MEDIUM-TERM IN VITRO STORAGE OF MATURE PISTACHIO MICROSHOOTS

Yelda Özden Çiftçi[1]* , Hülya Akdemir [1], Veysel Süzerer[1], Engin Tilkat[2], Ahmet Onay[3]

[1] *Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400 Gebze, Kocaeli*

[2] *Batman University, Faculty of Science and Literature, Department of Biology, 72100, Batman*

[3]*Dicle University, Faculty of Science and Literature, Department of Biology, 21280, Diyarbakır*

**ozden@gyte.edu.tr*

Abstract: The most common way of in vitro conservation of plant species concerns the usage of slow growth storage techniques in which temperature is reduced together with decrease of light intensity. Thus, in vitro proliferated microshoots of mature male "Atlı" and female "Siirt" were conserved in low temperature in dark for up to 12 months in this study in order to reveal out optimum storage period. Following the transfer of shoot apices excised from conserved microshoots to standard in vitro conditions, 97% proliferation was achieved even after the longest tested storage period. In addition, 2.3 and 2.6 microshoots per explant were also obtained in "Atlı" and "Siirt" cultures, respectively, with 12 months of storage. The relatively greater proliferation rates together with the higher number of shoots proliferated per explant indicated that in vitro-grown pistachio microshoots could be stored for longer period at low temperature without requiring subculture. The optimized technique has potential to be used for development of effective medium-term conservation protocols for other *Pistacia* species.

Key words: "Atlı", low temperature, medium-term storage, pistachio, "Siirt"

Olgun Antepfıstığı Mikrosürgünlerinin Orta Süreli
In Vitro Saklanması

Özet: Bitki türlerinin in vitro koşullarda saklanmasının en yaygın yolu ışık şiddeti ile birlikte sıcaklığında azaltıldığı yavaş büyüme saklanması tekniğinin kullanımıdır. Bu nedenle, in vitro çoğaltılan olgun erkek "Atlı" ve dişi "Siirt" mikrosürgünleri optimum saklama süresinin belirlenmesi için bu çalışmada 12 ay düşük sıcaklıkta saklandı. Saklanan mikro gövdelerden alınan gövde uçlarının standart in vitro koşullara aktarılmalarını takiben denenen en uzun saklama süresinden sonra bile %97 proliferasyon elde edildi. Buna ek olarak, "Atlı" ve "Siirt" çeşitlerinde 12 ay saklama sonrasında eksplant başına sırasıyla 2.3 ve 2.6 mikrosürgün oluştu. Görece yüksek proliferasyon oranları ile birlikte eksplant başına oluşan fazla sürgün sayısı, in vitro büyütülen antepfıstığı gövdelerinin alt kültürlenmeye gereksinimi olmadan düşük sıcaklıkta daha da uzun süre saklanabileceğini göstermektedir. Geliştirilen saklama tekniğinin diğer *Pistacia* türlerinin orta süreli in vitro saklanması için etkin protokollerin geliştirilmesinde kullanılma potansiyeli bulunmaktadır.

Anahtar Kelimeler: antepfıstığı, "Atlı", düşük sıcaklık, orta süreli saklama, "Siirt"

1. INTRODUCTION

Pistachio is grown in Iran, United States, Turkey, Syria, China, Greece, Afghanistan,Tunisia and Italy [1]. However, genetic erosion of pistachio together with other wild *Pistacia* species is evident in Central and West Asia and North Africa and the Mediterranean due to the usage of a few commercial cultivars in orchards and the destruction of tree's natural habitat [2] by severe anthropogenic pressure. Thus, in recent years several attempts were made in conventional conservation as well as unconventional biotechnological conservation methods including micropropagation, slow growth storage and cryopreservation. Among them, successful micropropagation procedures were developed especially for mature male and female pistachio trees [3, 4]. A detailed information on micropropagation of both juvenile and mature pistachio was also recently reviewed [5]. However, it should also be noted that long-term micropropagation of explants is labor-intensive and increases the cost of micropropagation [6]. Moreover and more importantly, maintenance of in vitro shoot cultures with frequent subculture could result in loss of genetic stability of the propagated material. Therefore, development of effective medium-term conservation strategies is important not only to avoid these problems but also to preserve its germplasm.

Slow growth storage in which microshoots are cultured at decreased temperature and nutrient composition sometimes together with reduced light intensity, is the most preferred technique for medium-term conservation of plant species. In the case of pistachio, Barghchi [7] utilized this technique and stored microshoots in medium containing varying levels of abscisic acid (0.25-4 mg L^{-1}) and mannitol (2.5-4.0 mg L^{-1}) up to 18 months at 4^0C. As the data on recovery rate of microshoots and optimum duration of conservation were not presented in those study, the success of this technique should be investigated further to reveal out also if these growth retardants must be included to basal culture medium. Thus, slow growth storage technique was applied to both female ("Siirt") and male ("Atlı") mature pistachio microshoots to find out if the previously reported growth retardants were essential for successful medium-term conservation of germplasm.

2. MATERIAL AND METHODS

2.1. Plant Material

Vigorous shoots of male "Atlı" and female "Siirt" mature trees growing at Pistachio Research Institute, Gaziantep, were truncated in April. Then, apical shoot tips (1-2 cm, in length) were surface disinfected in 10% (v/v) sodium hypochloride (NaOCl) for 30 min, rinsed in sterile distilled water for 3 times and washed twice for 1h and transferred to modified 1 mg L^{-1} benzyladenine (BA), 0.5 mg L^{-1} gibberelic acid (GA_3), 30 g L^{-1} sucrose and 7 g L^{-1} agar containing modified fresh MS medium [8] with Gamborg vitamins [9]. Cultures were maintained at 25^0C under 16 h photoperiod of 36 µmol m^{-2} s^{-1} of light intensity. The proliferated microshoots were subcultured every 3-4 weeks until they were assessed for slow growth storage technique.

2.2. Medium-term in vitro storage of microshoots via slow growth storage

Microshoots (approx. 1 cm, in length) were transferred to above-mentioned medium and cultured at 4^0C in the dark for 3, 6, 9 and 12 months. Following the each storage period, status of the microshoots were checked and shoot tips were excised from conserved microshoots and transferred to fresh medium. Then, shoot tips were cultured at 25^0C under 16 h photoperiod of 36 µmol m^{-2} s^{-1} of photosynthetic photon flux provided by cool-white fluorescent lamps to reveal out optimum storage period.

2.3 Rooting and acclimatization

Shoot tips proliferated from conserved microshoots were subcultured at least 3 times prior to rooting. After, microshoots were rooted in MS medium supplemented with 2 mg L^{-1} indole butyric acid (IBA) for 4 weeks. Rooted plants were rinsed with tap water and planted on plastic pots containing a mixture of sterile sand, soil and peat (1:1:1 in v/v/v) and successfully acclimatized to greenhouse conditions.

2.4. Statistical analysis

Statistical analysis of percentages were assessed by using the test for homogeneity of proportions whereas significant treatment differences were evaluated via a non-parametric Post Hoc Multiple Comparisons Test [10] at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

In the present study, pistachio male and female microshoots were conserved in media containing 30 g L^{-1} sucrose as the sole carbon source. The status of the microshoots were good as 100% of "Siirt" and 91.3% of "Atlı" cultures were green even after the longest storage period tested (Figure 1). However, relatively higher values were scored in both of the tested cultivars that were conserved up to 9 and 12 months at 4^0C when compared with the 3 and 6 month stored microshoots. These results could be due to the better adaptation of microshoots to low temperature conditions in prolonged culture period.

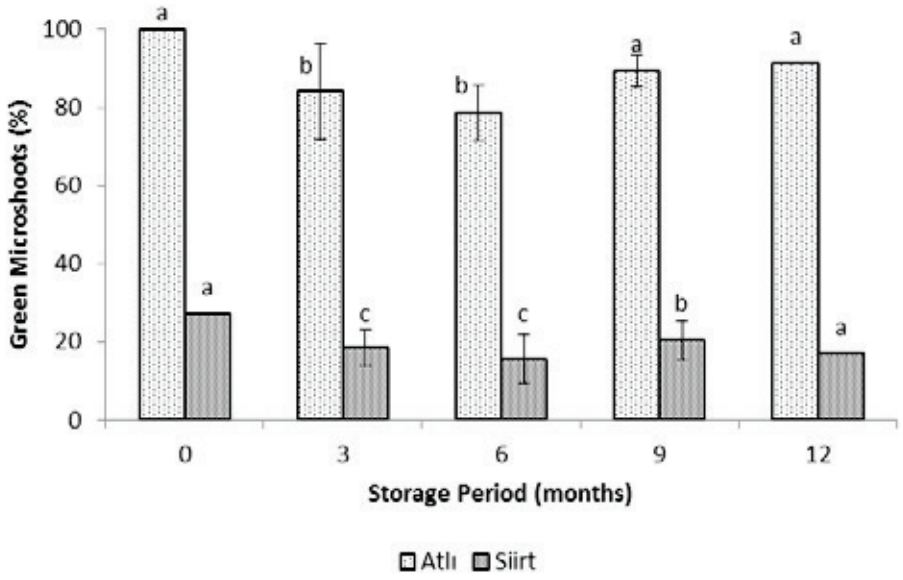

Figure 1: The influence of storage period on formation of green microshoots. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

The occurence of shoot tip necrosis is one of the most important obstacle faced in pistachio microshoots that cause rapid loses of cultures. However, with the culture of microshoots at low temperature, no significant differences were occurred in the formation of this symptom in both tested cultivars (Figure 2). This result also was not depended on the storage period. Thus, our results showed that the occurence of shoot tip necrosis was not related with at least tested storage period.

Figure 2: The influence of storage period on occurence of shoot tip necrosis. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

When the storage period was prolonged to 12 months, etiolated shoots started to be seen in both of the cultivars (Figure 3). The occurence of etiolated shoots was 60% in "Siirt" whereas it was quite low (2.5%) in "Atlı" cultivar. Thus, the frequency of occurence of etiolated shoots seemed to be varied with the storage period and tested cultivars. Nevertheless, the formation of etiolated shoots evidenced the adaptations of microshoots to low temperature.

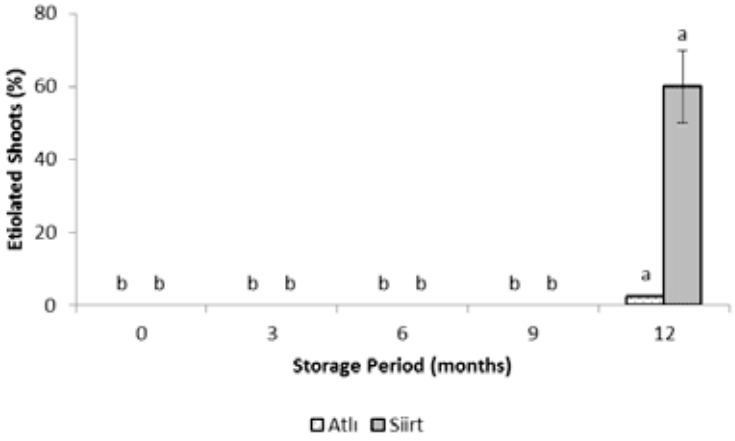

Figure 3: The influence of storage period on formation of etiolated microshoots. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

As regard the maintenance of proliferation capacity of shoot apices after storage at low temperature, Figure 4 showed that no differences were found in proliferation rates of explants. Although a decline was seen in proliferation rates of shoot tips excised from 9 or 12 month stored microshoots of "Atlı" and 12 month stored microshoots of "Siirt", these results were not significantly different. Moreover, 2.3 and 2.6 shoots were proliferated per explants from 12 month stored microshoots of "Atlı" and "Siirt", respectively (Figure 5). Thus, relatively higher shoot forming capacities were obtained in those explants (Figure 6).

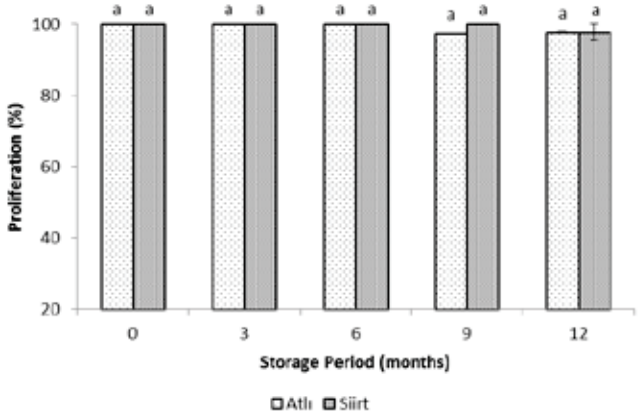

Figure 4: The influence of storage period on proliferation of shoot tips excised from in vitro stored microshoots. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

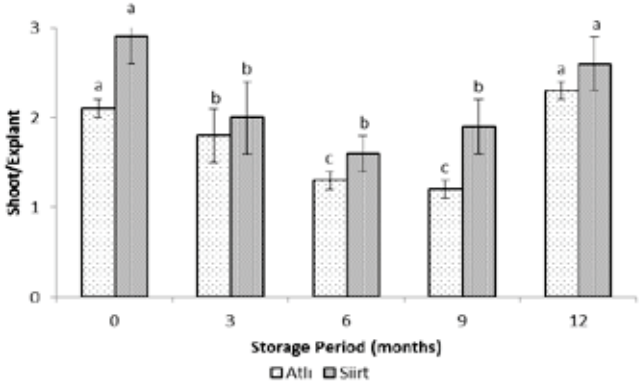

Figure 5: The influence of storage period on number of shoots proliferated per shoot tip excised from in vitro stored microshoots. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

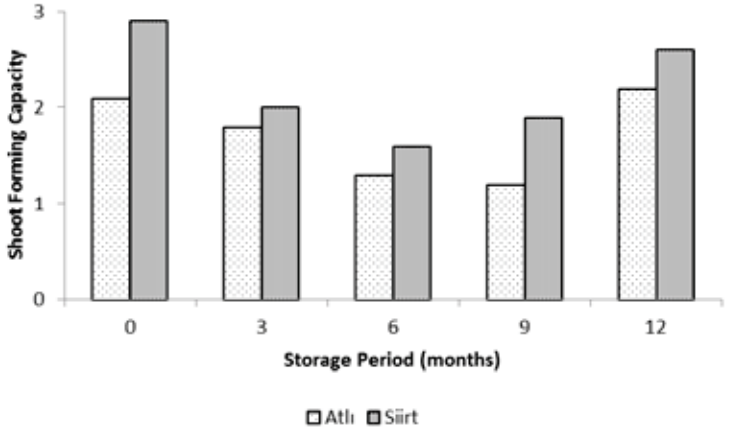

Figure 6: The influence of storage period on shoot forming capacity of shoot tips excised from in vitro stored microshoots. Means followed by the different letters in each cultivar are significantly different at P≤0.05.

With the transfer of rooting media, 80% rooting was achieved in both cultivars (data not shown) and plantlets were successfully acclimatized to greenhouse conditions.

The overall results showed that slow growth storage technique could be applied successfully to mature pistachio microshoots for medium-term conservation of cultures. The optimized protocol determined that the metabolism of pistachio microshoots could be reduced to acceptable level with the prolongation of storage period and light intensity without the requirement of addition of any growth retardants to storage medium. In contrast, the storage medium utilized in this study involved BA and GA_3 that could increase the metabolism. However, with the maintenance of cultures at low temperature and totally abolishment of the light, it was possible to conserve mature pistachio microshoots at least up to 12 months. The optimized protocol was not cultivar dependent so could be also applied to different pistachio cultivars. Moreover, the optimized protocol could reduce the cost of micropropagation and the occurence of somaclonal variation as the cultures maintained at low temperature did not require the transfer of shoots to fresh media every 3-4 weeks.

4. ACKNOWLEDGEMENT

This study was funded by a grant (209T030), from TUBITAK, the Scientific and Technological Research Council of Turkey. The study was also partially funded by a grant (2009-A10) from Gebze Institute of Technology.

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