Pak. J. Bot., 36(4): 763-768, 2004.

ADVENTITIOUS ROOT FORMATION IN LEAF-BUD CUTTINGS OF TEA (*CAMELLIA SINENSIS* L.)

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Abstract

Anatomical changes during adventitious root initiation are described in cuttings with single leaf and node of Turkish tea (*Camellia sinensis* L.) clone Fener-3. In this clone no preformed root initials or primordia were found. The rooting process consists of these stages; cuttings exhibited meristematic activity of vascular zone (especially, phloem parenchyma), root initial formation, differentiation of root primordia, and root elongation. Thus, adventitious root primordia arose from near the vascular cambium and secondary phloem parenchymatous tissue also contributed. Continuous cell division, elongation and differentiation within callus gave rise to formation of root primordia. Presence of perivascular fibers and sclereids did no mechanical barrier in inhibition of root formation and to retard rooting. The development of adventitious roots is directly correlated with the appearance of root initials. According to the process of root initiation, cuttings of Fener-3 Turkish tea clone may be described as easy to root.

Introduction

Asexual propagation of woody plants is an important commercial method of regenerating large quantities of genetically uniform plant materials. A lot of woody plants are commonly propagated by different stem cuttings and therefore adventitious roots must be formed successfully. Adventitious root formation arising from any part of the plant other than normal root development is difficult in some woody plants and easy in some ones. Various plants have the ability of adventitious root formation (Hartmann *et al*., 1997). Adventitious roots can either form naturally on the stem cutting or develop in response to wounding effect on the cutting. Some anatomical changes occur during adventitious root formation such as dedifferentiation of specific differentiated cells, formation of root initials arising from certain cells adjacent to vascular bundles or tissues gaining meristematic characteristic by dedifferentiation, changing into root primordia and establishment of vascular connection adventitious root and between vascular tissues of the cutting, respectively. In addition, adventitious root in stem cuttings of many woody plants may originate from various tissues such as living parenchyma cells, young secondary phloem, vascular rays, cambium, phloem, lenticels and pith (Esau, 1976; Hartmann *et al*., 1997). Rodriguez *et al*., (1988) pointed out that adventitious roots in hazelnut cuttings have their origin in the proliferation zones of the phloem parenchyma. Adventitious root development of the apple rootstock M.26 has been described by Mackenzie *et al*., (1986) as root primordia differentiated remote from the existing vascular tissue of the wound in winter cuttings. Hubl *et al*., (1984) concluded that root initials are localized in the area of the cambium which has divided intensively in softwood cutting of *Prunus cerasus* and *P. domestica.* The origin of adventitious roots from stem cuttings has been studied in plum rootstock and cultivars by Skolidis *et al*.

(1990). In these cuttings, callus developed as a first step in process of root regeneration, and root initials or primordias inducted, indirect regeneration occurred and the root emerged. Koyuncu & Tekintaş (1999) reported that adventitious roots in hazelnut hardwood cuttings originated in young secondary phloem with vascular cambium and rays. Adventitious roots are able to originate from callus tissue formed at the base of cutting, although connection with the main vascular system of these initials was extremely difficult (Davies *et al*., 1982; Koyuncu & Tekintaş, 1999).

C. sinensis L., is commercially cultured in the East Black Sea Region of Turkey. Tea culture is currently done in the gardens established with the superior clones selected from native population. Despite the amount of researches devoted to rooting ability of tea cuttings (Özbek *et al*., 1961; Ayfer *et al*., 1987a, b; Şen *et al*., 1988; Chen *et al*., 1990; Şen *et al*., 1991; Cheng & Yang, 1995; Altındal & Balta, 2002) there is no detailed information on the origin and development of adventitious roots in this species. Our main objective was to determine the anatomical structure of adventitious root formation in the Turkish tea clone Fener-3.

Materials and Methods

Tea (*Camellia sinensis* L.) cuttings of Fener-3 clone were taken from Atatürk Tea and Horticultural Research Institute of Rize Turkey. Stock clone plants before cutting preparation were hard-pruned in March so, the clone could develop healthy annual shoots. The single leaf-bud cuttings (lamina, petiole and 7-8 cm of shoot with an axillary bud) were prepared from middle portions of these shoots in September. These cuttings were planted on a rooting medium of sterilized mix of agriperlite and forestry soil (1:1) (v/v) in the black polyethylene pots with 20x15 cm dimensions and 7-8 drainage hole near bottom side under mist-propagation system, and placed in a greenhouse shaded with jute cloth. For histological studies cuttings were harvested from greenhouse at 15-days intervals during rooting period, and 2 cm basal portions of them were fixed in 5:5:90 FAA (v/v/v) (formalin/glacial acetic acid/ethanol). The samples were washed in distilled water for 48 h and sectioned to a thickness of 20-30 µm using a sliding microtome. The sections were stained with 1% safranin solution in 50% ethanol, followed by 1% fast green in absolute alcohol. The sections were observed in a Nikon Model Eclipse E 600 microscope with Nikon FDX-35, Nikon H-III photomicrographic equipment and microphotographs were taken with Inford black-white film. Line-drawings were made on the basis of complete serial section using Macromedia Flash MX software program.

Results and Discussion

Fig. 1a shows the general cross-sectional anatomy of tea cutting base. The cortex was composed of unorganized parenchyma cells. The lignified phloem fibres were found to be distributed in groups to form a discontinuous ring just outside the phloem. Phloem tissue consisted of cell layers which were arranged in irregular rows and various shapes. Vascular cambium adjacent to phloem cells had 3-5 cell layers arranged in radial rows. In tea cuttings recognizable root primordia or preformed root initials were not observed (Fig. 1a). Many researchers also recorded that cuttings of some fruit species such as apple, plum, peach and sour cherry had not preformed root initials (Hubl *et al*., 1984; Skolidis *et al*., 1990; Pfeiffer *et al*., 1991; Qrunfleh *et al*., 1992).

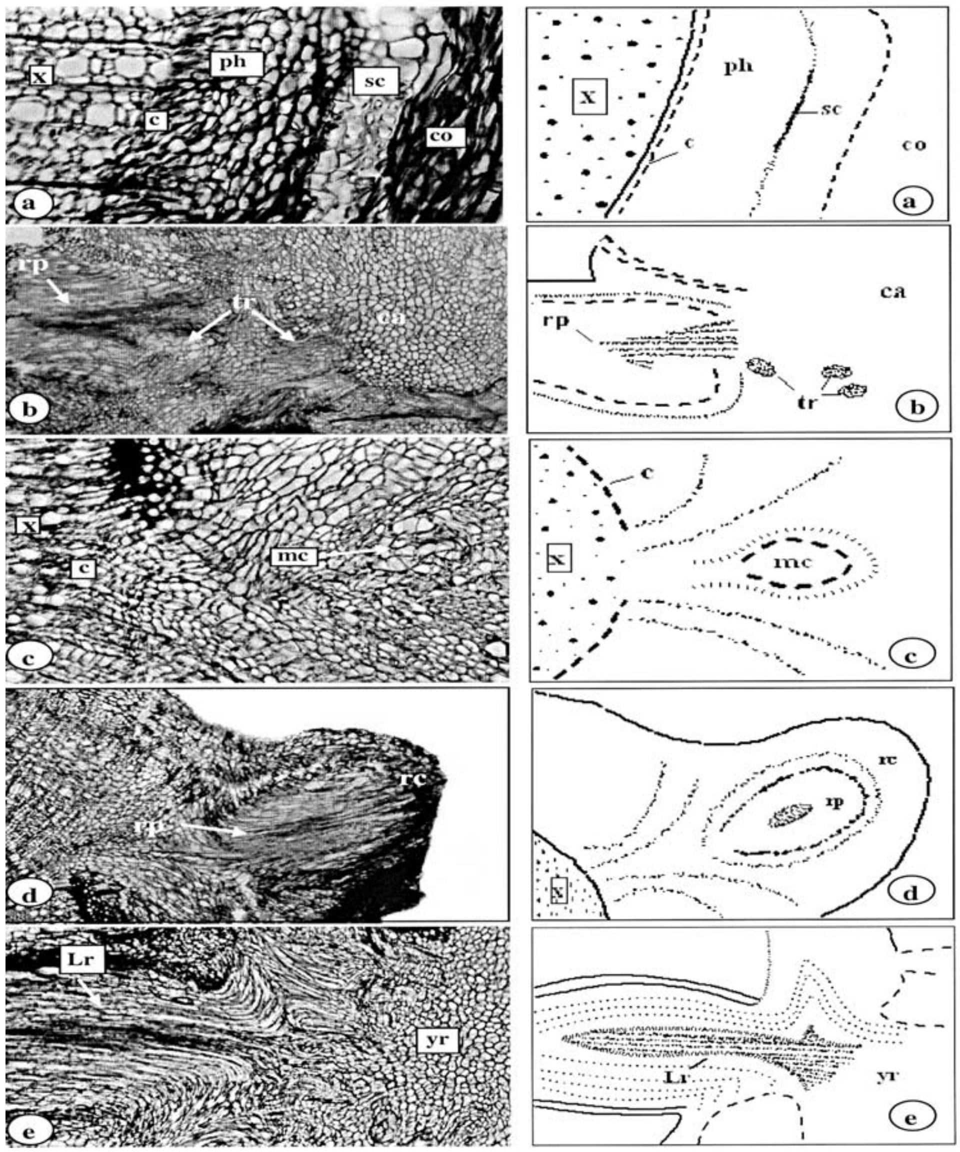

Fig. 1. Anatomical changes of tea cuttings undergoing adventitious root development with cross-sections (a), general anatomy of the internode stem; close up of a vascular bundle and sclerenchymatic tissue x 1760. (b), differentiation within callus originated from cambium and formation of root primordia x 550. (c), organized meristematic cells groups arising from vascular zone (vascular cambium, rays or/and undifferentiated secondary phloem) becoming a root primordia x 550. (d), a well developed root primordia almost near a node x 88. (e), showing complete differentiation of lateral root originated from pericycle of young root x 88. (co: cortex, c: cambium, ph: phloem, sc: sclerenchyma, x: xylem, rp: root primordia, tr: tracheary elements, ca: callus, mc: meristematic cells, rc: root cap, yr: young root, Lr: lateral root)

In cutting of *C. sinensis* L., adventitious roots commonly formed at basal and of cuttings, callus or sometimes at node above the base. The rooting zone in cuttings showed extensive cell division. Tissue swelling at the base of the cutting was observed after 10 days in the rooting branch. Microscopic investigations showed that meristematic activity accompanied this swelling. The callus formed cells proliferated mainly from vascular cambium and in part from phloem and xylem parenchyma. This newly formed callus consisted of irregular unspecialized parenchymatous cells. Adventitious roots frequently emerged through the callus, leading to the belief that callus formation is essential for rooting because differentiation within callus led to formation of root initials (Hartmann *et al*., 1997). Our study revealed that adventitious roots can initiate in callus, thus callus formation must precede root initiation. However, cuttings with excellent callus often failed to rooting, but many cuttings with poor callus developed root primordia and elongated. Thus, root initiations within callus led to formation of root initials in tea cuttings (Fig. 1b). Similar observations were reported for Douglas-fir stem cuttings (Bhella & Roberts, 1975) and *Ficus pumila* L., leaf bud cuttings (Davies *et al*., 1982). Continuous cell division and differentiation of these initials may occur at root primordia having complete vascular connection with the main system. On the other hand, root primordia might originate in different tissues such as vascular cambium and secondary phloem cells in tea cuttings. The adventitious root primordia were initiated by divisions and massing of undifferentiated parenchyma cells near the vascular zone. This formation was regarded as meristematic cells groups indicating emergence of root initials. Phloem rays around these cell groups were closely pressed toward sides and adjacent cells were also accompanied by root initial cells (Fig. 1c). These initials dividing and under-going meristematic activity, formed organized cell groups and differentiated into root primordia which elongated into cortical region. Primordia through continuous cell divisions and elongations developed adventitious root having vascular connections with stem vascular system. This primordia passed to periphery by extending through phloem and sclerenchyma fibers and then to emerge from the bark (Fig. 1d). In many studies, it was reported that adventitious roots originated from the area of the cambium which has divided intensively in 'Schattenmorelle' and 'Nancy Mirabelle' cuttings (Hubl *et al*., 1984) from cambium and phloem rays in kiwi hardwood cuttings (Messina & Testolin 1984), from vascular tissues in M.26 apple rootstock (Mackenzie *et al*., 1986; Mackenzie *et al*., 1988), from secondary phloem cells adjacent to cambium in hardwood cuttings of filbert (Koyuncu & Tekintaş, 1999), and from phloem near the cambium in ficus hardwood cuttings (Seferoğlu & Tekintaş, 1997). These findings almost conform to our findings. The new adventitious roots formed in cuttings were succulent, white coloured and very brittle. They developed rapidly, became brown, hard, less brittle and produced lateral roots in the pericycle of young roots (Fig. 1e).

According to our results it is possible to conclude that 1) adventitious roots might originate from vascular zone (cambium, rays, secondary phloem parenchyma) or basal callus; 2) sclerenchymatic cells in phloem were not a mechanical barrier in formation of root primordia; 3) Turkish tea clone Fener-3 may be described as easy to root.

Acknowledgment

We thank to Atatürk Tea and Horticultural Research Institute (Rize-Turkey) for supplying the plant material used in this work.

References

Altındal, E. and F. Balta. 2002. Comparison of rooting capabilities of Turkish tea clones. *Turkish Journal of Agriculture and Forestr,.* 26**:** 195-201.

Ayfer, M., M. Çelik, H. Çelik, M. Erden, T. Tutgaç and H. Mahmutoğlu. 1987a. Farklı köklendirme yöntemleri ve ortamlarının çay çeliklerinin köklenmeleri üzerine etkileri. Uluslararası Çay Sempozyumu Bildirileri, s. 16-25, 26-28 Haziran, Rize.

Ayfer, M., M. Çelik, H. Çelik, H. Vanlı, T. Tutgaç, T. Turna and H. Dumanoğlu. 1987b. Farklı gölgeleme materyalleri, çelik alma zamanları ve çelik tiplerinin çay çeliklerinin köklenmeleri üzerine etkileri. Uluslararası Çay Sem. Bildirileri, s. 26-34,26-28 Haziran, Rize.

Bhella, H.S. and A.N. Roberts. 1975. Seasonal change in origin and rate of development of root initials in Dauglas-fir stem cuttings. *J. Amer. Soc: Hort. Sci.,* 100(6): 643-646.

Chen, J.S., F.M. Thseng, and W.H. KO. 1990. Improvement of survival and subsequent growth of tea cutting. *Hortscience***,** 25(3): 305-306.

Cheng, W. and W. Yang. 1995. Investigation on cuttings from upper mature shoots of tea. *J. of Tea Sci.,* 15(1): 77-78.

Davies, F.T.Jr., J.E. Lazarte and J.N. Joiner. 1982. In initiation and development of roots in juvenile and mature leaf bud cuttigs of *Ficus pumila* L. *Amer. Journal Botany*. 69(5): 804-811.

Esau K. 1976. *Anatomy of Seed Plants*, John Wiley and Sons. Inc. 2nd Edition, Newyork and London.

Hartmann, H.T., D.E. Kester, Jr. F.T. Davies and R.L. Geneve. 1997. *Plant Propagation Principles and Practices,* (sixth edition) Upper Saddle River, New Jersey.

Hubl, D., W. Hartmann and R. Stosser. 1984. Anatomisch-histologische untersuchungender wurzelbildung bei grünstecklingen von *Prunus cerasus* L., *P. domestica* L., gartenbauwissenschaft, 49(5/6): 193-199.

Koyuncu, F. and F.E. Tekintaş. 1999. Fındık çeliklerinde köklenmenin anatomik ve histolojik olarak incelenmesi üzerine araştırmalar. Türkiye III. Ulusal bahçe Bitkileri Kongresi, Ankara. s; 201-207

Mackenzie, K.A.D., B.H. Howard, and R.S. Harrison-Murray. 1986. The anatomical relationship between cambial regeneration and root initiation in wounded winter cuttings of the apple rootstock M. 26. *Annals of Botany*, 58: 649-661.

Mackenzie, K.A.D., B.H. Howard, and R.S. Harrison-Murray. 1988. Anatomical features of rooting in wounded winter cuttings of the apple rootstock M.26*. Acta Horticulturae*, 227: 217-223.

Messina, R. and R. Testolin. 1984. Indagini anatomiche Sull' origine delle radici avventizie in talee di actinidia "Actinidia chinensis Pl.". Riv. Ortoflorofrult. It. 68.

Özbek, S., M. Özsan and M. Yılmaz. 1961. Çay çeliklerinin köklenmeleri üzerine muhtelif hormonların tesiri. *A.Ü.Z.F. Yıllığı*, 11(2): 175-224, Ankara.

Qrunfleh, M.M., M.M.S. Aratch and D.M. Al-Eisawi. 1992. Nodal anatomy of two Low-Chill Peach cultivars as related to adventitious root formation. *Advances in Horticultural Science,* 6: 129-133.

Pfeiffer, A., W. Hartmann and R. Stosser. 1991. Anatomisch-histologische untersuchungen der adventivwur zel bildung am astring einjähriger langtriebe von apfel und pflaume mitteilungen klosterneuburg 41: 119-126.

Rodriguez, A., M. Albuverne and R. Sánchez-Tamés. 1988. Rooting ability of *Corylus avellana* L. macromorphological and histological study. *Scientia Horticulturae*, 35**:** 131-142.

Seferoglu, G. and F.E. Tekintaş. 1997. Anatomical and histological development of rooting on the fig hardwood cuttings. Proc.of 1st IS on Fig. *Acta Hort*., 480: 115-117.

Skolidis, K., W. Hartman and R. Stosser. 1990. Histogische untersuchung der wurzelbildung an steckhölzern von pflaumenunter lagen und-sorten. *Gartenbauwissenschaft,* 55(4): 151-154.

Şen, S.M., S. Uzun, Y. Boz, H. Vanlı, T. Tutgaç and T. Turna. 1988. Çay klonlarının aşı ve çelik tiplerinin köklenmeye etkileri üzerinde araştırmalar. *O.M.Ü. Ziraat Fakültesi Dergisi,* 3(1): 13-20.

Şen, S.M., S. Uzun, Y. Boz, H. Vanlı, T. Tutgaç and T. Turna. 1991. Çay klonlarının aşı ve çelikle çoğaltılması üzerine araştırmalar**.** *Y.Y.Ü. Ziraat Fak. Dergisi*., 1-3: 67-88.

(Received for publication 28 February 2004)