HortScience 40(5):1474–1477. 2005.

The Structure of Graft Unions in European Chestnut Using Different Grafting Methods

Umit Serdar,[1] Bulent Kose, and Fatma Yilmaz
Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, 55139 Kurupelit-Samsun, Turkey

Additional index words. chestnut, grafting, anatomy, juvenile grafts, graft healing

Abstract. **We studied the anatomical structure of graft unions in European chestnut using several grafting methods. The work was done in the greenhouse during 2003–04. The grafting methods epicotyl, hypocotyl, and inverted radicle were used. The grafts were made with scions of clone SA 5-1 on clone SE 21-9 rootstock. The samples for examination were taken from the graft unions 2, 6, and 12 months after grafting, and fixed in a formalin–acetic acid–alcohol solution. The observation of the anatomical structure of the graft union area revealed that new cambium, xylem, and phloem tissues were formed in the samples two months after grafting. Further, it could be also observed that 6 months were necessary for continuous cambial connection.**

Asexually reproduced cultivars of chestnut are propagated by various methods such as grafting, rooted cuttings, root collar division, or tissue culture. The healing progress of the graft union area may vary according to stock-scion combinations, propagation techniques, and environmental conditions during and following grafting, growth activity of stock plant, insect and disease contamination, and use of plant growth regulators (Hartmann et al., 1990).

Some of the budding and grafting methods available can be used on seeds at a more or less advanced stage of germination. Such grafting methods offer several advantages compared with the conventional field grafting method such as: stock trees are not necessary, grafting can be done indoors, timing is not critical, scions with a small diameter can be used (Keys, 1978; McKay and Jaynes, 1969).

In anatomical and histological studies of graft unions in hypocotyl grafting of chestnut, Park (1968) reported that the callus tissue was formed from both stock and scion 10 d after grafting, and callus formation was completed 25 d after grafting. Using inverted radicle grafting, root development and graft healing were each completed in 9 stages. These stages were protoxylem, procambium, cambium ring, root primordia, caryptrogen, verticilate roots, growth in thickness, geotropism of verticilate roots, and growth vigor (root development), and callus, new cambium, phloic procambium, xylaric procambium, vascular bundle, epiderall cell, secondary xylem, connected union of both protoxylem of verticilate root and new protoxylem of incision face, completed union of both from scion callus, and new protoxylem of inverted radicle stock (graft healing) (Park, 1972). Vieitez and Vieitez (1981) reported perfect cambial and vascular connection between stock and scion in graft unions using hypocotyl grafting.

Many studies have been conducted on anatomical and histological structures in graft unions of chestnut using conventional budding and grafting methods (Balta et al., 1993, 1996; Seferoglu and Ertan, 2003; Serdar and Soylu, 2004; Ufuk and Soylu, 1999). However, there have been no attempts to investigate the anatomical structure in unions using different juvenile grafting methods. The main objective of this study was to investigate the anatomical and histological structure of epicotyl grafts, hypocotyls grafts and inverted radicle grafts in chestnut with samples taken 2, 6, and 12 months after grafting.

Received for publication 11 Jan. 2005. Accepted for publication 10 Feb. 2005.
[1]Corresponding author; e-mail userdar@omu.edu.tr.

Materials and Methods

This study was carried out in the greenhouse in Samsun, Turkey, during 2003–04. Epicotyl, hypocotyl, and inverted radicle grafts were used. Grafts were made on 17 May 2003. During the first month after grafting, mean temperature and relative humidity were 20.3 °C and 72.3% respectively, and the mean values for the 6-month period after grafting were 22.6 °C and 57.0%. Newly germinated chestnut seeds and young seedlings of clone SE 21-9 (Soylu and Serdar, 2000) were used as rootstock, and sprouts with dormant buds of clone SA 5-1 (Serdar, 1999) were used as scion materials.

Chestnut seeds weighting 11 to 13 g were taken from an orchard in the province Sinop, Turkey in October 2002, stored at 0 ± 0.5 °C, and stratified at 4 ± 0.5 °C for 2.5 months. The scions were taken from an orchard in the province Samsun, Turkey in February 2003 and were stored at 0 ± 0.5 °C until they were used for grafting.

In the inverted radicle grafting method, germinated seeds with a radicle length of 8.7 ± 0.9 cm were used. The radicle tip was cut off at 4 to 5 cm and split. Then the bottom of the scion was cut into a wedge-shape and inserted into the radicle tip as in a normal cleft graft (Park, 1972). In the hypocotyl grafting method, germinated seeds were planted in a peat medium and 16 to 20 d later young seedlings with 2 to 4 leaves and epicotyl lengths of 7.5 ± 1.5 cm were used. The epicotyl was cut off at the cotyledons and the hypocotyl was split. Then the bottom of the scion was cut into a wedge-shape and inserted into the hypocotyl as in a normal cleft graft (Vietiez and Vieitez, 1982). After grafting, the graft union was tied with parafilm (4 to 6 mm width) in both of the methods. Grafted seeds or young seedlings were planted in pots (30 × 40 cm) filled with a medium containing 3/4 soil + 1/4 ground pine needles. Some peat was added to the pots at planting, especially around the graft area. Then, grafting wax was applied to tips of the scions. In the epicotyl grafting method, germinated seeds were planted in pots of the same size containing the same medium. Young seedlings with 6 to 9 leaves, with stems 20.7 ± 1.5 cm long were used. The epicotyl was cut off at 4 to 7 cm and split. Then the bottom of the scion was cut into a wedge-shape and inserted into the epicotyl as in a normal cleft graft (Sawano et al., 1983). After grafting, the graft union was tied with plastic tape-which was left in place for two months.

To examine the anatomical structure of the graft unions, samples were collected 2, 6, and 12 months after grafting. In each period five randomly selected samples were taken from each graft combination. The samples were fixed in a FAA solution [formalin (37%): 10%, glaciyal acetic acid: 5%, ethanol (96%): 50%, distile water: 35%] and were stored at 3 to 5 °C. Before cutting the sections, the fixed samples were stored in 70% alcohol for at least 1 d. The tissues were cut in cross sections with a sliding microtome at 30 to 60 µm thickness (Ufuk and Soylu, 1999).

Results and Discussion

Callus formation was complete 2 months after grafting, and cambial tissue at the graft union was differentiated from the callus tissue (Fig. 1A and B). New cambium and vascular tissues were formed at the graft union. In all grafting methods, cambial connections were initiated and partially completed on both sides of the graft union. Some necrotic layers were observed in the xylem tissue of the stocks and cortex tissues. When different grafting methods were used under controlled conditions (Balta et al., 1993), and when T budding was done in the fall (Seferoglu and Ertan, 2003) cambial differentiation was initiated within the callus tissue, and the first new vascular components begun to form in samples one month after grafting.

The vascular connection was established successfully between rootstock and scion 6 months after grafting (Fig. 2A and B). A few necrotic layers were observed at the graft union but cambial connection was observed in all methods. In sections made 12 months after grafting, perfect cambial connections were found. Some researchers have stated that cambial connections formed within 45 to 60 d (Balta et al., 1993; 1996; Seferoglu and Ertan, 2003). However Ufuk and Soylu (1999) and Serdar and Soylu (2004) reported cambial connections 12 months after grafting. It is possible that this variation may arise from the use of different stock–scion combinations and environmental factors.

In this study, generally we used scions and

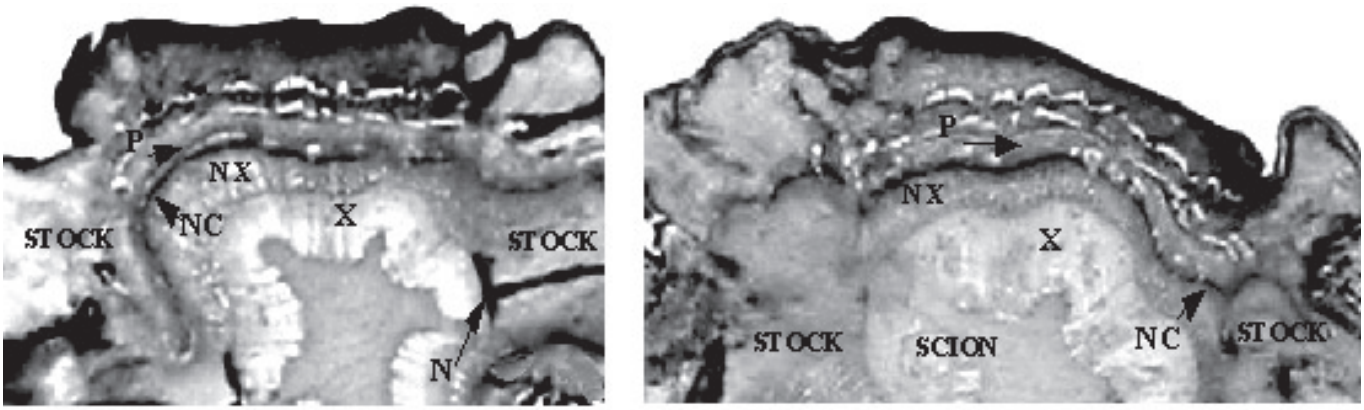

Fig. 1. Views of the tissues at the graft union in samples, 2 months after grafting; NC = new cambium, N = necrotic areas X = xylem, NX = new xylem, P = phloem. (**A**) The inverted radicle graft (**B**) The hypocotyl graft.

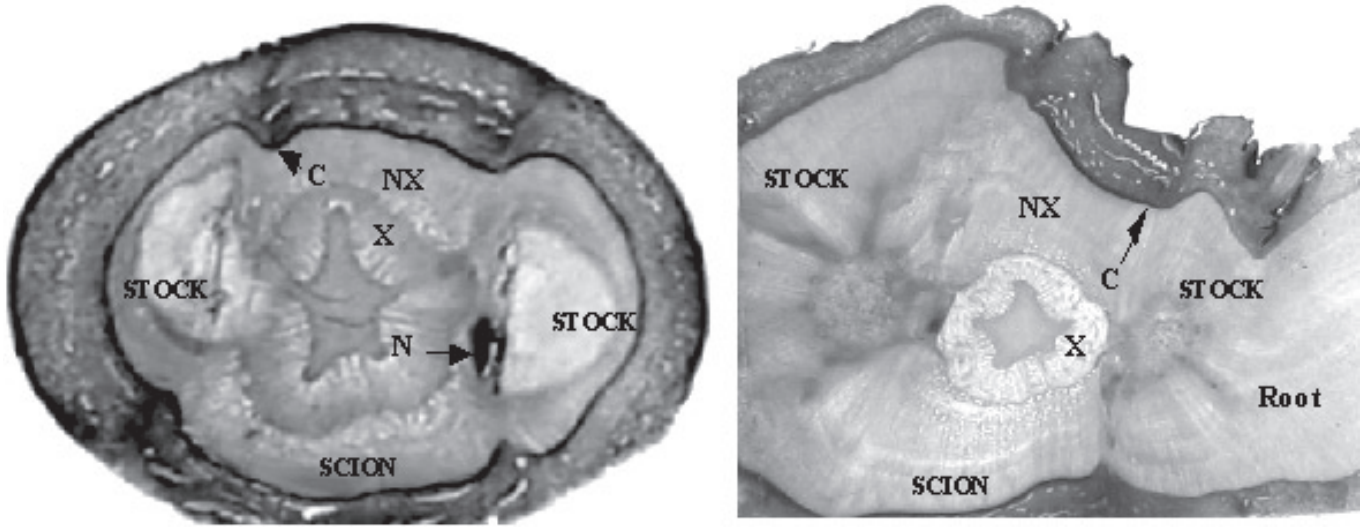

Fig. 2. Views of the tissues at the graft union in samples, 6 months after grafting; C = cambium, N = necrotic areas X = xylem, NX, new xylem, P = phloem. (**A**) The epicotyl graft (**B**) The inverted radicle graft.

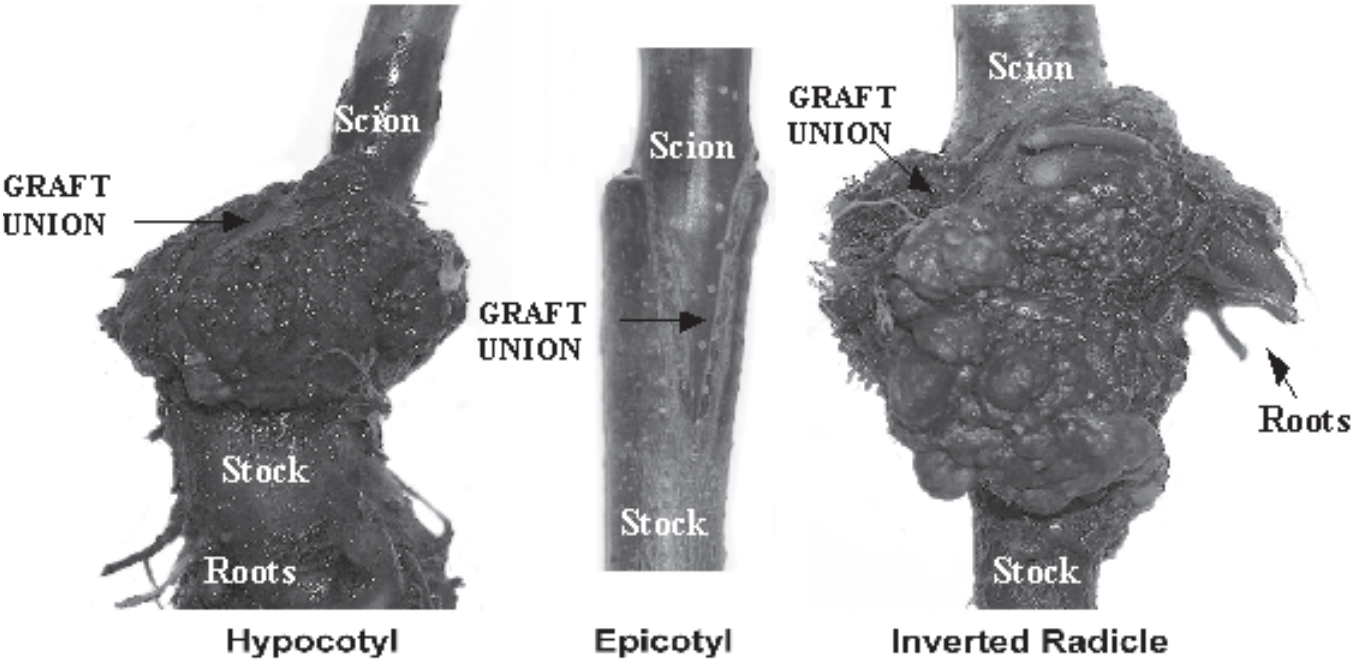

Fig. 3. Views of the graft unions in the hypocotyl, epicotyl and inverted radicle grafting methods, 6 months after grafting.

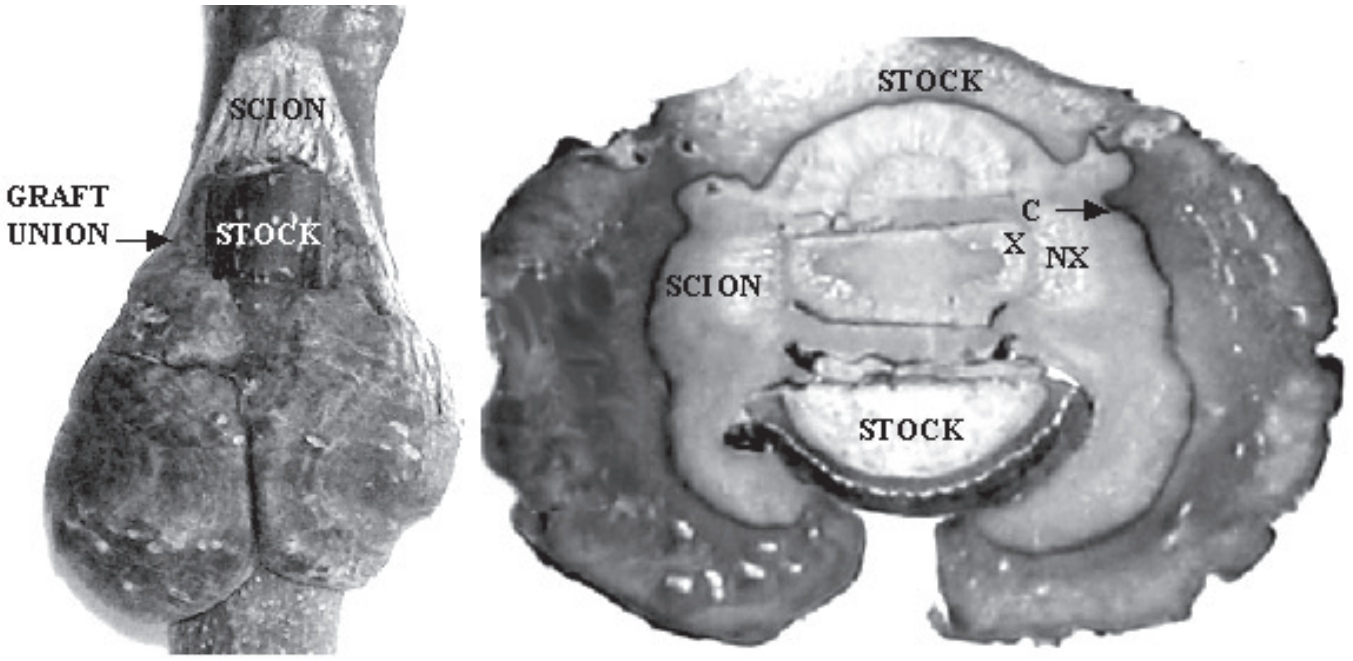

Fig. 4. Views of the tissues at the graft union in the abnormal sample taken from the epicotyl graft, 6 months after grafting; C = cambium, X = xylem, NX = new xylem.

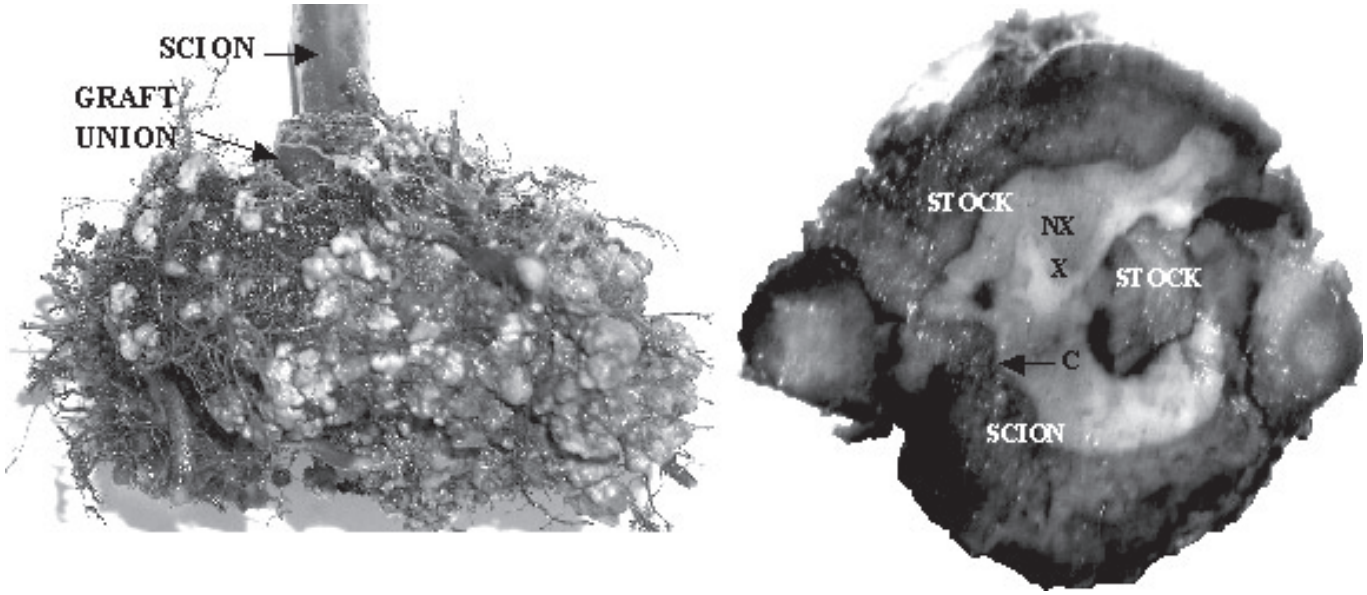

Fig. 5. Views of the tissues at the graft union in the abnormal sample taken from the hypocotyl graft, 6 months after grafting; C = cambium, X n= xylem, NX = new xylem.

stocks with the same diameter when making grafts and perfect graft unions were completed six months after grafting (Fig. 3). However, when scions thicker than stocks were also used, the cambium layers matched at only one surface and at those surfaces good cambial and vascular connections were observed. However, at the unmatched surface, excessive callus and swellings from the scion were formed (Figs. 4 and 5). The swelling around the graft union can be considered as one symptom of incompatibility. In addition, dense necrotic layers acting as barriers between the stock and scion tissues have been reported (Craddock and Bassi, 1999; Desvignes, 1999; Santamour, 1988; Huang et al., 1994; Serdar and Soylu, 2004). In the present study, no dense necrotic layers were observed between the stock and scion tissues. The swelling may be simply a result of mis-matched stock and scion sizes, or this may result from the genetic interaction of different stock-scion combinations.

Literature Cited

Balta, F., T. Karadeniz, M.A. Askın, and T. Yarılgac. 1996. Application of the micro-wave method in sectioning the chestnut (*Castanea sativa* Mill.) grafts, p. 353–361. (in Turkish with English summary). The Hazelnut and Other Nuts Symposium. Samsun, Turkey, 10–11 Jan.

Balta, F., T. Karadeniz, F.E. Tekintas, and S.M. Sen. 1993. Investigations on anatomical and histological development of the graft formation in chestnut (*Castanea sativa* Mill.), p. 231–234. Proc. 1st Intl. Cong. on Chestnut. Spoleto, Italy, 20–23 Oct.

Craddock, J.H. and G. Bassi. 1999. Effect of clonally propagated interspecific hybrid chestnut rootstock on short-term graft compatibility with four cultivars of Italian "Marrone". Proc. 2nd Intl. Symp. on Chestnut. Acta Hort. 494:207–212.

Desvignes, J.C. 1999. Sweet chestnut incompatibility and mosaics caused by the chestnut mosaic virus (ChMV). Proc. 2nd Intl. Symp. on Chestnut. Acta Hort. 494:451–458.

Hartmann, H.T., D. Kester, and F.T. Davies. 1990. Plant propagation principles and practices. 5th ed. Regents/Prentice Hall, Englewood Cliffs, N.J.

Huang, H., J.D. Norton, G.E. Boyhan, and B.R. Abrahams. 1994. Graft compatibility among chestnut (*Castanea*) species. J. Amer. Soc. Hort. Sci. 119 (6):1127–1132.

Keys, R.N. 1978. Prospects of vegetative propagation in the genus *Castanea*, p. 10–16. In: W.L. MacDonald, F.C. Cech, H. Luchok, and C. Smith (eds.). Proc. Amer. Chestnut Symp. 4–5 Jan., West Virginia.

McKay, J.W. and R.A. Jaynes. 1969. Chestnuts, p. 281–285. In: R.A. Jaynes (eds.). Handbook of North American nut trees. N. Nut Growers Assn., Knoxville, Tenn.

Park, K.S. 1968. Studies on the juvenile tissue grafting of some special use trees III. On the modified nurse seed grafting of some crop tree species (chestnut, ginkgo and oak). Res. Rpt. Inst. For. Gen. Korea 6:89–104.

Park, K.S. 1972. Histological study of the polaritically oriented differentiation in the inverted radicle grafting of chestnut. Res. Rpt. Inst. For Gen. Korea 9:39–49.

Santamour, F.S. 1988. Graft incompatibility related to cambial peroxidaz isozymes in Chinese chestnut. J. Environ. Hort. 6(2), p. 33–39.

Sawano, M., T. Ichii, and T. Nakanishi. 1983. Shortening of nursery period by a novel method of grafting on green wood stock of chestnut. Sci. Rpt. Fac. Agr. Kobe Univ. 15:241–246.

Seferoglu, G. and E. Ertan. 2003. Callus and graft union formation in Turkish chestnut selections. Bul. Pure Applied Sci. 22B (2):95–104.

Serdar, U. 1999. Selection of chestnut (*C. sativa* Mill.) in Sinop vicinity. Proc. 2nd Intl. Symp. on Chestnut. Acta Hort. 494:327–332.

Serdar, U. and A. Soylu. 2004. Investigation of anatomical structure of graft union for T and inverted T buddings and whip grafting in chestnut. Proc. of the 3rd Intl. Symp. on Chestnut. 20–23 Oct., Chaves, Portugal (in press).

Soylu, A. and U. Serdar. 2000. Rootstock selection on chestnut (*Castanea sativa* Mill.) in the middle of Black Sea Region in Turkey. Proc. EUCARPIA Symposium on Fruit Breeding and Genetics. Acta Hort. 538:483–488.

Ufuk, S. and A. Soylu. 1999. Researches on stock-scion compatibility between some important chestnut cultivars and hybrid rootstocks. Proc. of the 2nd Intl. Symposium on Chestnut. Acta Hort. 494:223–229.

Vieitez, M.L. and A.M. Vieitez. 1981. Injerto en hipocotilo de plantulas de castagno. Anales de Edafologia y Agrobiologia. Tomo XL. 3–4:647–655.

Vieitez, M.L. and A.M. Vieitez, 1982. Observaciones sobre el injerto juvenil del castano. Anales de Edafologia y Agrobiologia 41(9–10):1999–2002.