Meristem culture of two fig cultivars in Turkey

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Abstract — Introduction. Two Turkish fig cultivars, Alkuden and Bursa Siyahi, were propagated by meristem culture to eliminate the fig mosaic virus. The technique of dsRNA analysis was conducted on the *in vitro* propagated plants to test for virus-free status. **Materials and methods**. Four different Murashige and Skoog (MS) media complemented with different concentrations of growth hormones (GA_3, BA and IBA) were compared to study the cultured meristems' survival rate, shoot formation and rooting. Short- and long-term thermotherapy treatments were also applied. **Results**. For survival rate, 0.2 mg $GA_3 \cdot L^{-1}$ + 0.5 mg $BA \cdot L^{-1}$ gave the most favorable results; for shoot formation, it was the medium with 0.2 mg $GA_3 \cdot L^{-1}$ + 2.0 mg $BA \cdot L^{-1}$ which was the best, while rooting rate was the highest for meristems cultured on MS medium with only 0.1 mg $GA_3 \cdot L^{-1}$. **Conclusion**. Meristem culture, together with thermotherapy treatment, is recommended to obtain virus-free fig plant material. Although cultured plants seemed to be very healthy, dsRNA virus tests are recommended for sensitive evaluation of the sanitary status of the plants obtained.

Turkey / *Ficus carica* / micropropagation / meristem culture / plant growth substances / heat therapy / plant viruses

Culture de méristèmes de deux cultivars de figuier en Turquie.

Résumé — Introduction. Deux cultivars de figuier turcs, Alkuden et Bursa Siyahi, ont été multipliés par culture de méristèmes afin de les débarrasser du virus de la mosaïque du figuier. Une analyse de l'ARN bicaténaire a été conduite sur les plants obtenus *in vitro* pour tester leur statut vis-à-vis du virus. **Matériel et méthodes**. Quatre milieux différents de Murashige et Skoog (MS), complétés par diverses concentrations d'hormones de croissance (AG_3, BA et IBA), ont été comparés pour étudier le taux de survie des méristèmes mis en culture, la formation de tiges et l'enracinement. Des traitements de thermothérapie court et long ont été également testés. **Résultats**. Pour le taux de survie des méristèmes mis en culture, l'association d'hormones 0,2 mg $AG_3 \cdot L^{-1}$ + 0,5 mg $BA \cdot L^{-1}$ a été la plus favorable ; pour la formation de tiges, c'est le milieu avec 0,2 mg $AG_3 \cdot L^{-1}$ + 2,0 mg $BA \cdot L^{-1}$ qui a été le meilleur, alors que le taux d'enracinement a été le meilleur pour des méristèmes cultivés sur milieu MS avec seulement 0,1 mg $AG_3 \cdot L^{-1}$. **Conclusion**. La culture de méristèmes couplée à un traitement de thermothérapie est recommandée pour obtenir des plants de figuiers indemnes de virus. Bien que les plants obtenus semblent être sains, des mesures d'ARN bicaténaire sont recommandées pour évaluer leur statut sanitaire.

Turquie / *Ficus carica* / micropropagation / culture de méristème / substance de croissance végétale / thermothérapie / virus des végétaux

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1. Introduction

Fig belongs to the genus *Ficus* of the Moraceae family. *Ficus carica* is the most important species among the 600 species of this family [1]. The high adaptability characteristics of figs allow them to be grown mainly in areas of the world with Mediterranean climates. However, fig is mainly cultivated in the Mediterranean countries, which are the most important cultural centers of fig. The main fig-growing countries in the world are Turkey, Egypt, Greece, Iran and Morocco, in that order. Approximately 280 000 t of the 1 070 676 t of world fig production is supplied by Turkey [2]. This country is also one of the centers of fig origin, and fig is grown in the Aegean, Mediterranean and South-East Anatolian regions of the country.

Fig is an economically important crop in Turkey. The Aegean region mainly produces dry figs, while the Mediterranean and Marmara regions produce table figs. Establishment of new commercial fig orchards is increasing because of export opportunities. However, figs are prone to fig mosaic, an endemic disease that is widely distributed in most varieties and countries where figs are cultivated [3]. Fig mosaic can cause symptoms in both leaves and fruits. Infected leaves consist of various degrees of mosaic accompanied by yellow-green chlorotic lesions and deformation. Infrequently, similar chlorotic lesions also appear on immature fruits. As the disease progresses, fruits begin to drop prematurely and, in some cases, affected trees eventually die [4]. To introduce fig cultivars that are susceptible to fig mosaic to establish new orchards, there is a need for certified healthy propagation material. A viable system for fig propagation through tissue culture has already been reported [5–8]. Meristem culture has been used for production of virus-free plant material in many species [9–11], but in woody species, especially in fig, its use is rather limited [3]. Sometimes, meristem culture fails to result in virus-free plants, so it should be combined with thermotherapy as an antiviral treatment. Thermotherapy has been successfully used in virus eradication in many plant species [12–14].

In our study, an attempt was made to obtain mosaic virus-free fig plant material through thermotherapy and meristem culture of two fig genotypes, 'Bursa Siyahi' and 'Alkuden'. This study included *in vitro* culture of shoot tips developed during the treatments, rooting and root formation. The plantlets obtained by this method were transplanted into vials and stored under high humidity conditions. The healthy status of the plants was tested by dsRNA tests.

2. Material and methods

2.1. Plant material

The fig cultivar Bursa Siyahi is a black-fruited cultivar with large fruit size [(75–80) g], black fruit flesh and a short neck, dense fruit flesh good for transportation, easy peeling, and a small ostiolum. The fruit does not show any cracking, has a pH of 4.55, contains 0.19% total acidity, 17.52% reduced sugars, and needs 32.000 growing degree-hours to ripen [15–19].

The fig cultivar Alkuden is a yellow-fruited cultivar with big (65–70) g fruit, yellow fruit flesh, easy peeling, and a closed ostiolum. Its fruit does not show any cracking, and contains 15–16% reduced sugars [16, 19, 20].

2.2. Medium and explant establishment

Meristems of both fig cultivars were isolated (0.5–0.8 mm) in a sterile cabinet under a binocular microscope and transferred into 2.5 cm × 15 cm tubes. To avoid interference from phenolic compounds, meristems were kept in the dark for 1 week and transferred weekly. After 8 weeks, meristems were transferred into a shoot development medium. In this medium, new shoots occurred in 6 weeks, and then they were transferred into a rooting medium. Plants were kept in this medium for 4 weeks. In the fifth week, the covers of the tubes were opened to acclimatize the young plants issued from meristem culture to outdoor

conditions, and then they were transferred to the soil.

In the experiment, Murashige and Skoog (MS) medium [21] was used with the addition of various hormones [(gibberellic acid (GA_3), benzyladenine (BA) and indole-3-butyric acid (IBA)] (*table I*) for meristem development, proliferation and rooting.

Table I.
Hormones added to a Murashige and Skoog medium in order to achieve meristem culture of fig (Turkey).

Medium used for meristem culture		Gibberellic acid	Benzyladenine	Indole-3-butyric acid
		($mg \cdot L^{-1}$)		
Growing medium	1	0.1	0.2	0.1
	2	0.1	0.5	0.1
	3	0.2	0.2	0.1
	4	0.2	0.5	0.1
Shoot development medium	1	0.1	1.0	0.1
	2	0.1	2.0	0.1
	3	0.2	1.0	0.1
	4	0.2	2.0	0.1
Rooting medium	1	0.1	0.0	0.0
	2	0.1	0.0	1.0
	3	0.1	0.0	2.0
	4	0.0	0.0	0.0

2.3. Application of thermotherapy

Two different thermotherapy methods were applied before surface sterilization:

– short-term thermotherapy, where shoot tips were kept in water at 50 °C for 10–12 min and washed with tap water for 3–4 min, before the meristems were isolated,

– long-term *in vitro* thermotherapy, where shoot tips were kept at 38 °C and 70% relative humidity for 45 days.

Control meristems were cultured without any thermotherapy application.

2.4. Virus detection

In Alkuden and Bursa Siyahi explants that were infected with fig mosaic virus, dsRNA analysis was done in order to determine the effects of different thermotherapy and meristem culture treatments on virus elimination.

In the application of this test, leaf samples known to be infected with fig mosaic virus were collected and used as a positive control. Agarose gel electrophoresis results of the samples taken from plants produced from seeds and obtained by meristem culture, and thought to be free of the virus, resembled each other. In electrophoresis results, bands belonging to plant nucleic acids with almost 23 bp molecule weight were achieved.

2.5. Plantlet establishment

Rooted shoots from the cultures were planted in a [50% peat / 50% Perlite] mixture in plastic trays. Relative humidity was adjusted to 96–99% during the first week before being gradually reduced over the next 2 weeks. Later, the plants were acclimated to the external environment by gradually opening the plastic tunnels that covered the trays.

2.6. Statistical analysis

Analysis of variance of the data was conducted as appropriate for a completely randomized experimental design with three replications, each replicate containing five meristems for *in vitro* micropropagation. Different groups of means were separated by the Tukey test. All statistical analyses were performed with MSTAT.

3. Results

3.1. Viability rates of meristems

For the Bursa Siyahi cultivar, different benzyladenine (BA) concentrations did not significantly affect the viability of meristems. Averaged across all thermotherapy treatments, the best result (79.1%) was obtained on medium 4 (0.2 mg $GA_3 \cdot L^{-1}$ + 0.5 mg $BA \cdot L^{-1}$ + 0.1 mg $IBA \cdot L^{-1}$), while the lowest

viability rate (62.2%) was obtained on medium 2 (0.1 mg $GA_3 \cdot L^{-1}$ + 0.5 mg $BA \cdot L^{-1}$ + 0.1 mg $IBA \cdot L^{-1}$) (*table II*). On the other hand, for the Alkuden cultivar, the best result was found on medium 3 (0.2 mg $GA_3 \cdot L^{-1}$ + 0.2 mg $BA \cdot L^{-1}$ + 0.1 mg $IBA \cdot L^{-1}$), while the lowest rate was found on medium 2 (0.1 mg $GA_3 \cdot L^{-1}$ + 0.5 mg $BA \cdot L^{-1}$ + 0.1 mg $IBA \cdot L^{-1}$) (*table III*).

Table II.
Meristem viability (%) in the Bursa Siyahi fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	73.3	73.3	80.0	86.7	78.3 a
MC + short-term thermotherapy	53.3	46.7	53.3	73.3	56.7 b
MC + long-term thermotherapy	100	66.6	77.7	77.7	80.5 a
Mean	75.5	62.2	70.3	79.1	–

MSD 5%: not significant for (treatment × medium), and for (medium) D 5% (treatment): 17.0.

Table III.
Meristem viability (%) in the Alkuden fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	73.3	40.0	46.7	46.7	51.7
MC + short-term thermotherapy	60.0	46.7	80.0	60.0	61.7
MC + long-term thermotherapy	33.3	66.6	66.6	50.0	54.1
Mean	55.5	51.1	64.4	52.2	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

Table IV.
Meristem shoot formation (%) in the Bursa Siyahi fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	44.4	63.9	58.9	70.0	59.3
MC + short-term thermotherapy	50.0	44.4	50.0	47.2	47.9
MC + long-term thermotherapy	50.0	72.2	55.5	72.2	62.5
Mean	48.1	60.2	54.8	63.1	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

For the Bursa Siyahi cultivar, thermotherapy treatments caused statistically significant differences (*table II*). Meristem culture on medium without thermotherapy (78.3%) and meristem culture + long-term thermotherapy treatment (80.5%) gave similar results and were placed in the same statistical group. Alkuden did not show statistically significant differences for the viability rate of meristems. The best result (61.7%) was found with meristem culture + short-term thermotherapy treatment (*table III*).

3.2. Shoot formation rates of meristems

For Bursa Siyahi, different BA and GA_3 applications did not significantly affect the shoot formation; however, the highest shoot development (63.1%) was obtained on medium 4 with 0.2 mg $GA_3 \cdot L^{-1}$ + 2 mg $BA \cdot L^{-1}$, then (60.2%) with the medium 2 (0.1 mg $GA_3 \cdot L^{-1}$ + 2 mg $BA \cdot L^{-1}$). BA had a greater effect than GA_3 on shoot development. Long-term thermotherapy-treated meristems showed the highest shoot formation (*table IV*). However, for Alkuden, the highest shoot development (59.2%) was obtained on medium 3 (0.2 mg $GA_3 \cdot L^{-1}$ + 1 mg $BA \cdot L^{-1}$) (*table V*).

3.3. Rooting

For root formation, there were no significant differences between the applications. However, the highest root formations, 55.6% for Bursa Siyahi' and 53.7% for Alkuden, were obtained on medium 1 without BA or IBA (*tables VI, VII*). Meristem culture on medium without thermotherapy treatment showed better root formation for Bursa Siyahi (44.4%) and for Alkuden (44.5%) than treatments with thermotherapy.

3.4. Root formation per plant

In both cultivars, control plants cultured on medium without thermotherapy showed

better rooting than the others. Among the treatments, medium 1 (basic culture medium with only 0.1 mg $GA_3 \cdot L^{-1}$) showed the highest root formation. The rooting rates were 4.6 roots per plant for Bursa Siyahi and 2.6 roots per plant for Alkuden (*tables VIII, IX*).

4. Discussion

In our experiment, for Bursa Siyahi, the shoot formation rate was the highest in medium 4 with 2 mg $BA \cdot L^{-1}$ and 0.2 mg $GA_3 \cdot L^{-1}$ and a long-term thermotherapy treatment, and, for Alkuden, it was the highest in medium 3 with 1 mg $BA \cdot L^{-1}$ and 0.2 mg $GA_3 \cdot L^{-1}$ but without thermotherapy treatment. Researchers such as Günver and Ertan [6], Demiralay *et al.* [5] and Barbosa *et al.* [22] stated that shoot formation was high in medium containing 1 mg $BA \cdot L^{-1}$, while other researchers such as Kumar *et al.*, [23] and Brum *et al.* [24] found the best effect with 2 mg $BA \cdot L^{-1}$.

According to our results, the best medium for rooting rate was medium with no IBA for both genotypes. A similar result was found by Demiralay *et al.* [5]. Some researchers reported that root formation is high in media containing 2.5 µM IBA (Hepaksoy and Aksoy [8]), or 2 mg $IBA \cdot L^{-1}$ (Kumar *et al.* [23] Günver and Ertan [6], and Yancheva *et al.* [25]). As a result, medium 4 gave better results than the others.

As Açikgöz and Dökmen [4] stated, bands were not observed between 6.6 kbp and 0.6 kbp molecular weights belonging to the fig mosaic virus. Furthermore, no bands were observed in the results of dsRNA analysis done of infected samples and fig mosaic virus taken from the field. Korkmaz [26], in his collected work, stated that a lot of potyvirus could not produce large amounts of dsRNA and, for this reason, its diagnosis was not possible with dsRNA techniques. Also, in some situations (age, climate, etc.), field samples contain a small amount of dsRNA.

In vitro thermotherapy permits the acceleration of the process of obtaining healthy plants because treatments can be carried out all year round and many plants can be treated at the same time, thus increasing the chances of survival of healthy clones [3].

Table V.
Meristem shoot formation (%) in the Alkuden fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	63.9	70.0	44.4	50.0	56.9
MC + short-term thermotherapy	44.4	50.0	50.0	50.0	41.7
MC + long-term thermotherapy	50.0	17.0	83.3	33.3	37.5
Mean	47.2	45.7	59.2	44.4	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

Table VI.
Meristem rooting (%) in the Bursa Siyahi fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	66.6	44.4	44.4	22.2	44.4
MC + Short-term thermotherapy	50.0	33.3	50.0	33.3	41.7
MC + Long-term thermotherapy	50.0	17.0	50.0	33.3	37.5
Mean	55.6	31.5	48.2	29.7	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

Table VII.
Meristem rooting (%) in the Alkuden fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	44.0	83.3	33.4	16.7	44.5
MC + short-term thermotherapy	50.0	50.0	27.8	33.4	40.3
MC + long-term thermotherapy	66.7	–	33.4	33.3	37.5
Mean	53.7	44.5	31.5	27.8	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

Table VIII.
Number of roots per plant issued from meristems of the Bursa Siyahi fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	6.3	5.3	4.3	1.6	4.4
MC + short-term thermotherapy	3.6	2.3	2.3	1.6	2.5
MC + long-term thermotherapy	4.0	1.0	1.3	1.6	2.0
Mean	4.6	2.9	2.6	1.6	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

Table IX.
Number of roots per plant issued from meristems of the Alkuden fig cultivar cultured on four media containing a Murashige and Skoog medium with various concentrations of growth hormones (*table I*) (Turkey).

Treatment	Media				Mean
	1	2	3	4	
Meristem culture (MC)	3.0	4.0	0.7	1.3	2.3
MC + short-term thermotherapy	2.3	2.3	1.6	1.0	1.8
MC + long-term thermotherapy	2.6	–	1.3	1.0	1.2
Mean	2.6	2.1	1.2	1.1	–

MSD 5% not significant for (treatment × medium), (medium) and (treatment).

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Cultivo de meristemas de dos cultivares de higuera en Turquía.

Resumen — Introducción. Dos cultivos de higuera turcos, Alkuden y Bursa Siyahi, se multiplicaron mediante cultivo de meristemas con el fin de deshacerlos del virus del mosaico de la higuera. Se llevó a cabo un análisis del ARN en plantones obtenidos *in vitro* con el fin de testear su estado frente al virus. **Material y métodos**. Cuatro medios diferentes de Murashige y Skoog (MS), completados por diversas concentraciones de hormonas de crecimiento (AG_3, BA y IBA), se compararon con el fin de estudiar el porcentaje de supervivencia de las meristemas puestas en cultivo, la formación de tallos y el enraizamiento. Asimismo se aplicaron tratamientos de termoterapia corta y larga. **Resultados**. Para el índice de supervivencia de las meristemas puestas en cultivo, la asociación de hormonas más favorable fue 0,2 mg $AG_3 \cdot L^{-1}$ + 0,5 mg $BA \cdot L^{-1}$; para la formación de las tallos, el mejor medio fue con 0,2 mg $AG_3 \cdot L^{-1}$ + 2,0 mg $BA \cdot L^{-1}$; mientras que el índice de enraizamiento más alto se alcanzó para meristemas cultivadas en medio MS con sólo 0,1 mg $AG_3 \cdot L^{-1}$. **Conclusión**. El cultivo de meristemas asociado con un tratamiento de termoterapia se recomienda para obtener plantones de higueras indemnes de virus. A pesar de que los plantones parecieran sanos, se recomiendan medidas de ARN bicatenario con el fin de evaluar su estado sanitario.

Turquía / *Ficus carica* / micropropagación / cultivo de meristemas / sustancias de crecimiento vegetal / termoterapia / virus de las plantas