

ELIMINATION OF FIG MOSAIC FROM FIG SHOOT-TIP CULTURES BY THERMOTHERAPY

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Abstract

Apical buds (0.5-0.7 cm) of fig tree (*Ficus carica* L.) of cvs. "Urdana", "Napolitana" "Tiberio" and "Villalba" with evident mosaic symptoms were cultured in MP solid medium (Pontikis and Melas, 1986) and then subjected to an alternating, high temperature regime with 16 h light (5.000 lux) at 37°C followed by 8 h dark at 34°C. These *in vitro* techniques resulted in fig plants with no external symptoms of fig-mosaic disease after a year of pot cultivation in the greenhouse. Since *F. carica* is tolerant to high temperature treatments, the *in vitro* thermotherapy with alternating temperature is an appropriate method to eliminate the fig mosaic. This method allows production of a large number of treated shoot tips with high survival and regeneration rates. Indexing showed an absence of fig mosaic disease in all the plants obtained by this treatment.

1. Introduction

The fig tree (*Ficus carica* L.) is a typical species of Mediterranean countries. In the last years, as a result of the current crisis in agriculture and the increasing demand for diversity in agricultural products by consumers, this species has been considered as a potential alternative fruit crop for some agricultural areas in Spain. However, establishing new commercial orchards requires the study of different aspects, like plant material, methods of propagation, pruning, fertilization, fresh fruit conservation, etc. which has already been initiated (Toribio, 1993). Another of these aspects could be the sanitary selection, because figs are prone to fig mosaic, an endemic disease that is widely distributed in most varieties and countries where figs are cultivated. This disease, first described by Condit and Horne in 1933, mainly affects leaves, young branches and fruits. The symptoms are expressed as very evident mosaic lesions on the leaves. These lesions can also appear as diffuse areas of chlorosis, often with well-defined margins. In addition, this disease induces distortions in leaf shape and reductions in leaf area (Casalicchio, 1964). Because there are no field treatments, the most effective strategy is the production of healthy plants to be planted in new commercial orchards. Moreover, the production of healthy plants also allows the study of how the fig mosaic affects production, fruit quality and conservation.

Although the success depends on many factors (Nemeth, 1986), thermotherapy - high temperature treatments (35-37 °C) for certain periods of time - has been applied in many species against viruses in woody plants. For this reason, different authors have used an *in*

vitro system to obtain healthy plants of species that are sensitive to high temperatures, like cherry (Snir and Stein, 1985; Deogratias *et al.*, 1989; Gella *et al.*, 1995) or peach (Stein *et al.*, 1991).

Healthy fig plants were obtained by traditional thermotherapy, but there were plant regeneration problems (Martelli, 1966; Savio, 1978). Later on, Muriithi *et al.*, (1982) tried to produce mosaic-free plants by promoting the elongation of apices developed *in vitro* under traditional thermotherapy, without micropropagating the material obtained. Pontikis and Melas (1986) micropropagated fig shoots, but they did not study the sanitary status.

In the present study, fig shoot-cultures infected with fig-mosaic were established with the objective of obtaining mosaic-free figs by *in vitro* therapy. This study included *in vitro* culture of shoot-tips developed during the treatment, micropropagation of regenerated shoots, rooting, plant acclimatization and indexing.

2. Materials and methods

2.1. Plants

Fig tree plants with evident mosaic symptoms of cvs. "Urdana", "Napolitana", "Tiberio" and "Villalba" were propagated by hardwood cuttings and were grown in pots under greenhouse conditions. These plants were utilized as source plants for *in vitro* propagation.

2.2. *In vitro* propagation and heat therapy

Apical buds (0,5-0,7 cm) were sterilized in a solution of sodium hypochlorite (5 g.l-1 available Cl) and 0,01% Tween-20 for 20 minutes and rinsed 4 times in sterile distilled water.

Buds were cultured in the solid medium (MP) described by Pontikis and Melas (1986). Shoot cultures were maintained in a controlled environment chamber at $22 \pm 2^\circ\text{C}$ with a light intensity of 2.500-3.000 lux and a long day photoperiod (16 h light/ 8 h dark). Shoots were subcultured every 4 weeks and transferred to fresh medium every 4 weeks for 8 months.

Heat treatments were given to the plant material in tissue culture: microcuttings (1,5-2 cm) with 2 or 3 axilar and devoid of terminal apex were subjected to the following alternating temperature regime: 16 h light (5.000 lux) at 37°C followed by 8 h dark at 34°C .

The heat treatments were finished when shoots started to display high temperature effects (chlorotic leaves, low shoot growth, necrotic tips, etc.). At this moment, the tips (1-2 mm) developed under these treatments were excised and in-vitro cultured on MP medium.

2.3. Rooting and acclimatization

In vitro and *ex vitro* rooting ability of fig (*Ficus carica* L.) microcuttings was previously studied in our laboratory with the objective of establishing the most effective method (Lopez Corrales and Gella, 1994). *Ex vitro* rooting was carried out in the greenhouse: microcuttings (2-2,5 cm) were placed directly into Jiffypots with the substrate in absence of hormonal solution. Immediately afterwards, they were covered with a plastic tunnel, which was opened daily during increasing periods of time. After the development of roots (4 weeks), the plants were transplanted into bigger pots containing peat and vermiculite (1:1).

2.4. Indexing

The presence or absence of fig mosaic in plants obtained after *in vitro* therapy was quantified by an indexing protocol developed for this species (Lopez Corrales, 1996). This protocol consists in chip budding in virus-free indicator plants, which were obtained from seeds of cv. "Tiberio" and propagated *in vitro*. Temperature and two successive cutting-backs were the two most important factors that had to be optimized to obtain at least 60% of symptom transmission ratio into indicator plants.

The indexing blocks consisted of: 5 indicator plants inoculated with a bark piece from only one thermotreated plant. The positive controls were 5 indicator plants by each three treatments inoculated with a bark piece of fig mosaic infected plants. The negative controls consisted of 5 indicator plants, inoculated with their bark, by each 3 treatments.

3. Results

3.1. *In vitro* culture and heat therapy

Fig tree plants were easily cultivated *in vitro*, although some phenolic compounds appeared on the base of the explants during the establishment of the culture.

Fifteen days after heat therapy treatments, the shoots of the different cultivars showed good aspects as leaves of intense green colour, shoot multiplication and development of axilar buds were observed. Afterwards, the shoots became chlorotic and the growth rate decreased rapidly.

All cultivars appeared to be tolerant to heat therapy, and there was no difference in tolerance between cultivars. In general, shoots with bad aspects and little vigor are more sensitive to thermotherapy.

The number of treatment days was large for all cultivars.

At the end of the heat treatments, shoot-tips (1-2 mm) excised from fig tissue culture were of a pale green colour, but when they started to grow the leaves exhibited a deep green colour and great survival rates during the first subculture (Table 1)

High percentage of ex vitro rooting and acclimatization was obtained and the plants were of a high quality and normal appearance.

None of the regenerated plants had shown symptoms of fig mosaic after a year in pots in greenhouse.

3.2. Indexing

The indexing protocol showed an absence of the fig mosaic in all the plants obtained by thermotherapy.

4. Discussion

This study demonstrates that *in vitro* cultivated fig shoots are tolerant to prolonged heat treatments. Also, *in vitro* culture of tips excised after thermotherapy is a good method for plant regeneration due to their micro propagation ability.

This method gave better results than those utilized by Martelli (1966) and Savio (1978). It is an easier method than the traditional thermotherapy, with a very high survival rate of shoot-cultures. Also, *in vitro* culture allows for the control of certain factors (like temperature, lighting and humidity) that affects the success of plant regeneration. Barba *et al.* (1992) regenerated plums and peach plants after thermotherapy by *in vitro* culture of tips, but they obtained low rooting and decreased healthy plant rates.

In vitro thermotherapy treatments with alternating temperature regime (16 h light at 37°C followed by 8 h dark at 34°C) was an effective method and resulted in elimination of fig mosaic and greater survival rates of shoot-cultures. This temperature regime was successfully applied to shoot-tip cultures of PNRSV-infected cherries (Gella *et al.*, 1995).

Alternating temperatures were used for inactivation of PNRSV in cultured tissue of *Prunus persica* L. (Stein *et al.*, 1991). However, in other species (*Musa acuminata*) a constantly high temperature regime was more effective against other viruses (Wu and Su, 1991).

In vitro thermotherapy permits 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. Also, the indexing protocol allows for the screening of the presence of the fig mosaic in plants obtained by thermotherapy.

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Table 1 - Tips obtained by *in vitro* thermotherapy and survival

Cultivar	Heat-treatment days	Number of tips excised	Number of survival tips
Urdana	24	36	33
Napolitana	33	15	12
Napolitana	36	6	6
Napolitana	40	27	22
Tiberio	36	30	24
Tiberio	36	30	27
Villalba	30	40	39
Villalba	42	15	15