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BRIEF COMMUNICATION

Propagation of *Ficus carica* L. clones by *in vitro* culture

S. HEPAKSOY* and U. AKSOY

Department of Horticulture, Faculty of Agriculture, Ege University, İzmir 35100 Turkey

Abstract

This experiment is designed to determine the most suitable conditions and media for propagating three selected fig (*Ficus carica* L.) clones through tissue culture. The clone 37 displayed a higher performance than clones 50 and 82. As the multiplication medium, the Murashige and Skoog (MS) medium containing 1 mg dm^{-3} α-indole-3-butyric acid (IBA), 1 mg dm^{-3} gibberellic acid and 5 mg dm^{-3} 6-benzyladenine were the best, whereas, MS medium complemented with 1.2 and 2.5 μM IBA or 1-naphthalene acetic acid (NAA) were better in respect to rooting. Peat followed by volcanic tuff gave the best performance for acclimatization to outdoor conditions.

Additional key words: fig, micropropagation, shoot tip.

Fig (*Ficus carica* L.) is one of the traditional Mediterranean crops, and cv. Sarilop, is known on the world market for its big size, light colour, soft texture and sweet taste. Because cv. Sarilop can be propagated by cuttings very easily like other fig cultivars, the cultivar has conserved most of its superior properties for centuries. There are some clonal variations present among Sarilop populations thus to benefit from this variation and obtain mass and rapid propagation *in vitro* techniques can be utilized. This experiment is designed to determine the most suitable conditions and media for propagating three Sarilop clones, clone 37, 50 and 82.

Shoot tips (3 - 4 cm long) were taken from 20-year-old trees of *Ficus carica* L. cv. Sarilop clones 37, 50 and 82 in May or June. The tips were washed under running tap water, kept for 20 min in 2 % (v/v) sodium hypochlorite with 1 or 2 drops of *Tween 20,* and then rinsed with sterile distilled water three times for 5 min (Muriithi *et al.* 1982, Ranjit and Kester 1988).

Solid Murashige and Skoog medium (1962; MS) supplemented with 1 mg dm^{-3} α-indole-3-butyric acid (IBA), 1 mg dm^{-3} gibberellic acid (GA_3) and 5 mg dm^{-3} 6-benzyladenine (BA) were used to obtain a sufficient number of shoots. These *in vitro* shoots were cultured on five different nutrient media, both liquid and solid as follows: M1 (MS + 0.1 mg dm^{-3} IBA + 0.1 mg dm^{-3} GA_3 + 0.5 mg dm^{-3} BA), M2 (MS + 1 mg dm^{-3} IBA + 1 mg dm^{-3} GA_3 + 5 mg dm^{-3} BA), M3 (MS + 0.2 mg dm^{-3} 1-naphthalene acetic acid, NAA + 2 mg dm^{-3} BA), M4 (MS + 0.18 mg dm^{-3} NAA + 0.03 mg dm^{-3} GA_3 + 0.1 mg dm^{-3} BA) and M5 (MS + 1 mg dm^{-3} IBA + 0.5 mg dm^{-3} GA_3 + 2.5 mg dm^{-3} BA). As another variable, 89 mg dm^{-3} phloroglucinol (PG) was added to all solid media, except M1, to investigate its effect on growth rate and multiplication of explants. Solid media were solidified with 0.7 % *Difco bacto* agar, and the pH was adjusted to 5.7 prior to autoclaving for 20 min at 121 °C. Cultures were kept at 24 ± 1 °C under 16-h photoperiod at an irradiance of 40 μmol m^{-2} s^{-1} on the culture surfaces provided by cool white fluorescent lamps.

At the stage of rooting, solid MS media with the addition of IBA or NAA at 0, 0.5, 1.2, 2.5 and 5.0 μM were used. Rooting was studied only with clone 37 which showed the highest performance in respect to growth and multiplication. According to the rooting results of this clone, rooting studies were conducted for other two clones in media that gave good results. After rooting period, all rooted explants were thoroughly washed with water to remove agar medium from the roots and then transferred into pots filled with peat, volcanic tuff, *Perlite* or mixture of sand, soil and cow manure (1:1:1). These pots were covered with polyethylene sheets to ensure

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Abbreviations: BA - 6-benzyladenine; GA_3 - gibberellic acid; IBA - α-indole-3-butyric acid; MS medium - Murashige and Skoog medium; NAA - 1-naphthalene acetic acid; PG - phloroglucinol.

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* Corresponding author; fax: (+90) 232 388 18 65, e-mail: serra.hepaksoy@ege.edu.tr

higher humidity around the plants. After a month, polyethylene sheets were removed and plantlets were acclimatized for 15 d. At the end of the sixth week of acclimatization, rooted plantlets were removed from the acclimatization media to evaluate their growth performance, and root and shoot lengths and leaf numbers were determined. The values before transplanting substracted from the values obtained after six weeks of acclimatization were used to indicate the growth in different media.

The experiment was conducted as a completely randomized design with a simple factorial arrangement with four replications. The data were subjected to analysis of variance (*ANOVA*). All the percentage values were transformed by arcsin transformation prior to *ANOVA*. All the statistical analyses were performed with *SPSS* (*SPSS Inc.*, *Ver. 11.0*, Chicago, USA).

The clone 37 showed better growth than the other two. In clone 37, 75 % of the explants survived in May and 73 % in June on MS medium containing 1 mg dm^{-3} IBA, 1 mg dm^{-3} GA_3 and 5 mg dm^{-3} BA. Survival rates of the clones 50 and 82 were close to each other, 63 and 58% during the first period and 58 and 62 % in the second period, respectively.

In vitro shoots were cultured on five MS media. Clone 37 showed the highest performance on M2 solid nutrient medium, followed by M4 solid medium. The lowest ratio was obtained on the M1 liquid and solid media (Table 1).

Table 1. Growth [% of responding explants] of fig clones in five different nutrient media; solid and liquid. $LSD_{0.05}$ were 0.92, 1.23 and 1.68 for clones, media composition and solid or liquid media, respectively.

Media	Clone 37		Clone 50		Clone 82	
	solid	liquid	solid	liquid	solid	liquid
M1	41.7	33.3	35.4	27.1	28.9	24.4
M2	76.7	58.3	68.8	58.3	48.9	42.2
M3	65.0	56.7	60.4	54.2	44.4	35.6
M4	68.3	51.7	58.3	47.9	42.2	37.8
M5	58.3	46.7	52.1	39.6	35.6	24.4

The nutrient medium in which clone 50 showed the highest performance was M2 solid medium. This medium was followed by M3 and M4 solid media, and M2 and M3 liquid media. Clone 50 showed the lowest stand rate on M1 liquid and on solid nutrient media as it was the case for clone 37. The highest performance of clone 82 was seen on M2 solid medium, as was the case for other clones, and it was followed by M3 and M4 solid, and M2 liquid media. The lowest ratios were observed on M1 and M5 liquid media.

Observations were made on explants at 20-d intervals after planting, and shoot length and leaf numbers were determined. Clone 50 showed higher growth at the beginning of the period compared to the other two clones while clone 37 was ranked the first after the fortieth day. The reason for this was not only that the growth of clone 37 was better but also due to dead shootlets of clone 50 on some media. When the mean growth rates in both liquid and solid media were taken into consideration, shoots belonging to clone 37 reached the mean length of 44 mm on M2 at the end of the 60-d period while to the length of 22.17 mm on M5 medium. Leaf numbers on the same media were 7.60 and 2.83, respectively. On the other hand, clone 50 showed its highest growth rate on M2 medium at the end of the 60-d period. Even if its growth on other media showed a good growth until the 40^{th} day, thenafter it got worse and died. Clone 82 showed the best growth on M3 and M4 media at the end of the 60-d period. Although the growth of the other two clones were better on M2 medium, this clone did not grow on M2, and some shootlets died after the 40^{th} day. The growth of surviving plantlets was good, and especially the number of leaves increased. For this reason, mean number of leaves was comparatively higher than on other media. At the end of the 60-d period, all surviving cultures were healthy, and formation of callus at the bottom of the plants did not occur or if did, it was in small amounts.

Clone 37, which performed the best in terms of multiplication starting from the beginning of the experiment, was higher than the other two clones and statistically separated from clones 50 and 82. On the other hand, the difference between clones 50 and 82 was not significant. Average of shoot numbers per explant in clone 37 was 2.69, while this ratio was 1.13 for clone 50 and 1.10 for clone 82 (Table 2).

Table 2. Multiplication (number of shoots per eplant) of three clones on the different MS solid media without or with phloroglucinol (PG). $LSD_{0.05}$ were 0.13, 0.15 and 0.25 for clones, media composition and effect of PG, respectively.

Media	Clone 37		Clone 50		Clone 82	
	-PG	+PG	-PG	+PG	-PG	+PG
M2	6.33	9.17	1.75	4.91	2.33	4.78
M3	1.75	2.93	0.83	2.36	0.67	1.28
M4	1.50	2.53	1.17	2.77	1.08	1.15
M5	1.17	2.00	0.75	1.71	0.33	0.99

Addition of PG increased both the proliferation and multiplication of explants. Proliferation of clones was higher on M2 and M3 media supplemented with PG than M4 and M5 with PG. All clones showed the highest proliferation and multiplication on M2 medium with PG (Table 2).

Although the effect of interaction between the media and clones on multiplication was found statistically significant, M2 was the best medium for all three clones. It performed better with PG than without PG. Clone 37 had the highest multiplication for all the media compared to the other two clones.

At the end of the fifth week in the rooting media, there was a significant effect of the auxin concentration

on the rooting percentage of clone 37 plantlets. The highest (88.9 %) rooting percentage of this clone occurred in MS nutrient medium containing 2.5 μM of IBA. This was followed by media containing 1.2 μM of IBA, and 1.2 and 2.5 μM of NAA. High rooting percentages were obtained in media containing 1.2 and 2.5 μM of IBA or NAA, while the lowest rooting percentages were observed in media not containing any hormones. On the other hand, media containing 0.5 and 5.0 μM of IBA or NAA were placed in between these two media (Table 3).

Table 3. Rooting percentage of fig clones in different concentrations of IBA and NAA. $LSD_{0.05}$ were 2.84 and 2.48 for growth regulators and clones respectively.

Conc.	Clone 37		Clone 50		Clone 82	
[μM]	IBA	NAA	IBA	NAA	IBA	NAA
0.0	55.5	55.5	53.3	53.3	46.7	46.7
1.2	72.3	72.3	73.3	66.7	66.7	66.7
2.5	88.9	72.3	80.0	80.0	73.3	73.3
5.0	61.1	61.1	-	-	-	

Since the lowest rooting percentage were obtained in media containing the lowest (0.5 μM) and the highest (5.0 μM) IBA or NAA, these media were not used for rooting studies of the other two clones. Although the rooting percentage was low in the control medium, it was included in the study to compare the effect of the plant growth regulators. The rooting of clones 50 and 82 were the highest on media supplemented with 2.5 μM IBA or NAA, but the performance was not as high as clone 37. Rooting percentage of clone 50 was 80.0 % and clone 82 was 73.3 % on media containing 2.5 μM both IBA and NAA. No statistically significant differences were found between the addition of 2.5 or 1.2 μM concentrations of IBA or NAA to the nutrient medium (Table 3). The rooting percentages of these two clones in control media were similar to that of clone 37. In general, the root number and lengths were higher on media that gave higher rooting percentages.

Survival of the rooted plantlets was high when transferred to a mist system for hardening and acclimatization, especially in peat. There was no significant difference among clones in terms of survival ratio of the rooted plantlets, and average ratios were 63.3, 62.5 and 60.4 %, respectively for clones 37, 50 and 82. Among tested acclimatization media, the highest survival ratio was in peat, for clones 37, 50 and 82 as 86.7, 83.3 and 83.3 %, respectively. Volcanic tuff was the second among the acclimatization media. The lowest survival ratios of the rooted plantlets were obtained in *Perlite* and mixture of sand, soil and cow manure (1:1:1). Survival ratios of clones ranged between 46.7 and 50.0 % in *Perlite* and between 41.7 and 50.0 % in the mixture medium (Table 4).

The rooted plantlets continued to grow during the acclimatization stage, produced new leaves, elongated and formed an efficient root system. The highest growth rate was in peat and the lowest one was in mixture of sand, soil and cow manure (1:1:1), paralel to the survival ratios. This difference is especially evident in the growth of shoots. Plants grown in peat and volcanic tuff were healthier.

Table 4. Survival ratio and growth rate of rooted plantlets in the different acclimatization media. $LSD_{0.05}$ 3.08 and 0.72 for clone and media, respectively.

Clone	Media	Survival [%]	Root length [mm]	Shoot length [mm]	Leaf number
37	volcanic tuff	73.3	66.5	80.2	2.3
37	*Perlite*	46.7	66.0	70.0	2.2
37	peat	86.7	75.2	109.5	3.0
37	mixture	46.7	63.2	69.5	2.2
50	volcanic tuff	63.3	72.5	85.0	2.5
50	*Perlite*	75.0	71.0	77.5	2.2
50	peat	50.0	79.2	99.0	3.0
50	mixture	83.3	70.2	70.5	2.3
82	volcanic tuff	58.3	69.5	79.2	2.7
82	*Perlite*	50.0	69.0	75.2	2.5
82	peat	83.3	80.5	92.2	2.8
82	mixture	50.0	70.5	71.5	2.3

In terms of explant development, no differences were found between sampling times, and similar survival rates were obtained. This result reveals that May and June are suitable months for taking explants. About 45 d after the bud-burst in the spring, appropriate period for *in vitro* propagation during which shoot tips are cultured on the medium starts. Shoot tips can be taken from trees and cultured on the nutrient media, until shoot growth stops in mid-June. MS medium containing 1 mg dm^{-3} IBA, 1 mg dm^{-3} GA_3, 5 mg dm^{-3} BA and 89 mg dm^{-3} PG, is a suitable nutrient medium for *in vitro* propagation of Sarilop fig clones. Both the concentration of plant growth regulators and the balance among them is important for survival, growth and multiplication of explants. The obtained results showed that, a reduction in the concentration of plant growth regulators, or changes in ratios, exerted negative effects on survival and multiplication. Solid media gave better results compared with the liquid ones. Explants showed higher survival ratios and multiplication on all tested media with PG compared to those that without PG. Some researchers (Pontikis and Melas 1986, Muriithi *et al*. 1982) suggested that PG is added to the culture medium to prevent negative effect of polyphenols from the explants. In this study negative effect of polyphenols on growth and multiplication was not observed even if PG increased the survival ratio and multiplication of explants.

The effect of auxin type on rooting of clones was not significant. Similarly, Hu and Guo (1994) determined that there was no difference between *in vitro* rooting of media containing IBA and NAA. NAA was found to be

more effective in *in vitro* culture of some fruit cultivars (Cozza *et al*. 1997, Rugini 1986). Apart from the genetic preference of a cultivar to a specific auxin, NAA is considered to be more effective in inducing rooting under *in vitro* conditions mainly due to its greater stability under light (Stasinopoulos and Hangarter 1990).

Addition of auxins, mainly of IBA and NAA, into the rooting media with different concentrations, increased rooting ratios. Low concentrations as 0.5 μM were not so effective and concentrations over 5.0 μM had adverse effects on rooting of fig clones. 1.2 and 2.5 μM auxin were determined to be the most suitable concentrations for rooting. Nobre and Romano (1998) claim that fig cvs. Lampa Branca and Berbera had the highest rooting ratios in MS medium containing 2.5 μM IBA and the lowest rooting ratios in MS media which did not contain any hormones. Similarly, Murithii *et al.* (1982) recommended medium containing 0.5 mg dm^{-3} NAA or IBA for rooting. These concentrations are more or less equal to 2.5 μM IBA and NAA. In general, the media with an auxin concentration that is suitable for rooting, also gave the best results in respect to root number and length.

Although some researchers like Dunstan (1981) and Arena and Caso (1992) stated that callus formation is high in media containing NAA, this was not the case in the current study. In case, callus formation occurs at the bottom of the shoots, survival is affected negatively because the roots were initiated from this part. This negative event also created problems during acclimatization to outdoor conditions.

The last and most important phase of propagation through tissue culture is the stage of transferring rooted plantlets to the outdoor conditions. For acclimatization, it is necessary to transfer shootlets rooted under *in vitro* conditions to an environment containing 100 % relative humidity and then to normal atmospheric conditions. The overall high survival ratio of the rooted plantlets could be ascribed to the acclimatization under mist or fog, which prevents high transpiration.

According to the results of the study, it can be said that peat is the most suitable medium in transferring the plantlets into the outer conditions. Besides Perlite, volcanic tuff can be used for this purpose. To use the mixture of these two media in future studies can be recommended.

Among the three Sarilop clones of which the *in vitro* propagations were investigated, it was found that clone 37 has a higher ability in both propagation and growth compared with the other two clones. Kuşaksız (1999) also stated that the rooting percentage of clone 37 cuttings is higher than the other 10 Sarilop clones, and that taller nursery trees were obtained after a one year growth and that rooting performances of the cuttings of clones 50 and 82 were close to each other. Besides, orchard observations of trees belonging to these three clones suggested better growth for clone 37. In propagation of these clones either by tissue culture through shoot tips or by cuttings, similar results were obtained both in terms of rooting and growing.

As a result, it was put forward that Sarilop clones selected for their high performance can be propagated by tissue culture within a short time and in great quantities. The plants propagated *in vitro* did not show any detectable variation in respect to growth or morphological characteristics when compared with the mother plant, but the fruiting characteristics and further developments of these plants need to be observed.

References

Arena, M.E., Caso, O.H.: Factors affecting the *in vitro* shoot multiplication of *Prunus* rootstocks. - Phyton **53**: 29-38, 1992.

Cozza, R., Turco, D., Briccoli, Bati C., Bitonti, M.B.: Influence of growth medium on mineral composition and leaf histology in micropropagated plantlets of *Olea europaea*. - Plant Cell Tissue Organ Cult. **51**: 215-223, 1997.

Dunstan, D.I.: Transplantation and post-transplantation of micropropagated tree-fruit rootstocks. - Proc. int. Plant Propag. Soc. **31**: 39-45, 1981.

Hu, J., Guo, J.: A study on tissue culture of *Ficus carica*. - J. Nanjing Forest. Univ. **18**: 73-76, 1994. [In Chinese.]

Kuşaksız, K.E.: [Research on some selected fig cv. Sarilop (=Calimyrna) clones.] - PhD Thesis, Ege University, İzmir 1999. [In Turkish.]

Murashige,T., Skoog, F.: A revised medium for rapid growth and bioassays with tabacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Muriithi, L.M., Rangan, T.S., Waite, B.H.: *In vitro* propagation of fig through shoot tip culture. - HortScience **17**: 86-87, 1982.

Nobre, J., Romano, A.: *In vitro* cloning of *Ficus carica* L. adult trees. - Acta Hort. **480**: 161-164, 1998.

Pontikis, C.A., Melas, P.: Micropropagation of *Ficus carica* L. - HortScience **21**:153, 1986.

Ranjit, M., Kester, H.: Micropropagation of cherry rootstocks. I. Response culture. - J. amer. Soc. hort. Sci. **113**: 146-149, 1988.

Rugini, E.: Olive (*Olea europaea* L.). - In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry. Trees I. Pp. 253-267. Springer-Verlag, Berlin - Heidelberg 1986.

Stasinopoulos, T.C., Hangarter, R.P.: Preventing photochemistry in culture media by long-pass light filters alters growth of cultured tissues. - Plant Physiol. **93**:1365-1369, 1990.