

## DETERMINATION OF GRAFT SUCCESS OF GRAPE CULTIVARS GRAFTED ON TWO DIFFERENT ROOSTOCKS

Oguz Dolgun<sup>1</sup>, Simin Saygac Ulas<sup>2</sup>, Turcan Teker<sup>2</sup>

<sup>1</sup> Adnan Menderes University, Aydin, Turkey

<sup>2</sup> Viticultural Research Station, Manisa, Turkey

**Abstract.** The aim of this study was to determine the graft performance and to follow the graft compatibility of Bornova Misketi, Trakya Ilkeren and Sultan 1 grape cultivars grafted on 5BB and 1103P rootstocks. Grafts were made by using omega technique. Following waxing, grafts were packed in plastic boxes filled up with fungicide humidified sawdust and chips (3:1) and were placed in callusing room (25°C, 85% relative humidity) for three weeks. Post callusing, grafts were evaluated for their callusing rate at graft union (0–4 scale), sprouting rate (%), rooting rate (%) and sapling yield (%). According to obtained data statistically significant differences were determined in all parameters except sprouting rate. Graft samples were taken each month for histological analysis during six months following callusing. No significant differences were observed among combinations in respect to histological development of graft unions. Callusing rate (3.73/5 BB–3.70/1103 P) and potted sapling yield (86.66/5 BB%–90.00/1103 P%) were the highest in Bornova Misketi among all combinations for two rootstocks.

**Key words:** grafting, omega technique, histology, graft compatibility

### INTRODUCTION

Turkey has optimum climate conditions for viticulture and also has an important position in the world in respect to raisin and table grape production [Miran 2005]. As many other grape producing countries, vineyards are established with grafted vines on phylloxera resistant rootstocks [Troncoso et al. 1999, Gambetta et al. 2009]. However, to choose the right rootstock it is also important to take into account the climate and soil

---

Corresponding author: Oguz Dolgun, Adnan Menderes University, Sultanhisar Vocational College, Department of Plant Production, Aydin, Turkey, e-mail: odolgun@gmail.com

© Copyright by Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie, Lublin 2016

conditions of the region and also rootstock-cultivar affinity [Bahar et al. 2007, Gargin and Altindisli 2014]. Well-adapted rootstocks positively affect the vigor, yield, quality, maturation time and resistance of the cultivars [Celik 2007, Gambetta et al. 2009] After grafting it is necessary to obtain a good compatibility between stock and scion tissues as they behave like a unique plant [Coombe and Dry 1992, Patil et al. 2005, Pellegrino et al. 2005, Basheer-Salima and Hamdan 2009] Omega technique is commonly used for grafting in viticulture. Bench graft method is faster and makes it possible to have a number of grafts in a shorter time [Korkutal et al. 2011]. By post grafting observations of the graft union, sapling yield may be estimated [Petkou et al. 2004, Gambetta et al. 2009, Gargin and Altindisli 2014].

In this study, histological progress of graft compatibility, callusing levels, rooting ratios, sprouting ratios and potted sapling yield were evaluated in Bornova Misketi, Trakya Ilkeren and Sultan 1 grape cultivars grafted on 5BB and 1103P rootstocks.

## MATERIAL AND METHODS

This research was conducted at Manisa Viticulture Research Institution Nursery (38°37'57,68"N, 27°24'00,76"E) in Turkey in the year 2014. 5BB and 1103P rootstocks were used. 5 BB (*Vitis riparia* Mich × *V. berlandieri* Planch) is suited for the wet and clay soils, has a good tolerance up to 20% active lime and nematodes but sensitive to salt. Rooting ability is quite high. 1103P (*Vitis berlandieri* Planch × *V. rupestris* sch.) depends on the region, has a good vigor on clay and limy soils. It has a tolerance to 7–18% active lime and 0.6 NaCl kg<sup>-1</sup>. It is generally suggested for dry soils. Graft affinity and rooting ability of this rootstock is quite high.

Grape cultivars of the study were Bornova Misketi, Trakya Ilkeren and Sultan 1. Bornova Misketi is a wine grape cultivar producing in Izmir province. Berries are round, green-yellow color, matures early-mid season, very sensitive to powdery mildew and prunes short-mix. It has a special Muscat aroma and one of the very important white wine cultivars of Turkey. It is used for producing dry, semi-dry and sweet wines (fig. 1).

Trakya Ilkeren is a seeded grape cultivar producing in Mediterranean, Aegean and middle and south Anatolia. Berries are round, blue-black color. Very early maturation time (end of June) is the characteristic of this cultivar. Although early maturation berries can stay on vine without any disorder (fig. 2).

Sultan 1 is a seedless cultivar producing for table and raisin grape in Aegean region. Berries are elliptic, green-yellow color. It matures second week (for table) and third week (for raisin) of August (fig. 3).

Research was designed as random plots with 3 repetitions and 20 grafts in each repetition. Scions taken from the cold room were dipped in a fungicide solution (200 g × 100 L mancozeb) for one day. To observe histological process, additional 60 grafts were also made. Scions height were 35–40 cm and 0.8–1 cm diameter. Before grafting all buds but the bottom ones were dampen by graft knives. Omega technique was used [Becker 1989, Korkutal et al. 2011]. Scion and stocks were chosen as the same diameter as much as possible. Grafts were coated with paraffin (55–65°C melting temperature) up to 2–3 cm under graft union and packed in plastic boxes filled up with sawdust and coarse chip

(3:1) right after grafting. Boxes were then placed in callusing room (25°C, 85% relative humidity) for three weeks and then transferred under net for acclimatization for one day.

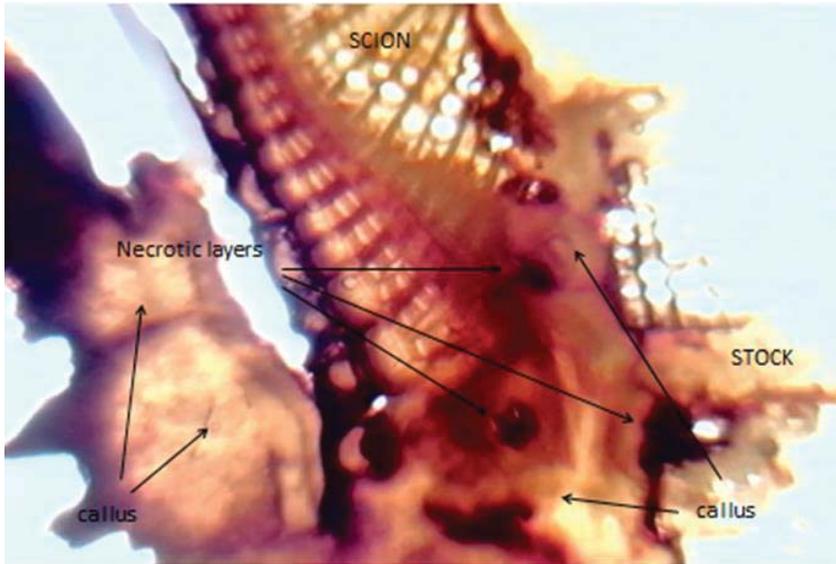


Fig. 1. The appearance of the tissues in sample Trakya Ilkeren/5BB taken from the callusing room,  $\times 60$  magnification

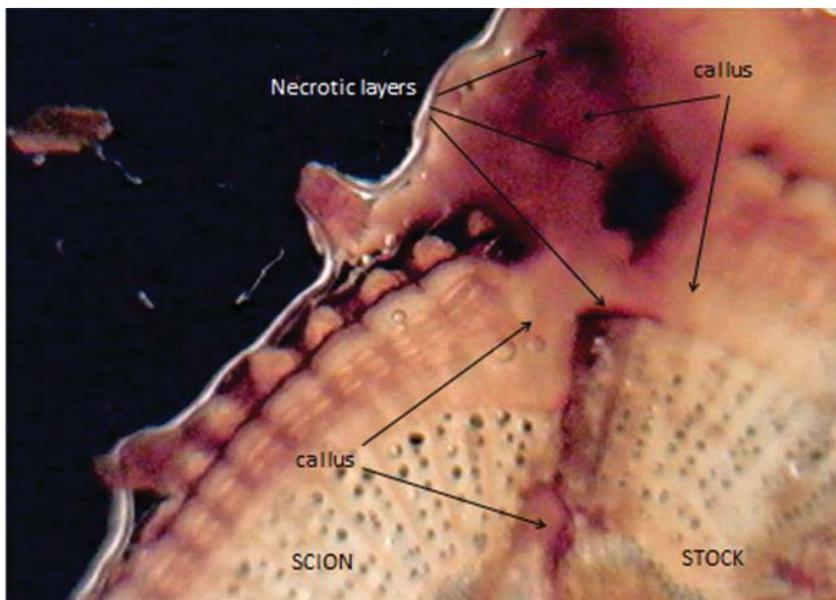


Fig. 2. The appearance of the tissues in sample taken one month after the Sultani 1/5BB combination transferred to bag,  $\times 50$  magnification

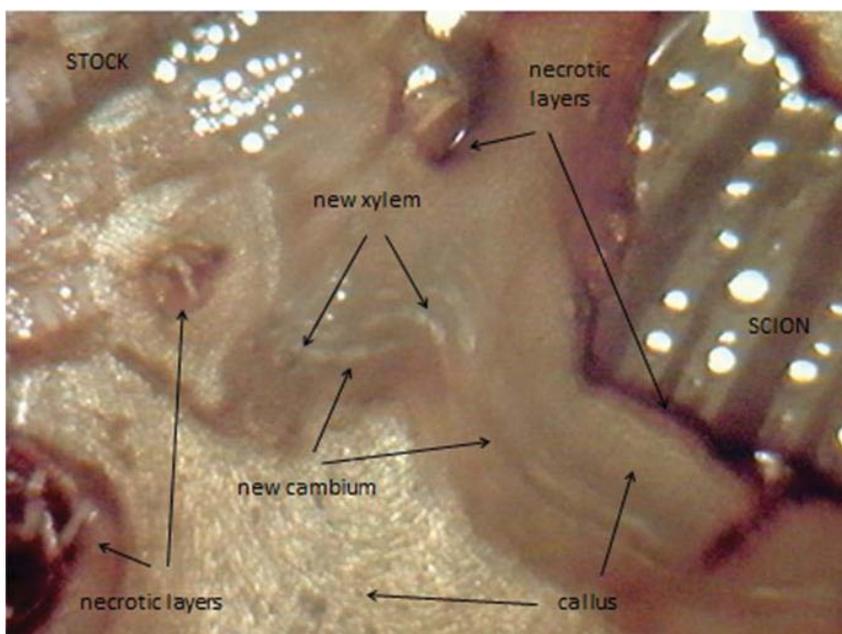


Fig. 3. The appearance of tissues in the sample Bornova Misketi/1103P taken 150 days after grafting,  $\times 80$  magnification

Callusing ratios in graft union were recorded according to 0–4 scale (4 – round and uninterrupted callus development, 3 – partially interrupted callus development, 2 – crescent shaped callus development, 1 – one sided and weak callus development, 0 – no callus development). Rooting and sprouting rates were recorded as to %. Before second paraffin application, grafts were dipped in a 2000 ppm IBA solution for 10 seconds. for better rooting. Paraffin (75–80°C) application was made on graft union. Grafts were planted in polyethylene bags filled with fine compost and soil and placed in a controlled greenhouse for 6 weeks and then transferred under shade for 2 weeks. Sapling yield was determined as to %. All data were analyzed using to SAS statistical program and Duncan multiple comparison test were used ( $P < 0.05$ ).

Graft samples were taken from each combination and stored in 70% ethanol for histological analyses until taken last samples at the end of fieldwork. Graft samples were cut in 0.2–0.5 mm thickness by using steel knife microtome and stored in 70% ethanol. Intact sections were not stained due to soft and perishable structures. Sections which can be examined were observed in different magnification levels until see tissues clearly by using digital microscope (Olympus Mic D, Tokyo, Japan).

## RESULTS AND DISCUSSION

**Histological development of graft union.** One month old grafts were not strong enough to cut and the sections were not intact. Microscopic observations of these bro-

ken samples revealed that parancimatic callus started to develop in the gaps between stock and scion. Dense necrotic layers were also observed on the cutting surfaces of both stock and scion (fig. 1). Many researchers who studied on graft compatibility have reported that, graft union between two plants was produced by phloem or close places to phloems of the stock and scion right after grafting [Moore and Walker 1981, Moore 1984] Thus, water and food transfer between graft members was provided in cellular level. In the following period, callus mass was increased and necrotic cells were broken and absorbed [Dolgun and Tekintas 1998, Yildirim et al. 2010]. All other periodic samples have revealed that the gaps between stock and scion were filled with a callus mass and there were a blackout on the outer surface of callus that contacted to the air and a thin layer of callus tissue has wrapped the outer surface. Irregular xylem masses were also observed in callus tissue (fig. 2). Researchers whom studied on graft compatibility between different vine rootstocks and cultivars reported that after grafting, callus had continued development thus the gaps between stock and scion were filled and necrotic layers were broken and disappeared [Balta et al. 1996, Cangı et al. 1999, Dolgun et al. 2008]. According to Cangı et al. [2000] in the situations where necrotic layers have not broken, cambial differentiation has took a long time, sapling vigor and yield have reduced.

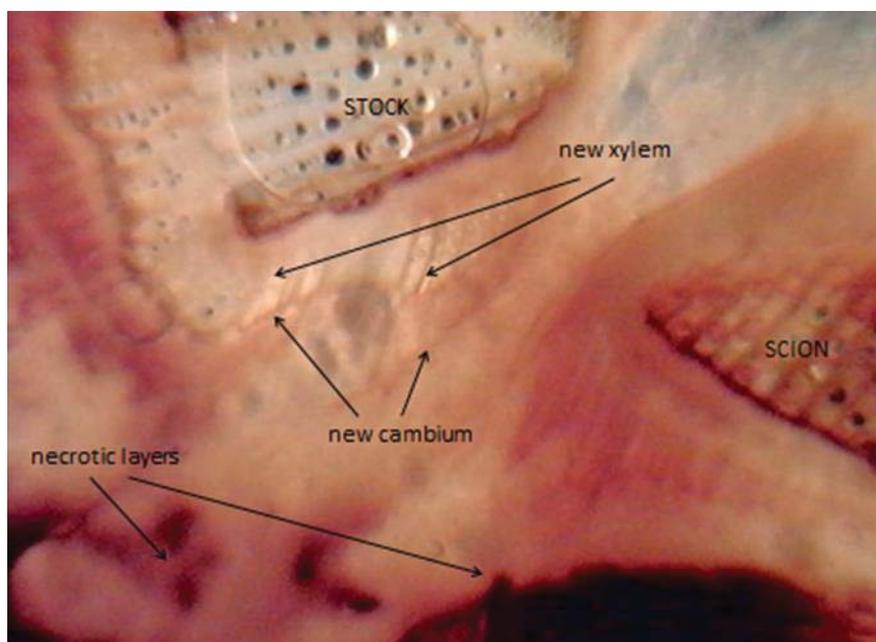


Fig. 4. The appearance of the tissues in sample Sultan1/1103P taken 180 days after grafting, ×60 magnification

The best graft sections were cut after 150 days from grafting. In transverse cuts of the samples revealed that the gaps between stock and scion were totally filled by callus formed near cambium tissues of stock and scion by the way spread into the gaps as growing clusters. Necrotic layers on cutting surfaces were enclosed by callus tissue by they still remained. There were partial necrotic layers in callus tissue and part of it was broken and absorbed as small pale colored necrotic masses while other part of it remained as very dark colored tissue (fig. 3). It was observed that vascular tissues between stock and scion had developed in 180<sup>th</sup> day sections of all combinations. Xylem cells were very distinctive as large and empty cells. On the other hand phloem tissues were formed by small and dense cells. Cambial continuity between stock and scion was clearly observed. Cambial tissue showed a wavy (alternating) development. Because of the necrotic layers in callus tissue, cambial tissue showed different levels of wavy development depending on combination but it was still observed that successful cambial continuity is formed. In grapes, as other fruit species, a strong contact between stock and scion is formed in further periods of grafting [Schoning and Kollmann 1997, Todici et al. 2005]. Initially unclear cambial continuity is become clearly observed in further periods of grafting and irregular mass of xylem cells formed a regular and strong xylem tissue [Cangi et al. 2000, Polat et al. 2010].

In Sultan 1/1103P combination graft union formation was more difficult than the other combinations because of less callus formation and dense necrotic layers caused more wavy cambial contact and relatively weak vascular tissues (fig. 4).

In the combinations where not formed enough callus tissue and have more necrotic layers causing late and wavy cambial continuity and weak vascular tissue and resulting low sapling yield [Balta et al. 1996, Hartmann et al. 1997, Cangi et al. 2000, Dolgun et al. 2008, Polat et al. 2010].

**Callusing Level.** After grafting it is expected to form enough callus between stock and scion. Thus the first contact between stock and scion is provided. Callus is a tissue produced to cover wound surfaces of the plant and formed by parancimatic cells differentiating from phloem zone near cambium. In further periods there are cellular differentiations in callus, some cells transform to produce cambium and cambium produces xylem and phloem. Thus vascular tissue between stock and scion is formed and water and food transfer is provided [Moore and Walker 1981, Moore 1984, Wang and Kollmann 1996, Hartmann et al. 1997, Cakir 2013]. In grafted vine production, uninterrupted and strong callus growth is aimed [Celik 2007, Gambetta et al. 2009]. According to Cangi [2000] graft union rate higher than 3.5 is necessary to have a successful graft. Kamiloğlu and Tangolar [1995] reported that the rates between 3–4 are sufficient.

In our study the highest results in respect to callusing level were obtained from Bornova Misketi/5BB (3.73) and Bornova Misketi/1103P (3.70) combinations. Contrarily, Sultan1/1103 combination has the lowest rate (2.96) (fig. 5). Sivritepe and Turkben [2001] reported that in their study where they search the sapling yields of Muskule grape variety grafted on different rootstock (Salt Creek, 1616 C, 1613 C, 5BB, 41B), 5BB had the lowest union ratio (2.88). But, in our study 5BB gave the higher results in all combinations, the lowest one (3.30) is Trakya Ilkeren in respect to union ratio. This result in agreement with Cakir et al. [2013]. On the other hand 1103P showed a good callusing level more than 3 with all cultivars except Sultan 1 (2.96). These results sup-

ported by the other studies revealing that different stock-scion combinations have different union ratios depending on the cultivars and rootstocks [Kamiloğlu and Tangolar 1995, Cangi et al. 2000, Sivritepe and Turkben 2001].

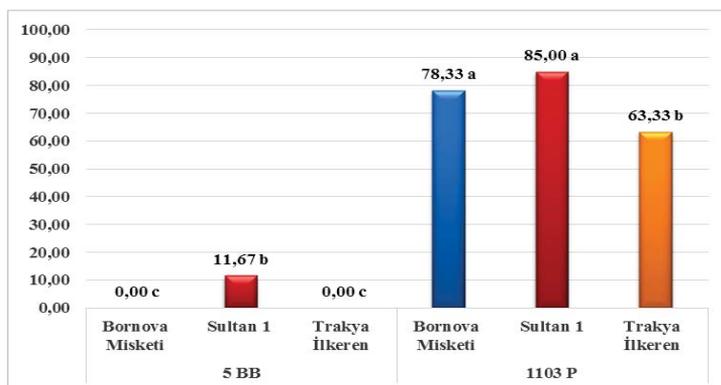


Fig. 5. Callusing levels of cultivar/rootstock combinations in callusing room, ( $p < 0.05$ )

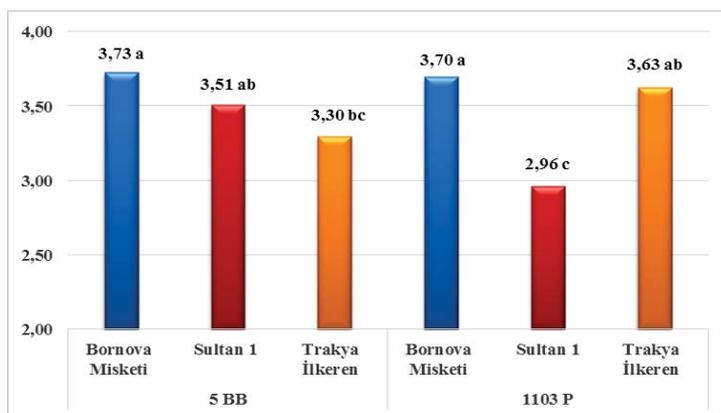


Fig. 6. Rooting ratio of cultivar/rootstock combinations in callusing room (%), ( $p < 0.05$ )

**Rooting ratio.** In callusing room, adventive roots could form together with callus. In our study there were statistically significant differences in rooting ratios of the combinations. There was no rooting in Bornova Misketi/5BB and Trakya İlkeren/5BB (0.00) combinations where in Sultan1/5BB combination there was very low rooting ratio (11.67). On the other hand in all combinations grafted on 1103P rootstock there was very high levels of rooting ratios (fig. 6). Sivritepe and Turkben [2001] reported that the lowest rooting ratios in 5BB rootstock in their study where they studied the

graft compatibility of Muskule grape variety with different rootstocks. Kamiloğlu and Tangolar [1995] reported that rooting ratios of King's Ruby grape cultivar grafted on 41B was 87% while on 5BB it was 60%. Eris et al. [1989] also reported 90% rooting ratio Hafizali and Hamburg Misketi grape cultivars grafted on 5BB.

**Sprouting ratio.** The buds on scion could sprout in callusing room where there is favorable temperature and moisture. But this is an unwanted situation in this period when there was no strong contact between stock and scion and no water transmission is possible via adventitious roots. Normally, leaves on shoots help root growth by making assimilation and producing food and growth regulators. In grafts however, sprouting is unwanted because of consuming reserve food necessary to produce callus in graft union.

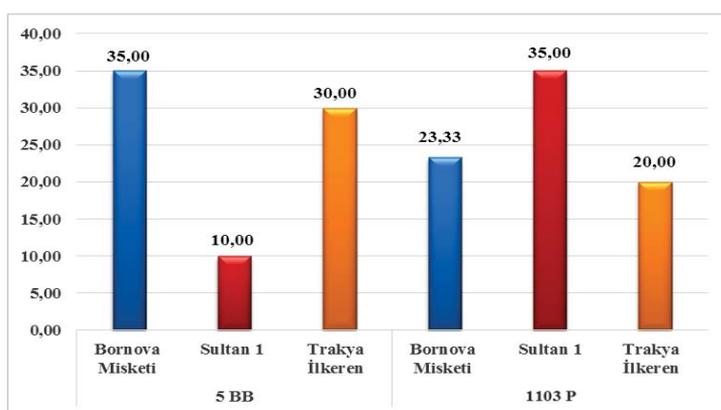


Fig. 7. Sprouting ratios of cultivar/rootstock combinations in callusing room (%), ( $p < 0.05$ )

In our study all combination produce shoot in callusing room. But there were no statistically differences among the combinations. Sultan1 gave 10% sprouting ratio on 5BB rootstock but 30% on 1103P. Still it is remarkable to observe the same cultivar has different sprouting ratios on different rootstocks (fig. 7). Sivritepe and Turkben [2001] found the west sprouting ratio (44.78) in Muskule/5BB rootstock.

**Sapling yield.** For the growers the most important factor in vine sapling production is to have high quality and in large quantities sapling. In our study there were statistical differences in respect to sapling yield. (fig. 8). Bornova Misketi gave the highest yield on both 5BB (86.66) and 1103P (90.00) rootstocks. Sultan1, on contrary gave the lowest results for rootstocks 5BB (66.66) and 1103P (50.00). Cangi et al. [1999] reported the higher sapling yield in 5BB than in 41B, among nine different grape cultivars grafted on 5BB and 41B rootstocks. On the other hand, Sivritepe and Turkben [2001] reported the lowest sapling yield in Muskule grape cultivar grafted on 5BB rootstock among all other rootstocks.

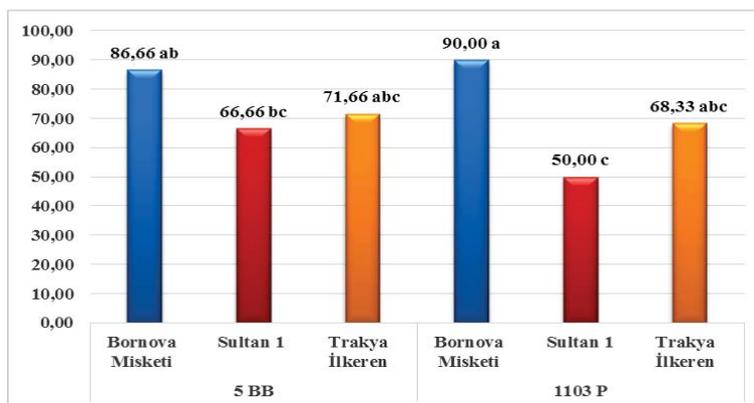


Fig. 8. Sapling yields of different cultivar/rootstock combinations (%), ( $p < 0.05$ )

In our study the highest sapling yield (90.00) was obtained from Bornova Misketi/1103P combination. Bornova Misketi/5BB (80.66), Trakya İlkeren/5BB (71.66), Trakya İlkeren/1103P (68.33), Sultan1/5BB (66.66), Sultan1/1103P (50.00) followed respectively.

## CONCLUSIONS

It is observed that Bornova Misketi and Trakya İlkeren cultivars are quite compatible with 5BB and 1103P rootstocks. In histological observations of these two cultivars, enough callus formation, less necrotic layers, and a wavy cambial continuity of new cambium were determined. Whereas in Sultan1/1103P combination where has the lowest sapling yield, relatively less callus formation, dense necrotic layers caused quite wavy cambial continuity result weak and late formation of vascular tissues.

Evaluating all results, in vine sapling production high amount of callus formation between stock and scion is a priority. It is also necessary to provide a strong graft union in a short time. Our results also revealed that different stock scion combinations gave different levels of callus formation, rooting and sprouting ratios and sapling yield compatible with the previous studies. For newly developed rootstocks and cultivars, histological progress of graft union should observed and sapling yield should estimated thus the most appropriate combinations should choose for the new climate and region.

## REFERENCES

- Bahar, E., Korkutal, I., Dirak, M. (2007). Performances of burst and unburst cuttings and scions during omega grafting. Turkey 5<sup>th</sup> National Horticulture Congress. Vegetable, Viticulture, Ornamental Plants 2, 447–450, 4–7 Sept., Erzurum.

- Balta, F., Cangi, R., Dogan, A., Karadeniz, T., Sen, S.M. (1996). Anatomical and histological investigations on graft union formation of iskenderiye misketi variety grafted on rupestris du lot rootstock. *Yuzuncu Yil University J. Agric. Sci.* 6(2), 201–208.
- Basheer-Salima, R.B., Hamdan, A.J. (2009). Assessment of Preliminary Grafting Compatibility-Incompatibility between Local Palestinian Table-Grapevine Cultivars and Different Phylloxera (*Daktulosphaira Vitifoliae*) Resistant Rootstocks. *An-Najah Univ. J. Res. (N. Sc.)* 23, 49–71.
- Becker, H. (1989). Situation der Deutschen Rebenunterlage. *Der Deutsche Weinbau*, 44, 55–60.
- Cangi, R., Kelen, M., Dogan, A. (1999). Vine sapling production facilities in cold climate conditions. Turkey 3<sup>th</sup> National Horticulture Congress, 430–435, Ankara.
- Cangi, R., Balta, F., Dogan, A. (2000). Anatomical and histological investigations on the effects of stratification substrates on final take and quality of grafted vines, *Turk. Agric. For.*, 24, 393–398.
- Cakir, A., Karaca, N., Sidfar, M., Baral, C., Soylemezoglu, G. (2013). Determination of grafting success of sultani seedless grape variety on different American Rootstocks. *Yuzuncu Yil University J. Agric. Sci.*, 23(3), 229–235.
- Celik, S. (2007). *Bagcilik (Ampeloloji)*, 1, Expanded 2<sup>nd</sup> Press. 428, Trakya Univ., Avci Ofset, Tekirdag.
- Coombe, B.G., Dry, P.R. (1992). *Viticulture*, 2, 376, Practices, Winetitles, Austria.
- Dolgun, O., Tekintas, F.E. (1998). Anatomical and histological investigation on propagation by cuttings and grafting of *Olea europe* L. Aegean Region I. *Agricultural Congress* 48–55.
- Dolgun, O., Tekintas, F.E., Ertan, E. (2008). an histological investigation of graft union in some plum varieties grafted on pixy rootstock. *J. Adnan Menderes Univ. Agric. Fac.*, 5(1), 1–4.
- Eris, A., Soylu, A., Turkben, C. (1989). Effects of some applications on callus formation and rooting at graft place in vine sapling production. *Bahçe*, 18(1–2), 29–34.
- Gambetta, G.A., Rost, T.L., Matthews, M.A. (2009). Passive pathogen movement via open xylem conduits in grapevine graft unions. *Am. J. Enol. Vitic.* 60 (2), 241–245.
- Gargin, S., Altindisli, A. (2014). A research on the affinity coefficients of Red Globe grape variety with 140 R, 41 B rootstocks, *BIO Web of Conferences* 3, 01004.
- Hartmann, H.T., Davies, D.E., Geneve, F.T. (1997). *Plant principles and practices*. 6<sup>th</sup> edn. Prentice Hall, Englewood Cliffs, NJ.
- Kamiloğlu, O., Tangolar, S. (1995). A research on the development of vine sapling production by grafted seedlings. Turkey 2<sup>nd</sup> National Horticulture Congress 2, 447–451.
- Korkutal, I., Kaygusuz, G., Bayram, S. (2011). Different effects of scion types on callusing in bench grafting. *Afr. J. Biotech.*, 10(67), 15123–15129.
- Miran, B. (2005). *Agriculture in Turkey*. In: *Agricultural condition and production*, Yavuz, F. (ed.). March 6.2016, [http://traglor.cu.edu.tr/objects/objectFile/turkiyede tarim 2008 01 18.pdf](http://traglor.cu.edu.tr/objects/objectFile/turkiyede%20tarim%202008%2001%2018.pdf) (in Turkish).
- Moore, R., Walker, B. (1981). studies of vegetative compatibility – incompatibility in higher plants. II. A structural study of an incompatible heterograft between *Sedum telephoides* (Crasulaceae) and *Solanum pennellii* (Solanaceae). *Amer. Jour. Bot.*, 68, 831–942.
- Moore, R. (1984). A model for graft compatibility and incompatibility in higher plants. *Amer. Jour. Bot.*, 71(5), 752–758.
- Patil, S.G., Karkamkar, S.P., Deshmukh, M.R. (2005). Screening of grape varieties for their drought tolerance. *Indian J. Plant Physiol.*, 10, 176–178.
- Pellegrino, A., Lebon, E., Simmoneau, T., Wery, J. (2005). Towards a simple indicator of water stress in grapevine (*Vitis vinifera* L.) Based on the differential sensitivities of vegetative growth components. *Aust. J. Grape Wine Res.*, 11, 306–315.

- Petkou, D., Diamantidis, G., Vassilakakis, M. (2004). Anionic peroxidase iso-form profiles from calli and barks of pear cultivars and of the quince rootstock EM. *J. Biol. Res.*, 2, 51–55.
- Polat, M., Dolgun, O., Yildirim, A., Askin, M.A., Gokbayrak, Z. (2010). Graft union formation of spur apple varieties grafted on different rootstocks. *J. Food Agric. Environ.*, 8(2), 490–493.
- Schoning, U., Kollmann, R. (1997). Phloem translocation in regeneration in vitro heterografts of different compatibility. *J. Exp. Bot.*, 48, 289–295.
- Sivritepe, N., Turkben, C. (2001). The effects of different rootstocks on grafting success and ratio of grafted-vine in muskule grapevine cultivar. *J. Agric. Fac. Uludag Univ.*, 15, 47–58.
- Todic, S., Beslic, Z., Kuljancic, I. (2005). Varying degree of grafting compatibility between cv. Chardonnay Merlot and different grapevine rootstocks. *J. Cent. Europ. Agric.*, 6(2), 115–120.
- Troncoso, A., Atte, C.M., Cantos, M. (1999). Evaluation of salt tolerance of in vitro-grown grapevine rootstock varieties. *Vitis*, 38(2), 55–60.
- Wang, Y., Kollmann, R. (1996). Vascular differentiation in the graft union of *in vitro* grafts with different compatibility. Structural and functional aspects. *J. Plant Physiol.*, 147, 521–533.
- Yildirim, A.N., Polat, M., Dolgun, O., Askin, M.A., Gokbayrak, Z., San, B. (2010). Graft formation in some spur and vigorous apple varieties grafted on ottowa 3 rootstock: A histological investigation. *J. Food Agric. Environ.*, 8(2), 512–514.

## OKREŚLENIE POWODZENIA SZCZEPIEŃ ODMIAN WINOROŚLI SZCZEPIONYCH NA DWÓCH RÓŻNYCH PODKŁADKACH

**Streszczenie.** Celem badania było określenie skuteczności szczepów oraz śledzenie zgodności odmian winorośli ‘Bornova Misketi’, ‘Trakya Ilkeren’ i ‘Sultan 1’ szczepionych na podkładkach 5BB i 1103P. Szczepienia dokonano przy użyciu techniki omega. Po woskowaniu, szczepy były zapakowane w skrzynie plastikowe wypełnione wiórami i trocinami nawilżonymi fungicydem (3:1) i umieszczone w pomieszczeniu kalusującym o temperaturze 25°C i względnej wilgotności 85% na trzy tygodnie. Po kalusowaniu, szczepy oceniano pod względem wskaźnika kalusowania szczepów (skala 0–4), wskaźnika kiełkowania (%), wskaźnika ukorzenienie (%) oraz plonu sadzonek (%). Według uzyskanych danych, określono statystycznie istotne różnice dotyczące wszystkich parametrów, z wyjątkiem wskaźnika kiełkowania. Próbkę szczepów po kalusowaniu co miesiąc (przez 6 miesięcy) były poddawane analizie histologicznej. Nie zaobserwowano żadnych istotnych różnic między kombinacjami w odniesieniu do histologicznego rozwoju szczepów. Wskaźnik kalusowania (3.73/5 BB–3.70/1103 P) oraz plon kiełków w doniczkach (86.66/5 BB%–90.00/1103 P%) spośród wszystkich kombinacji dla dwóch podłoży były najwyższe dla ‘Bornova Misketi’.

**Słowa kluczowe:** szczepienie, technika omega, histologia, zgodność szczepów

Accepted for print: 12.04.2016

For citation: Dolgun, O., Ulas, S.S., Teker, T. (2016). Determination of graft success of grape cultivars grafted on two different rootstocks. *Acta Sci. Pol. Hortorum Cultus*, 15(4), 135–145.