EFFECTS OF DIFFERENT ROOTSTOCKS IN MICROGRAFTING ON GROWING OF WASHINGTON NAVEL ORANGE PLANTS OBTAINED BY SHOOT TIP GRAFTING

G. Sertkaya
University of Mustafa Kemal, Faculty of Agriculture
Department of Plant Protection, 31034-Hatay, Turkey

ABSTRACT

*In this research, the effects of different rootstocks in micrografting on rapid production of virus-free Washington Navel orange (**Citrus sinensis** (L.) Osb.) plants obtained by thermotherapy (TT) and shoot tip grafting (STG) **in vitro** were studied. Shoot-tip grafted plants on Troyer citrange (**C. sinensis** X **Poncirus trifoliata**) were cultured in liquid Murashige and Skoog medium for 6 weeks and then micrografted onto Citrumelo (**C. paradisi X P.trifoliota**), Cleopatra mandarin (**C. reshni** Hart ex Tan), Macrophylla (**C. macrophylla** Wester.), Rough lemon (**C. jambhiri** Lush.), sour orange (**C. aurantium** L.), Troyer citrange (**C. sinensis X P.trifoliata**) and Volkamer lemon (**C. Volkameriana** Ten and Pas.). Micrografted plants were kept in greenhouse for 24 months at a temperature 26±2°C and under 16/8 hours photoperiod (day/night). The success rates of STG and micrografting were 42.0% and 80.4%, respectively. Diameters of graft-union, stock and scion trunks, survival rates of the plants micrografted onto different rootstock, and shoot length of scion were observed and measured. The shoot lengths of the plants micrografted onto Citrumelo, Cleopatra mandarin, Macrophylla, Rough lemon, sour orange, Troyer citrange, and Volkamer lemon were measured as 122.6, 121.3, 148.3, 162.4, 158.7, 144.3, and 151.7 in cm, 12 months after micrografting. Averages of graft-union-stock-scion trunk diameters of the plants micrografted onto Citrumelo, Cleopatra mandarin, Macrophylla, Rough lemon, sour orange Troyer citrange, and Volkamer lemon at 10 cm under and above the graft-union were found as 13.80-11.02-8.20, 12.55-10.20-8.45, 15.55-11.10-8.75, 14.95-11.90-8.63, 15.58-10.50-8.42, 15.24-10.77-9.72 and 17.23-11.30-9.90 in mm respectively, 24 months after micrografting. Many of plants had been reached to the size of indexing for main virus diseases in a short time (in 6-8 months) by micrografting method. The plants micrografted onto Rough lemon and sour orange produced the longest shoot. As a result of the study, it was concluded that sour orange could be suggested to use as a rootstock in micrografting studies for rapid development of plant obtained by TT+ STG.*

Introduction

A great number of viruses and other graft-transmissible infectious agents were known to affect and endanger the citrus industry worldwide (Salibe, 1986; Roistacher, 1991). Recently, a new virus disease has appeared in citrus plantations in Eastern Mediterranean Region of Turkey (3). Because of the using infected plant material to produce of citrus seedlings, some important virus dis-

eases of citrus have been presently spread out to new areas in Turkey.

Obtaining of pathogen-free citrus plants is one of the most important step in "Citrus Variety Improvement Program". Numerous methods have been developed to re-cover virus-free plants. Shoot-tip grafting (STG) *in vitro*, which was studied by Murashige et al (1972) and described in details by Navarro et al. (1975), is the most effective technique for elimination of all major virus and virus-like pathogens, including those not eliminated by thermotherapy. Plants obtained by STG are true-to type and they do not have juvenile characters. Thus, these plants could be used for budwood production after they are indexed (12).

In Turkey, virus-free citrus plants have been recovered successfully by using of standard procedure of shoot-tip grafting *in vitro* and indexing of that plants about 12-14 months after transplanting into special growing mixture (7, 17).

Transplanting the seedlings obtained by standard procedure of STG into soil mixture needs a long time (approximately 10-15 months). In order to shorten the growing period of plants for indexing stage, some modified procedures and methods, i.e. micrografting technique, have been developed (4, 8). Unfortunately, Rough lemon (*C. jambhiri* Lash.), which is used as rootstock in the micrografting studies, could be sensitive to *Phytophtora*, a soilborne fungus that has the capability to kill seedlings with in a few weeks, (19). In this case, there is a high risk to loss the plants obtained by a difficult and length procedures of STG *in vitro*.

This study was conducted to determine the most suitable citrus varieties as rootstock in micrografting technique and to short the period of the obtaining virus-free Washington Navel orange (*C. sinensis* (L.) Osb.) seedlings as budwood source.

Materials and Methods

All the mature leaves of 15 W. Navel orange plants grown in glasshouse were excised by cutting and the seedlings were kept in a climatical room at 32±2°C temperature and under 8/16 hours photoperiod (day/night) conditions for thermotherapy treatment for 3 months. The shoots from actively growing branches on the plants were used as source of shoot-tips in STG. After germination period in Murashige and Skoog (1962) culture solution solidified with 1% Bacto agar at 26±1°C in continuous darkness for 2 weeks, Troyer citrange (*C. sinensis* X *P. trifoliata*) seedlings which are widely used as rootstock for sweet orange in STG were removed from the germination medium and decapitated leaving 2-4 cm at the epicotyl and 2-4 cm of the root. Their cotyledons and axillary grafts were also removed. The excised shoot-tips of W. Navel orange composed of the apical meristem and 3 primordia (0.15-0.18 mm in height) were set on the cortex surface in an inverted-T incision (12, 13). The plantlets were cultured in liquid MS nutrient solution at a constant 27°C and exposed 16 hours daily to 1000 lux illumination for 4 weeks and than for 2 additional weeks under a higher light intensity (about 5000 lux). The plantlets, having at least 2-3 expanded leaves (**Fig. 1-B**), were micrografted on different rootstocks approximately 6 weeks after STG (De Lange, 1978). 12 plantlets (6 weeks-old) obtained by STG culture *in vitro* were micrografted on each 7 different rootstock species (9-10 months-old) on the date of 19.03.1997 (**Table 1**).

All the rootstocks were grown in plastic pots filled with 5 litre of sterilised mixture of 50% peat and 50% tuff in controlled greenhouse at 25±2°C temperature and under 8/16 photoperiod (day/night) conditions. The micrografted plants were also kept under the

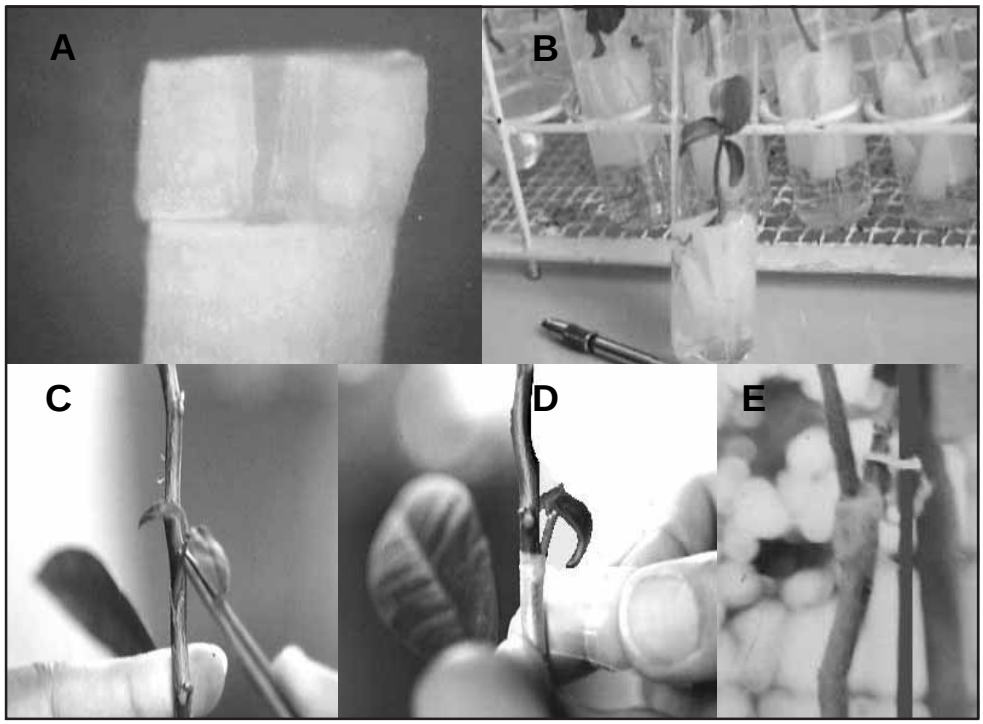

Fig.1-A. Shoot tip grafted plant; shoot tip of Washington Navel orange was placed on Troyer citrange rootstock by inverse T-insicion.
B. *In vitro* culture of shoot tip grafted plants.
C. Micrografting of Washington Navel orange plantlet on sour orange rootstock.
D. Binding of the micrografted plantlet with strech parafilm.
E. The development of bud union (Troyer citrange part of rootsock) and scion (Washington Navel orange) on micrografted sour orange rootstock.

same greenhouse conditions and fertilised every week by using fertigation system.

The upper part of STG plantlets with the first rootstock, Troyer citrange (in 10-15 mm length) were cut by an incision of inclined cut and placed into a T-incision on second rootstock (**Fig. 1-C**). The micrografted seedlings were bound with stretched parafilm (Figure 1-D), and covered by a transparent plastic bag until the scions were well adapted to the new conditions approximately 10 days after micrografting.

After the beginning of the shoot development on micrografted seedlings, the upper parts of rootstocks were cut at 2-3 cm above graft-union.

In order to determine the effect of the different rootstocks, width on width of graft-union, rootstock and scion stems of the micrografted plants had been measured by a calliper compass (in 1/20 sensitivity) during the period of 24 months at 4 week-intervals. Stock and scion diameters (mm) were measured at 10 cm under and above the graft-union. One-way analysis of variance was conducted on all data and Duncan's multiple range test was used to separate the means.

In order to determine the effect of the root-

TABLE 1

The citrus species used as rootstock in shoot tip grafting and micrografting experiments for obtaining of virus-free Washington Navel orange seedlings as source of budwood in a short time

Techniques	Rootstock Species
Shoot tip grafting	Troyer citrange (*Citrus sinensis* X *Poncirus trifoliata*)
Micrografting	Citrumelo (*C. paradisi* X *P. trifoliota*) Cleopatra mandarin (*C. reshni* Hart ex Tan) Macrophylla (*C. macrophylla* Wester.) Rough lemon (*C. jambhiri* Lush.) Sour orange (*C. aurantium* L.) Troyer citrange (*C. sinensis* X *P.trifoliata*) Volkamer lemon (*C. volkameriana* Ten and Pas.)

TABLE 2

The numbers and the success rates of micrografting on different rootstocks

Rootstock Species	No. of Survival Plants / No. of Grafted Plants	Success Rate of Grafting (%)
Citrumelo	10/12	83.3
Cleopatra mandarin	7/12	58.3
Macrophylla	9/12	75.0
Rough lemon	11/12	90.1
Sour orange	11/12	90.1
Troyer citrange	11/12	83.3
Volkamer lemon	10/12	90.1

stocks on length of shoots, the plants which were linearly grown as a single shoot had been measured from graft-union to top of the flush by using a tape measure (in cm) periodically for 12 months at 4 week-intervals.

Results and Discussion

In shoot tip studies, about 20 shoot tips had been grafted on Troyer citrange seedlings in one-hour period. Totally 228 plantlets were grafted by STG method. Shoot tips were survived on Troyer citrange rootstocks but shoot formation was not observed by 10% of grafted plantlets in 4th weeks after *in vitro* culture. So, the plantlets with shoot development were accepted as successfully grafted plant. The plantlets with 3-4 expanded leaves *in vitro* culture were used in micrografting on different rootstocks, 6 weeks after STG. Shoot regeneration was observed in all plantlets micrografted on Rough lemon seedlings. The success rates of micrografting on Rough lemon, sour orange, Volkamer lemon, Citrumelo, Troyer citrange, Macrophylla and Cleopatra mandarin were obtained by 90.1%, 90.1%, 90.1%, 83.3%, 83.3%, 75.0%, and 58.3% respectively (**Table 2**). Rough lemon had the largest rootstock growth, followed by Volkamer lemon, Mac-

rophylla, sour orange, Troyer citrange, Cleopatra mandarin and Citrumelo, respectively. Macrophylla, sour orange and Troyer citrange were not statistically different from each other in rootstock growth (**Table 3**).

Volkamer lemon was placed in the first group by the width of graft-union (**Fig. 1-E**). Sour orange and Macrophylla were in the second group, Troyer citrange, Rough lemon were in the third group, Citrumelo was in the fourth group and Cleopatra mandarin was in the fifth group.

Volkamer lemon rootstock was placed in the first group by the width of scion. Troyer citrange placed in the second group while Macrophylla and Rough lemon in the third group, Cleopatra mandarin, Sour orange and Citrumelo were placed in the fourth group.

The averages of shoot lengths of W. Navel orange on different rootstocks at 6-month intervals were shown in **Fig. 2**. Rough lemon and sour orange had the longest shoot length in cm followed by Volkamer lemon, Macrophylla, Troyer citrange, Citrumelo, and Cleopatra mandarin in 162.4, 158.7, 151.7, 148.3, 144.3, 122.6, and 121.3 respectively, 12 months after micrografting.

STG resulted in 42% of shoot formed plants. The success rate of grafting was similar with other studies (12, 13). The success rate of micrografting ranged from 58.3% (Troyer citrange) to 90.1% (Rough lemon). De Lange (1978) reported 90% of success rate with micrografting on Rough lemon. Although many of that rootstocks had been studied as rootstock in STG experiments (4, 5, 12, 13, 14), there was no study to compare the effects of these species on seedling development in micrografting studies.

The graft-union + rootstock (Troyer citrange) part of shoot tip grafted plantlets micrografted on different rootstocks were exhibited greater tissue development than both in rootstock and scion parts of seedlings. Especially in Volkamer lemon, a very clear overgrow was observed at the graft-union in all micrografted plants. This situation might be due to incompatibility or being of a few part of Troyer citrange which was used as rootstock in STG, between scion and new rootstock species which were used in micrografting experiments (Figure 1-E). Similarly, rootstocks with trifoliate orange parentage regularly overgrowth their scions was reported by Ferguson et al. (6). L. Navarro et al. (1975) reported that the success rate of shoot

TABLE 3

Graft-union, rootstock and scion growth of micrografted plantlets on different rootstocks

Rootstock Species	Diameter of Graft-union (mm)*	Diameter of Rootstock (mm)*	Diameter of Scion (mm)*
Citrumelo	13.80 c+	9.90 c	8.20 c
Cleopatra mandarin	12.55 d	10.20 bc	8.45 c
Macrophylla	15.55 b	11.10 ab	8.75 bc
Rough lemon	14.95 bc	11. 90 a	8.63 bc
Sour orange	15.58 b	11.05 ab	8.42 c
Troyer citrange	15.24 bc	10.77 ab	9.72 b
Volkamer lemon	17.23 a	11.30 b	9.98 a

tip grafting on Troyer citrange when lemon shoots were used as source shoot tip was lower in the STG studies.

The plants transferred to soilless mixture after STG culture are required 2 or 3 times longer growing period to reach a suitable size for indexing than micrografted ones (7, 13, 18). Especially plants grafted on sour orange and Rough lemon reached to optimum size (60-80 cm) for indexing within 6-8 months (Figure 2). Other rootstocks such as Macrophylla and Troyer citrange reached to satisfactory size within 8-10 months. In Citrumelo, Macrophylla and Volkamer lemon rootstocks, the shoot growth from rootstocks was at a high level. These shoots slowed down the growth of grafted plants (especially the part of scion). Moreover, two of plants grafted on Rough lemon dried due to foot rot (gummosis) and root rot.

Foot rot and root rot caused by *Phytophtora* spp. create major problems in nurseries. Some Rough lemon sources are highly susceptible to infection while Cleopatra mandarin and sour orange rootstocks are resistant or tolerant (2, 6, 19).

This situation creates risk of losing healthy plant candidates obtained from STG and micrografting which are detailed, difficult and time consuming methods. Sour orange is widely used (95%) rootstock in citriculture in Turkey (1). The results of our study showed that, besides Rough lemon, sour orange was found to be also suitable for micrografting studies for rapid development of plant after thermotherapy + STG *in vitro*. Reasons of this are as following;

1) Root and root neck problems are at a minimum level with sour orange rootstock.
2) Sour orange is commercially propagated in a large quantities in the controlled glasshouses and they are required lesser amount of time and labour when compared with the other rootstocks.
3) Sour orange rootstock could be obtained from nurseries growing rootstocks periodically by the quantities and ages (8-10 month) as they are requested.

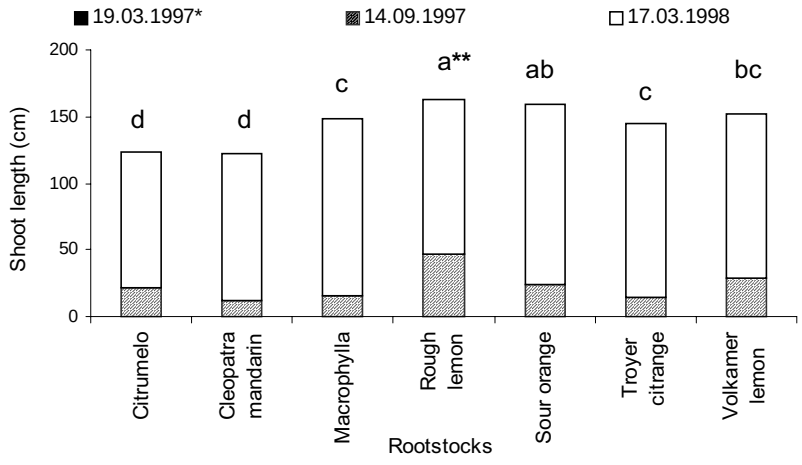

Fig. 2. Averages of shoot lengths of scion (Washington Navel orange) micrografted on different rootstocks (cm). *Shoot lengths in grafting stage were between 0.2-0.4 cm. **Means by the different letters within each column indicate significant differences (Pd : 0.01, n:7).

4) Other rootstocks require additional place time, labour and cost for the propagation, seed obtaining, seed storage and seed production. Also, periodical seeding is required to obtain rootstock at a suitable age with others.
5) Sour orange rootstock has advantages of high success rate of micrografting and plant growth.

Sour orange has been routinely used as rootstock in our micrografting studies to rapid growth virus-free citrus budwood sources.

REFERENCES

1. **Baloğlu S**. (1998) Proc. of the Mediterranean Network on Certification of Citrus (1995-1997), 27-35.
2. **Castle W.S., Tucker D.P.H, Krezdorn A.H., Youtsey C.O**. (1993) Rootstocks for Florida Citrus, Rootstock Selection: the first step to success. SP 42, Inst. of Food and Agricultural Sciences, Univ. Florida, 92.
3. **Çınar, A., Kersting U., Önelge N., Korkmaz S., Şàş G. (**1993). Proc. 12th IOCV Conf., IOCV Riverside, USA, 397-40.
4. **De Lange J.H. (**1978) Citrus and Subtrop. Fruit, **39**, p. 15.
5. **Edriss M.H., Burger D.W.** (1984). Sci. Horticult., **23**, 255-259.
6. **Ferguson L., Sakovich N., Roose M.** (1990) California Citrus Rootstocks. Division of Agriculture and Natural Resources, Univ. California. Public No. 21477, 18 p.
7. **Göçmen M.** (1992) Turunçgillerde *in vitro* sürgün ucu aşılama tekniğinde ve bu yolla elde edilen bitkilerin yetiştirilmesinde yapılan modifikasyonların aşılama başarısına ve bitki gelişimine etkisi. (Msc. Thesis), Çukurova Üniv. Fen Bil. Enst., 70 s.
8. **Hong-Ji S., Jan-Yang C.** (1984) Proc. Int. Soc. Citriculture, **1**, 332-334.
9. **Korkmaz S**. (1997) Doğu Akdeniz Bölgesi turunçgillerinde vektörlerle taşınan virüs benzeri bir hastalığın yayılması, taşınması, duyarlı tür ve çeşitlerin belirlenmesi ve tanısı üzerinde araştırmalar (PhD Thesis), Çukurova Üniv. Fen Bil. Enst. 70.s.
10. **Murashige T., Skoog F.** (1962) Phisiol. Plant, **15**, 473-497.
11. **Murashige T., Bitters W.P., Ragan T.S, Nauer E.M., Roistacher C. N., Holliday P. B.** (1972) Hort. Scien. **7** (2), 118-119.
12. **Navarro L.** (1984) Proc. FAO/NORWAY Symp. on plant tissue culture, technology and utilization, micropropagation of selected rootcrops, palms, citrus and ornamental plants. Plant Production and Protection Paper, **59,** 113-154.
13. **Navarro L., Roistacher C. N., Murashige T.** (1975) J. Amer. Soc.Hort. Sci., **100** (5), 471-479.
14. **Roistacher C.N., Kitto S.L.** (1977) Plant Disease Reporter, **61**, 594-596.
15. **Salibe A.A.** (1986) A programme for citrus improvement and protection in Turkey. Report to the Government of Turkey. FAO, Rome, p. 66.
16. **Şàş G.** (1991) Turunçgillerde GA_3 uygulaması ve *in vitro* sürgün ucu aşılama tekniği kombinasyonunun Psorosis grubu hastalık etmenlerinden arındırılmış üretim materyali elde etme oranına etkisi. (Msc. Thesis), Çukurova Üniv. Fen Bil. Enst. 97 s.
17. **Tamer Ş.** (1988) Virüs ve virüs benzeri hastalık etmenlerinin Navel portakallarından arındırılması. (PhD Thesis), Çukurova Üniv. Fen Bil. Enst.), 97 s.
18. **Taşdemir H.A., Davarcı T., Mermer S.** (1991) Shoot tip grafting in citrus and the use of micrografting for rapid growth of shoot tip grafted plants (Abstr). Derim, **8** (1), 24-29.
19. **Timmer L.W., Menge J.A**. (1988) In: Compendium of Citrus Disease. (J. O. Whiteside, S. M. Garnsey, L. W. Timmer, Eds.), APS, p. 23.