature and low soil moisture condition was reported to decrease the growing zone of root tip in the same material (Mohammad *et al*., 1999). The proximity of the hair zone to the root tip in this study therefore occurred due to the cessation of growth of roots.

The elongation of root hairs in the initial stage of stress induction was also distinct in our study. Root hair size was reported to vary with several environmental factors (Ewens and Leigh, 1985; Jaunin and Hofer, 1988; Marschner, 1995). Functional hairs increase the capability of roots to absorb water and solutes by increasing the root surface area (Marschner, 1995). Uptake efficiency is correlated with root hair length (Itoh and Barber, 1983). The elongation of hairs in the slightly modified roots was therefore thought as the adaptation strategy of roots under this stress condition. This means that the stressed roots initially trend to cope with low soil moisture condition by extending their absorption areas.

The shape and size of hairs varied considerably in all sampling dates. Uphof (1962) also noted different types of root hairs in different plants. However, initially the slightly modified hairs possessed little swollen tips. Meekes (1986) also showed similarly 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 treatment referred to as high temperature and low soil moisture condition in the present study therefore resulted this swollen root hair tips.

As the modification in root morphology increased in our study, the hair tips altered differently. Schnall and Quatrano (1992) also reported that the swelling of hair base was the characteristic changes in root hair development due to water stress. Along with these changes, the degradation of hair tips and finally their complete abolition appeared. This kind of extreme effect probably occurred due to the presence of high temperature accompanied low soil moisture condition as the acute stress situation for the growth of trifoliate orange root.

Although root hairs are short lived, there is distinct zone of hairs and within one zone other stages of hairs are not found. In our observation, we studied only the middle portion of hair zone where they should be normal. However, the gradual degradation of hairs in that portion led us to assume the treatment as severely adverse for root hair formation. Root hair is directly associated with the absorption by plants. In other words, the present stress condition is severely critical for the physiological activities in plants.

In conclusion, the high temperature and low soil moisture condition causes the gradual proximity of hair zone to the root tip. It enhances the elongation of hairs to a certain level followed by deformation of tips and finally their complete abolition. The condition should therefore be carefully handled as it is adverse to the successful cultivation of satsuma mandarin onto trifoliate orange.

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