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

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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 formaldehyde–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 at the graft union. Some necrotic layers were observed at the graft union. In all methods, cambial connections formed within 45 to 60 d after grafting, perfect cambial connections in all methods. In sections made 12 months 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 weighing 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 hypocotyl as in a normal cleft graft (Vieitez 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 the 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 ± 5.5 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%, glacial acetic acid: 5%, ethanol (96%): 50%, distilled 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 began 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.
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 Soyulu, 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.

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