# Research on shortening the nursery period in grafted chestnut

**E. Duman, U. Serdar**

*Department of Horticulture, Faculty of Agriculture, University of Ondokuz Mayis, Samsun, Turkey*

**ABSTRACT**: The objective of this study was to determine the effects of inverted radicle and hypocotyl grafting methods on potted nursery tree production in chestnut. The study was carried out in greenhouse conditions in 2003–2004. Newly germinated chestnut seeds and young seedlings of SA 5-1 genotype were used as rootstocks and sprouts with dormant buds of the same genotype were used as scions. Graftings were done at three periods from April to June. Graft success and scion shoot growth were examined to determine a suitable method and time of grafting. It was found in the study that potted chestnut nursery trees could be produced in one year using either of the grafting methods. Better graft success and survival ratio were obtained from inverted radicle grafting. In this method, graft success was 83.6% in 2003 and 96.7% in 2004.

**Keywords**: chestnut; *Castanea sativa*; inverted radicle grafting; hypocotyl grafting; nursery period; graft success; juvenile grafts

The vegetative propagation of chestnut can be accomplished by grafting, rooting of cuttings, layering and micropropagation. However, grafting has proved to be a more successful and feasible method for asexual propagation (Huang et al. 1994). In this type of vegetative propagation, different budding and grafting methods are used. In order to perform these methods, one- or two-year-old rootstocks are needed. But it increases the nursery period and production costs.

Different types of grafting have been tested on chestnut. Some of these are done on seeds at the more or less advanced stage of germination. The nursery seed grafting technique was described for the first time by Moore (1964) and newly taken into consideration with some changes by other authors (Jaynes 1965; Ackerman, Jayne 1980; Wang, Qian 1998; Greenwell 2002). The hypocotyl and inverted radicle grafting methods, modifications of the nursery seed grafting, were described by Park (1968a,b). These methods had more graft success than the previous one (Park 1968a,b; Vietiez, Vietiez 1981, 1982).

Juvenile graft methods offer several advantages over conventional field grafting. Some of them are: no stock trees are necessary, grafting can be done indoors, timing is not critical, scions having small diameters can be used (McKay, Jaynes 1969; Keys 1978).

Turkey is one of the important countries in chestnut production in the world. In Turkey chestnuts have usually been collected from naturally occurring seedling-origin trees scattered in forest areas, especially in the Black Sea, Aegean and Marmara Regions. Therefore no chestnut orchards have commonly been established yet. Chestnut orchards have to be established using previously selected cultivars/genotypes from the regions by Ayfer and Soylu (1995), Ozkarakas and Onal (1995), Serdar (1999) and Serdar and Soylu (1999) to increase fruit quality and yield. Hence, it is needed to produce a great number of grafted chestnut trees. Some researches carried out studies on propagation of chestnut by grafting in Turkey to produce grafted chestnut trees (Soylu 1982; Ozkarakas, Onal 1997; Serdar 2000; Tekintas et al. 2003). However, there was no attempt to test the inverted radicle and hypocotyl grafting methods on chestnut. This study aimed to determine the suitable juvenile grafting method and time to produce grafted chestnut trees.

## MATERIALS AND METHODS

The study was carried out in a greenhouse in Samsun province in 2003–2004. Newly germinated chestnut seeds and young seedlings of SA 5-1, selected by Serdar (1999) and Soylu and Serdar (2000),

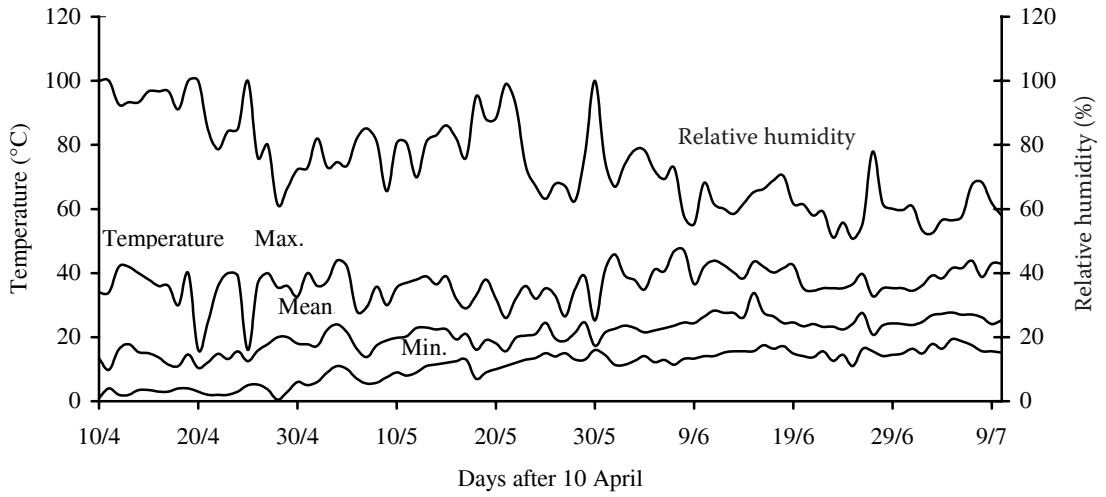


Fig. 1. Air temperature (°C) and relative humidity (%) in the greenhouse from the first grafting period in 2003 (10 April)

were used as rootstocks and sprouts with dormant buds of SA 5-1 were used as scion materials.

Two grafting methods were applied, namely, inverted radicle grafting and hypocotyl grafting. Graftings were done at three periods in 2003, namely 1st period on April 10–11, 2nd period on May 7–9 and 3rd period on June 6–9 and one period on April 29 in 2004. Temperature (°C) and relative humidity (%) were recorded during the experiment (Fig. 1).

Chestnut seeds with 11–13 g were taken from an orchard in Sinop province in October in 2002 to 2003, stored in perforated plastic bags (0.02 mm) at 0 ± 0.5°C and stratified at 4 ± 0.5°C for 2.5 months. Scions were taken from an orchard in Samsun province in February in 2003 to 2004 and stored at 0 ± 0.5°C until the grafting procedure.

In the inverted radicle grafting method, germinated seeds with radicles 6.6 ± 0.8 cm long and 4.9 ± 0.7 mm in diameter were used. The radicle tip was cut off at 4–5 cm and split. The scion was prepared with a wedge-shaped cut. Prepared scions were approximately 7 to 11 cm long and 4–6 mm in diameter. Then the scion and radicle were grafted as a normal cleft graft (Park 1972). After grafting, the graft union was wrapped tightly with a strip of parafilm 3–4 mm wide (Fig. 2).

In the hypocotyl grafting method, germinated seeds were sown in a medium of 3/4 soil + 1/4 ground pine needles in the first period in 2003 and

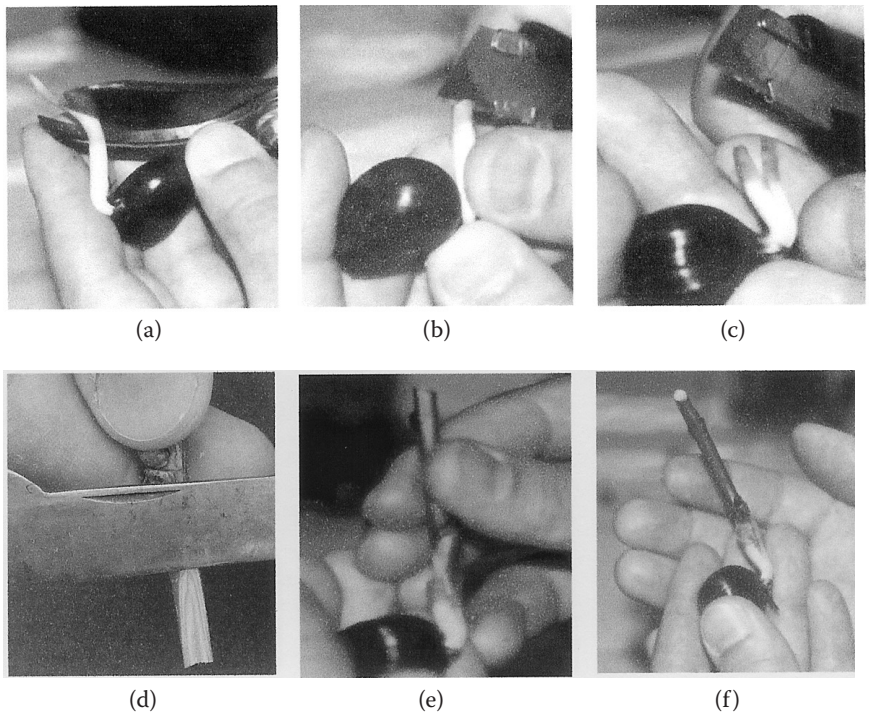


Fig. 2. The inverted radicle grafting method: (a) cutting of radicle tip, (b) splitting of radicle, (c) the radicle prepared as stock, (d) preparing of scion with a wedge-shaped cut, (e) grafting of scion on radicle, (f) grafted seed tied with parafilm

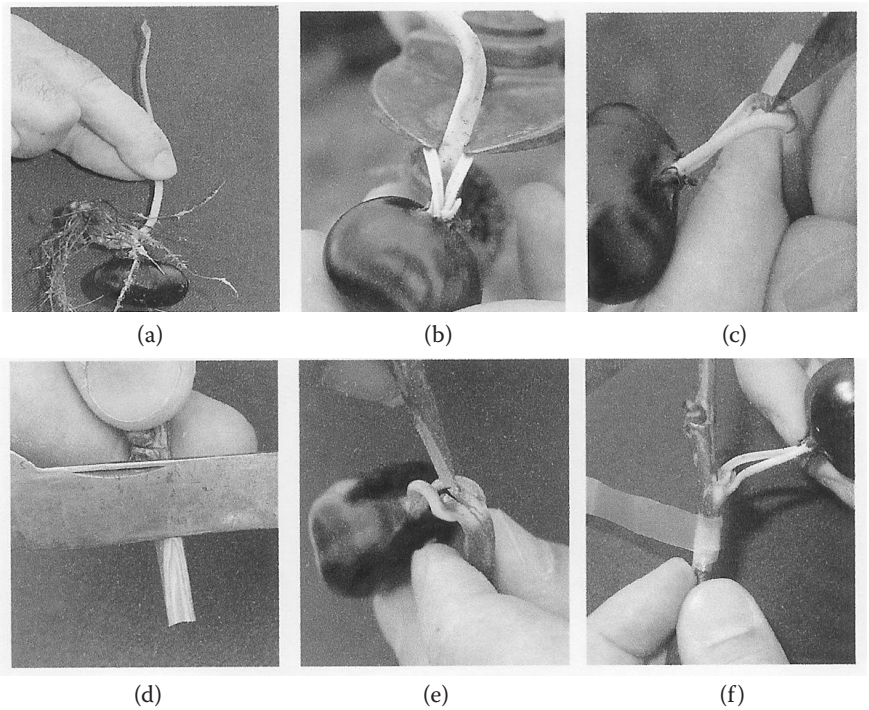


Fig. 3. The hypocotyl grafting method: (a) young seedling as stock, (b) cutting of epicotyl at level of cotyledon stalks, (c) splitting of hypocotyl, (d) preparing of scion with a wedge-shaped cut, (e) grafting of scion on hypocotyl, (f) tying the graft union with parafilm

in peat medium for the other periods, 16–20 days before grafting. Young seedlings having 2–4 leaves and epicotyls 7.5 ± 1.5 cm long were used for grafting. The epicotyl was cut off at the level of the cotyledon stalks and the hypocotyl was split. The scion was prepared with a wedge-shaped cut as described previously. Then the scion was grafted on the hypocotyl as a normal cleft graft (Vietiez, Vietiez 1982). After grafting, the graft union was wrapped tightly with a strip of parafilm 4–6 mm wide (Fig. 3).

In both methods, grafted seed or young seedlings were planted immediately after grafting in a medium of 3/4 soil + 1/4 ground pine needles in 30 × 40 cm pots. Some peat was added into the pots at the time of planting. Then grafting wax was applied to the tips of the scions.

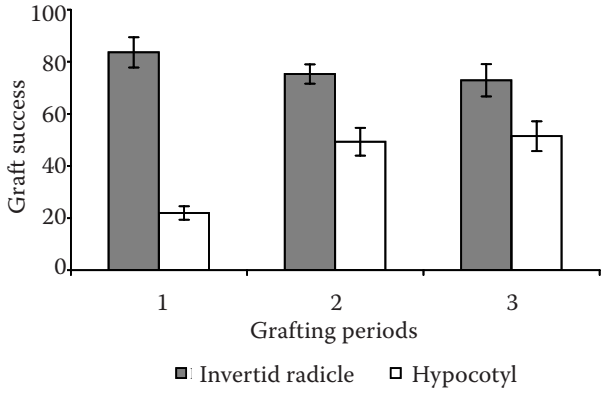


Fig. 4. Graft success (%) for different grafting methods and periods in 2003

To determine the suitable grafting method and time, graft success (sprouting) and survival ratios (%), scion shoot length (cm) and diameter (mm) were examined at the end of the vegetation period. The period from grafting to sprouting and scion shoot growth from one month after grafting to the end of the vegetation period were also determined in 2003.

The experimental design was a randomized complete block. Three replications and 20 grafts per replication were used. Vertical bars in the figures represent one standard error of each mean ($p < 0.05$).

## RESULTS AND DISCUSSION

### Graft success

The highest graft success (83.6%) was achieved with inverted radicle grafting in the first period in 2003 (Fig. 4). However, there were no significant differences between the grafting periods in inverted radicle grafting.

In the hypocotyl grafting, graft success was lower (21.9%) in the first period since germinated seeds were sown in a medium of soil + ground pine needles, contrary to the other periods. Thus it was very difficult to take out the young seedlings from this medium. Graft success for this method was 49% and 51% in the second and third period, respectively. Park (1968a) reported 76–100% and Vietiez and Vietiez (1981, 1982) reported 50–100% graft suc-

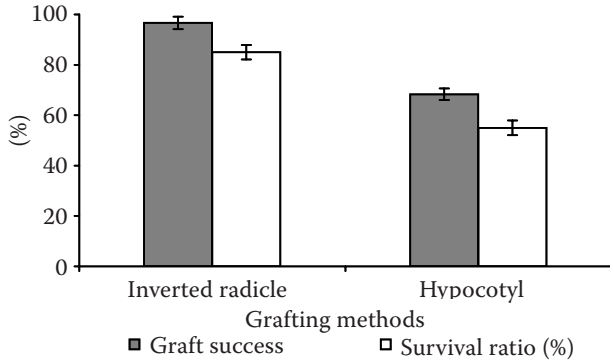


Fig. 5. Graft success and survival ratios (%) for different grafting methods in 2004

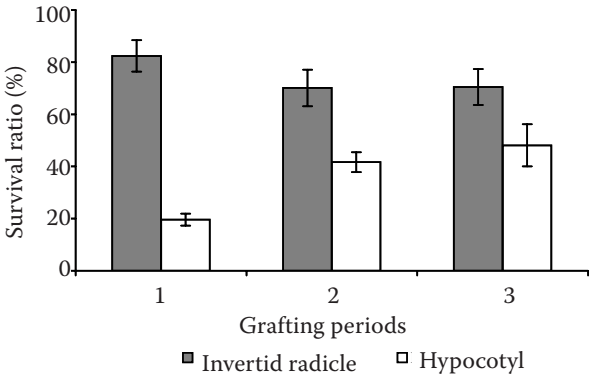


Fig. 6. Survival ratios (%) for different grafting methods and periods in 2003

cess depending on the combination of the scion and rootstock with hypocotyl grafting.

Because of insignificant differences between the grafting periods in the inverted radicle grafting and also grafting methods except the first period in 2003, all graftings were performed at the same time (April 29) in 2004. The graft success was higher for both methods in 2004 than 2003. The highest graft success (96.7%) was from the inverted radicle grafting as in 2003 (Fig. 5). This is essentially identical to the results of Park (1968b), who achieved 100% graft success in the greenhouse and 55% in the open field conditions using the inverted radicle grafting.

### Survival ratio (%)

Some scion shoots died after breaking at the graft union. In these grafts, a smaller graft union area was observed. Higher survival ratios were obtained from inverted radicle grafting in both years (70.1–82.4% in 2003 and 85.0% in 2004) (Figs. 6 and 5). However, there were no significant differences in survival between the grafting periods in the inverted radicle grafting in 2003 (Fig. 6).

### Scion shoot growth

The time required for scions to sprout varied from 12 to 23 days (Fig. 7). Mean temperatures for the 20 days after grafting for each grafting period were 14.7, 19.7 and 25.5°C, respectively (Fig. 1). In all graftings, the sprouting period was shortened with later grafting times because the rate of callus formation in the graft union probably increases with temperature. There was a negative relationship between temperature and sprouting period in the inverted radicle grafting ($r = -0.946^{**}$). This result is in accordance with the findings of Serdar (2000). The hypocotyl grafts sprouted more quickly than the inverted radicle grafts. It may be assumed that this case is a result of age and growth stage of rootstocks at the time of grafting.

The growth curve of the scion shoot is presented in Fig. 8. The scion shoot length was 5–7 cm in the 1st period, 9–14 cm in the 2nd period and 13–17 cm in 3rd period in one month after grafting. In July, scion shoot growth was slow in grafts performed in the first and second period. However, there was a rapid increase in scion shoot growth in the grafts

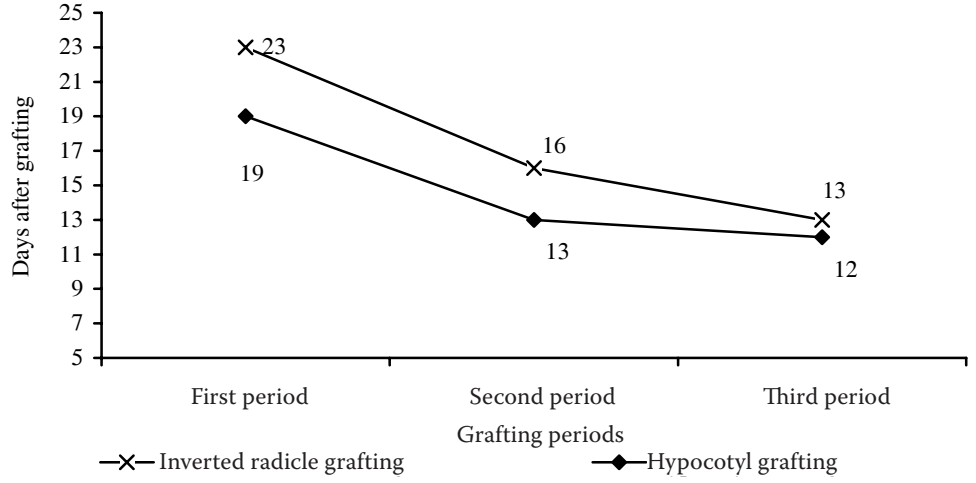


Fig. 7. Sprouting period of grafts for different grafting periods and methods in 2003

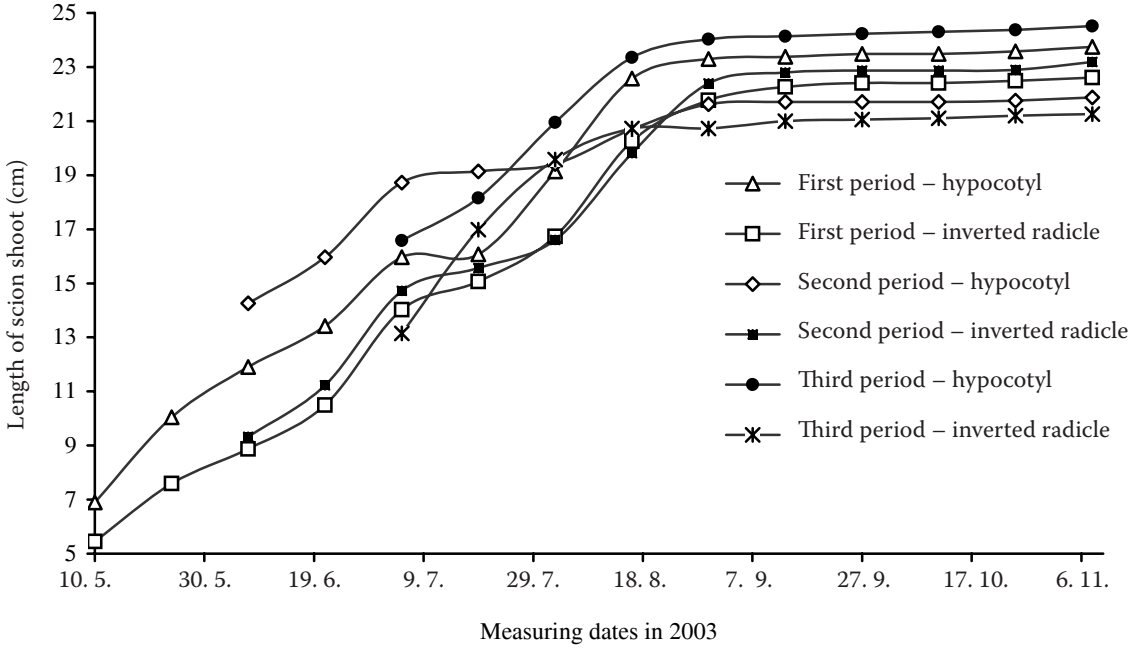


Fig. 8. Growth of scion shoot from one month after grafting to the end of vegetation period for different grafting periods and methods in 2003

performed in the third period. The hypocotyl grafts had longer scion shoots. Scion shoot growth was completed in early September. Although the shoot growth rate varied according to the grafting periods, lengths of scion shoots were generally similar at the end of the vegetation period.

At the end of the vegetation period in 2003, the growth of scion shoots was faster and their length and diameter were larger in the hypocotyl grafts (Figs. 9 and 10). In this method, the respective lengths of scion shoots from period 1, 2 and 3 were 24.2, 21.0 and 22.9 cm, and shoot diameters were 5.0, 5.1 and 4.6 mm, respectively. However, in both methods there were no significant differences between the grafting periods. In 2004, scion shoot growth was better than it was in 2003 for both methods (Fig. 11). However, differences between the grafting methods in the length and diameter of scion shoots were not significant.

In the present study, scion shoot growth was found to be faster than reported in previous studies performed with chip budding on the epicotyl (Soylu 1982), T and inverted T budding methods on one-year-old rootstocks in the pots (Serdar 2000).

## CONCLUSIONS

The inverted radicle grafting was found to be more suitable method to produce grafted chestnut tree in a one year. This method can be performed for different periods in spring, adjusting the stratification times. To obtain successful results from this method, the graft

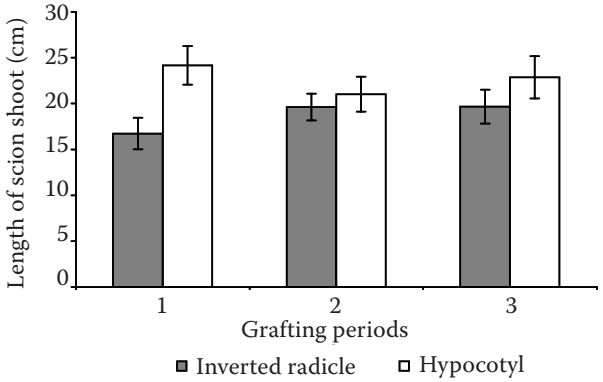


Fig. 9. Length (cm) of scion shoot for different grafting methods and periods in 2003

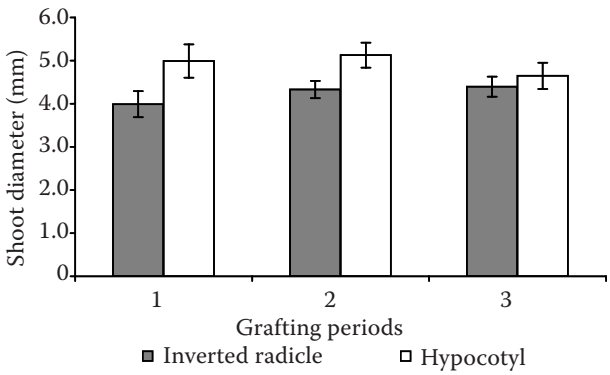


Fig. 10. Diameter (mm) of scion shoot for different grafting methods and periods in 2003

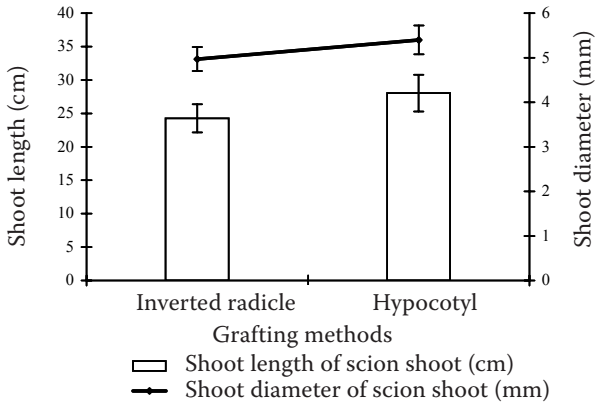


Fig. 11. Length (cm) and diameter (mm) of scion shoot for different grafting methods in 2004

union should have a long contact zone and must be wrapped tightly with parafilm when the grafted seed/seedlings are planted in the pots, some peat should be added in the graft union area. These methods should be studied in terms of effects of seed size, stock-scion combinations and grafting conditions characterized by different temperature and light intensity.

## References

ACKERMAN W.L., JAYNE H.T., 1980. Budding of epicotyls of sprouted chestnut seeds. HortScience, *15*: 186–187.

AYFER M., SOYLU A., 1995. Selection of chestnut cultivars (*Castanea sativa* Mill.) in Marmara Region of Turkey. In: Proceedings of the International Congress on Chestnut. October 20–23, 1993, Spoleto, Italy: 285–289.

GREENWELL E., 2002. Nutgrafting for American Chestnut Restoration. http://www.accf-online.org/chestnut/nutgrafting.htm.

HUANG H., NORTON J.D., BOYHAN G.E, ABRAHAMS B.R., 1994. Graft compatibility among chestnut (*Castanea*) species. Journal of the American Society for Horticultural Science, *119*: 1127–1132.

JAYNES R.A., 1965. Nurse seed grafts of chestnut species and hybrids. Proceedings of the American Society for Horticultural Science, *86*: 178–182.

KEYS R.N., 1978. Prospects of vegetative propagation in the genus *Castanea*. In: MAcDONALD W.L., CECH F.C., LUCHOK H., SMITH C. (eds.), Proceedings of the American Chestnut Symposium, January 4–5, West Virginia: 10–16.

McKAY J.W., JAYNES R.A., 1969. Chestnuts. In: JAYNES R.A. (ed.), Handbook of North American Nut Trees. The Northern Nut Growers Association, Knoxville: 281–285.

MOORE J.C., 1964. Propagation of chestnut and *Camellia* by nurse seed grafts. In: Proceedings of International Plant Propagation Social**,** *14*: 141–143.

OZKARAKAS I., GONULSEN N., ULUBELDE M., OZAKMAN S., ONAL K., 1995. Selection studies of chestnut cultivars (*Castanea sativa* Mill.) in Aegean Region. In: Turkey 2nd National Horticulture Congress, 3–6 October, Adana, *1*: 505–509 (in Turkish with English summary).

OZKARAKAS I., ONAL M.K., 1997. Researches on the determination of the most suitable budding methods and times in chestnut (*Castanea sativa* Mill.) propagation. Anadolu, *7*: 74–79.

PARK K.S., 1968a. 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). Research Report Institute of Forestry Genetic, Korea, *6*: 89–104.

PARK K.S., 1968b. Studies on the juvenile tissue grafting of some special use trees II. An experiment on inverted radicle grafting of crop tree species. Reports of Korean Journal of Botany, *11*: 8–17.

PARK K.S., 1972. Histological study of the polaritically oriented differentiation in the inverted radicle grafting of chestnut. Research Report Institute of Forestry Genetic, Korea, *9*: 39–49.

SERDAR U., 1999. Selection of chestnut (*C. sativa* Mill.) in Sinop vicinity. In: Proceedings of the Second International Symposium on Chestnut. Acta Horticulturae, *494*: 327–332.

SERDAR U., 2000. The Effect of Different Grafting Times and Methods on Grafted Nursery Tree Production in Chestnut. [Ph.D. Thesis.] Ondokuz Mayıs University, Institute of Science, Samsun, Turkey: 137.

SERDAR U., SOYLU A., 1999. Selection of chestnut (*C. sativa* Mill.) in Samsun vicinity. In: Proceedings of the Second International Symposium on Chestnut. Acta Horticulturae, *494*: 333–338.

SOYLU A., 1982. A study on budding of chestnut. Bahçe, *11*: 5–16.

SOYLU A., SERDAR U., 2000. Rootstock selection on chestnut (*Castanea sativa* Mill.) in the Middle of Black Sea Region in Turkey. In: Proceedings EUCARPIA Symposium on Fruit Breeding and Genetics. Acta Horticulturae, *538*: 483–488.

TEKINTAS F.E., SEFEROGLU G., ERTAN E., GUNVER G., 2003. Investigations on the seedling propagation in the controlled conditions on walnut and chestnut. http://www.agr.ege.edu.tr/~fitekno/Doc52.htm.

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

VIEITEZ M.L., VIEITEZ A.M., 1982. Observaciones sobre el injerto juvenil del castano. Anales de Edafologia y Agrobiologia, *41*: 1999–2002.

WANG B., QIAN Y., 1998. Study on germinated seed grafting in *Castanea mollissima*. Horticultural Abstract, *68*: 10249.

Received for publication April 19, 2005
Accepted after corrections May 6, 2005

# Výzkum zkracování školkařského cyklu u štěpovaného kaštanovníku

**ABSTRAKT**: Cílem práce bylo studium použitelnosti metod invertního štěpování kořínku a hypokotylu pro produkci kontejnerovaných školkařských výpěstků kaštanovníku. Výzkum probíhal ve skleníku v letech 2003 a 2004. Vyklíčená semena a mladé semenáčky genotypu SA 5-1 byly použity jako podnože a výhonky se spícími očky stejného genotypu byly použity jako rouby. Roubování se provádělo ve třech termínech v období od dubna do června. S cílem volby nejvhodnějšího postupu a doby roubování byl hodnocen újem a růst roubů. Bylo zjištěno, že prodejné kontejnerované školkařské výpěstky kaštanovníku lze během jednoho roku dopěstovat oběma metodami štěpování. Lepšího újmu a růstu výpěstků však bylo dosaženo použitím invertního štěpování kořínku. Při použití této metody bylo v r. 2003 docíleno výtěžnosti 83,6 % a v r. 2004 dokonce 96,7 %.

**Klíčová slova**: kaštanovník; *Castanea sativa*; invertní štěpování kořínku; štěpování hypokotylu; školkařský cyklus; výtěžnost; juvenilní rouby

*Corresponding author:*

Dr. Ümit Serdar, University of Ondokuz Mayıs, Faculty of Agriculture, Department of Horticulture,
55139 Kurupelit-Samsun, Turkey
tel.: + 90 362 457 6020, fax: + 90 362 457 6034, e-mail: userdar@omu.edu.tr