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Reproduction of Ficus pumila L. (Climbing Fig) with Tissue Culture

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ABSTRACT: ***Ficus pumila*** *L., which provides a green wall appearance with its very close texture, is not commonly grown in Turkey despite its significance. Among the studies published so far, there is no record of successful micropropagation of* ***Ficus pumila*** *species. Thus, the objective of the present study was to propagate* ***Ficus pumila*** *with tissue culture, and to expand the use of the species in Turkey. Various growth regulators and doses (IAA, BA, BA + Kinetin) were tested in studies where Murashige and Skoog (MS) compounds are used commonly as nutrient media. Plant leafs and leaf stems were used as explant. It was possible to obtain plants with different explant and nutrient media combinations. Cultures were kept in climate chamber at 23 ° C temperature and 70% humidity and in 16 hours light and 8 hours dark photoperiodic conditions. In the present study, it was observed that the combination of BA dose and cytokinin in BA and MS were successful in the findings. The highest shoot formation was obtained in MS medium supplemented with 1 mg / l BA doses and combinations.*

Keywords: ***Ficus pumila*** *L.,* ***in vitro****, climbing fig, Creeping fig, tissue culture.*

Ficus pumila L. (Tırmanıcı Kauçuk)'nın Doku Kültürü ile Çoğaltılması Üzerinde Çalışmalar

ÖZ: *Çok sık olan dokusuyla tamamen yeşil bir duvar görüntüsü veren* ***Ficus pumila*** *L., bu özelliği ile önem kazanasına rağmen ülkemizde bu türün kullanımı pek yaygın değildir. Bugüne kadar yayınlanan bilgiler arasında* ***Ficus pumila*** *türlerinde mikro çoğaltımın çok başarılı sonuçlar taşıdığına ilişkin bir kayıt bulunmamaktadır. Bu sebeple çalışmanın amacı* ***Ficus pumila****'nın doku kültürü ile çoğaltılmasını sağlamak, bununla birlikte ülkemizde kullanımını yaygın hale getirmek. Besin ortamı olarak Murashige ve Skoog (MS) bileşiminin yaygın olarak kullanıldığı çalışmalarda değişik büyüme düzenleyicileri ve dozları (IAA, BA, BA+Kinetin) denenmiştir. Eksplant olarak da bitkinin yaprak ve yaprak sapları kullanılmıştır. Farklı eksplant ve besin ortamı kombinasyonlarından bitki elde etmek mümkün olmuştur. Kültürler 16 saat aydınlık 8 saat karanlık fotoperyodik koşullarda, 23 °C sıcaklıkta ve % 70 nem ile iklim dolabında tutulmuştur. Bu çalışmada da ortaya konulan bulgular ışığında BA dozunun ve BA ile sitokinin kombinasyonlarında MS ortamında başarılı olduğu gözlemlenmiştir. En yüksek sürgün oluşumu 1 mg/l BA doz ve kombinasyonlarının eklendiği MS ortamında elde edilmiştir.*

Anahtar Kelimeler: ***Ficus pumila*** *L.,* ***in vitro****, sürünücü, tırmanıcı kauçuk, sürünücü, doku kültürü.*

INTRODUCTION

Creeping and climbing plants have fast-growing shoots and are easily used in several spaces due to this feature. They decorate walls or estrades very effectively. They also climb on enclosure elements such as wood and iron fences, covering their unwanted appearance and providing privacy. They provide shade by partially or completely covering architectural facilities such as pergolas, alcoves and arches.

The creeping and climbing plants are quite complacent compared to other ornamental plants. Their rapid growths even in adverse conditions are the reason for the selection of creeping plants (Güçlü, 1999, Tanrıverdi, 2001).

Ficus pumila L. (Climbing fig / Creeping fig) is a species with Chinese origins. This is a climber or creeping plant that grows on air roots. It is evergreen, and has small leaves in the form of a heart and they are 2.5 cm in length and 1.2 cm wide. It is a suitable species for sheltered places. The plant is resistant to drought and can also grow under extreme shadow. Fruit-bearing branches have broad leaves, but no air roots. It can also be grown indoors. In this case, the winter temperature should vary between 5º-8ºC. For propagation, head cuttings are used. Production; in hot, head steels. Vegetative cutting is used mainly in propagation; this imposes limitations for the breeding of high quality varieties. Sun-dried mature seeds which were traditionally used for propagation do not easily germinate. All of which are based on tissue culture as well as in this study. The *Ficus pumila* is very popular in Chinese but plantings are limited and do not meet the needs of growing markets (Hu and Liu, 1985; Hu *et al*., 1986; Chen, 1987; Pamay, 1993; Var, 2010).

MATERIALS AND METHODS

The study material *Ficus pumila* was procured from the Karadeniz Technical University, Faculty of Forestry breeding greenhouse.

The plant leaves and leave stems were used as explants. On the other hand, shoots of plantlets obtained from in vitro shoots were also considered as test material.

In the first experiment, leaves and leaf stalk explants (~ 1 cm in size) that were washed three times with distilled water were sterilized for 20 seconds in 70% ethanol and 30 minutes in 3% sodium hypochlorite. Thus, they were further sterilized for 2 minutes in 70% ethanol and 30 minutes in 3% sodium hypochlorite. In the last stage, they were washed in sterile distilled water and dried in sterile medium.

The experimental material and media were sterilized in the autoclave at 121 °C and under 1.05 kg/cm² pressure for 20 minutes, with the lids closed.

Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) which is the basic medium for in vitro shoot studies, was used based on the soil structure and material requirements in the natural plant habitat (Table 1).

Table 1. Basic nutrition media used in the study.
Çizelge 1. Çalışmada kullanılan temel besi ortamı.

Compound Bileşim	MS medium (mg/l) MS besi ortamı (mg/l)
Macro nutrient element Makrobesin elementleri	
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 . 2 H_2O$	440
$MgSO_4 . 7 H_2O$	370
KH_2PO_4	170
Micronutrient elements Mikrobesin elementleri	
H_3BO_3	6.200
$MnSO_4 . 4 H_2O$	22.300
$ZnSO_4 . 7 H_2O$	8.600
KI	0.830
$Na_2MoO_4 . 2 H_2O$	0.250
$CuSO_4 . 5 H_2O$	0.025
$CoCl_2 . 6 H_2O$	0.025
$FeSO_4 . 7 H_2O$	27.840
$Na_2EDTA . 2 H_2O$	37.240
Vitamins Vitaminler	
Nikotinik Asit	0.5
Thiamin HCl	0.5
Pridoksin HCI	0.5
İnositol	100.0
Glisin	2.0

For callus formation, 2 mg / l IAA, 1 mg / l BA, 3 mg / l BA + 1 mg / l Kinetin supplemented MS basic nutrient medium was used. The obtained calluses were renewed and placed in the same medium and the development of the shoots was observed. Callus became multiple shoots after 4 weeks and was rooted in the same medium after the fifth subculture.

6 g / l agar and 30 g / l sucrose were added to all media and the pH was adjusted to 5.7.

Germination experiments were conducted in a climatic chamber at a temperature of 25 ± 2 ° C for a 16/8 hour long day period.

Each experiment was set up in 3 replicates and 35 explants were used in total. At the end of the experiments, the obtained data were evaluated as percentage.

RESULTS AND DISCUSSION

Sterilized explants were taken to propagation experiments in a laminar flow sterile study cabinet.

Experiments were conducted in tubes and 50x93 mm culture jars. A total of 315 explants were tested in the MS medium. In the 2 mg / l IAA medium where 105 explants were tested, shoot formation was 67.6 ± 3.5%. In 1 mg / l BA medium where 105 explants were tested, shoot formation was 85.7 ± 2%. Furthermore, shoot formation in the 3 mg / l BA + 1 mg / l Kinetin medium with 105 implants was 83.8 ± 2.1% (Table 2).

Table 2. Shooting outcomes in different media.
Çizelge 2. Farklı ortamlarda görülen sürgün sonuçları.

Medium Ortam	Number of explants Eksplant sayısı	Shoot % Sürgün %
2 mg/l IAA	105	67.6 ± 3.5
1 mg/l BA	105	85.7 ± 2.0
3 mg/l BA+ 1 mg/l Kinetin	105	83.8 ± 2.1

The findings indicated that the best medium for *Ficus pumila* to develop shoots was MS medium that contained 1 mg / l BA (Figure 1). Kumar, *et al*. (1998), achieved 90% success in MS medium supplemented with 2 mg / l BA and 0.2 mg / l NAA on *Ficus carica*. These results support our work.

Healthy calli were formed and regeneration was observed in the subculture process in the same medium. The regenerated rootless plantlets that were cultivated in the subculture developed shoots in the same medium. Repeated subculturing may change the physiological state and gradually rejuvenate the shoot, which in turn promotes better rooting (Economou and Read, 1986). However, when these plantlets were kept in the subculture for more than 2 weeks, they faded out.

In the present study, callus and root formation was more common in cytokine-containing medium when compared to the auxin-containing medium. The initial explant hormone levels might have increased to desired levels during the subculture cultivation period. Thus, if the cytokine content in the culture medium is excessive, subculture cultivation without changing the media components might result in callus formation, regeneration and rooting, consecutively (Figure 2).

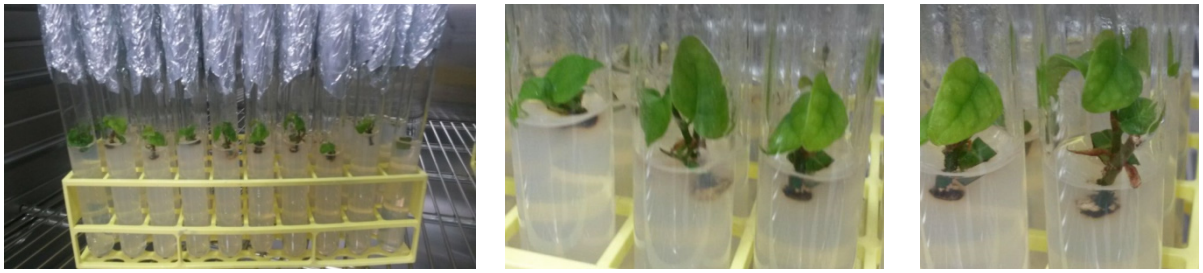

Figure 1. *Ficus pumila* explants forming shoots on MS medium.
Resim 1. *Ficus pumila* eksplantlarının MS ortamında oluşturduğu sürgün oluşumu.

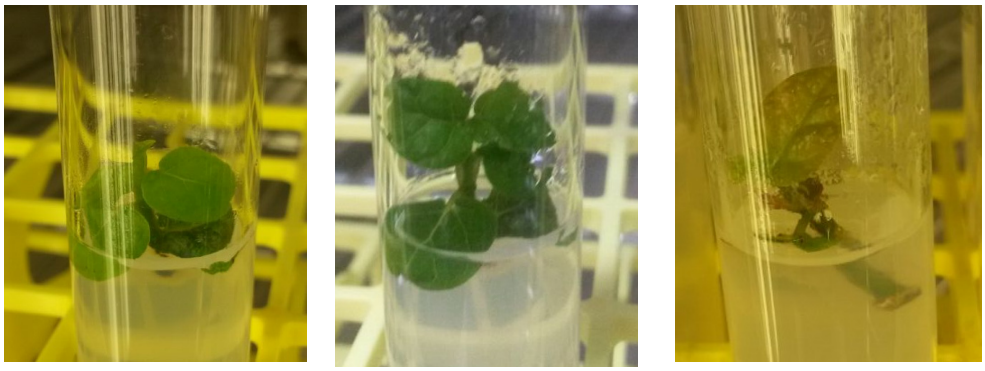

Figure 2. Shoot growth occurred in medium containing BA.
Resim 2. BA hormonu içeren besi ortamında gözlemlenen sürgün gelişimi.

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