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Investigation of the growing performance of promising almond genotypes obtained through selection breeding under *in vitro* conditions

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Abstract

Almond is one of the economically important nut species for Türkiye. There are many genotypes adapted to different regions of Türkiye. The propagation of these genotypes through plant tissue culture methods is essential. Among nut species, almonds are classified as recalcitrant plants that respond poorly to plant tissue culture techniques. In this study, the development of eight genotypes obtained through selection was examined under *in vitro* conditions in full and ½ NRM media containing different concentrations of IBA (0, 1, 2 mg/L). As a result of the study, the longest plant height was observed in genotype 48-6 (2.04 cm), while the highest number of leaves was obtained in genotype 33-B-44 (12.26 leaves). Rooting was observed in genotype 33-B-54, which was selected from Mut, Mersin, in a ½ NRM rooting medium supplemented with 2 mg/L IBA. The results are considered significant since these genotypes have been selected for the first time.

Keywords: Almond, *In Vitro*, Plant Tissue Culture, NRM

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INTRODUCTION

Almond belongs to the Rosales order and the Rosaceae family and is a nut species (Özbek, 1978). Almond, an economically important fruit species, is in increasing demand worldwide. Consequently, almond production areas have recently expanded in Türkiye and globally (Gök et al., 2020). Although production varies by year and climatic conditions, Türkiye ranks among the leading almond-producing countries. According to FAO data, the total global in-shell almond production in 2022 was 3.6 million tons. The highest almond-producing country was the United States, with 1,858,010 tons, followed by Australia, 360,328 tons, and Spain, 245,990 tons. Türkiye ranked fourth with a production volume of 190,000 tons (Anonymous, 2024a).

Almonds have been an essential food source in Asia and Europe for thousands of years. Natural selection and environmental adaptation efforts have continued, alongside challenges such as major diseases and insect pests, with farmers routinely selecting the best wild plants (Grasselly, 1972; Socias i Company, 2002; Gradziel & Martinez-Gomez, 2012). Superior genotypes have been identified and vegetatively propagated. In the Mediterranean region, Asia, and California, most modern cultivars have been developed more recently through clonal selection (Gradziel, 2003; Gradziel & Martinez-Gomez, 2012). Türkiye is known to have a rich genetic diversity of almonds, and selection studies on almonds are widespread (Şimşek, 2015).

Plant tissue culture plays an indispensable role in modern agriculture and plant breeding. Tissue culture methods enable various applications, from propagating plants at the cellular level to making genetic modifications. It is effectively used for preserving genetic diversity, developing disease-resistant plants, increasing productivity,

and supporting sustainable agricultural practices. Additionally, it plays a significant role in conserving endangered species. Plant tissue culture has great potential for increasing agricultural production and developing plants resistant to environmental conditions. Combined with genetic engineering and selection methods, it helps shape the future of agriculture (Yücel, 2022).

Numerous studies have established micropropagation protocols for *Prunus* species and hybrids by employing various nutrient media and concentrations of plant growth regulators. For example, Ünek et al. (2011) employed Murashige and Skoog (MS) medium with varying concentrations of 6-benzyleaminopurine (BAP), kinetin (Kin), 2-isopentenyladenine (2iP), and thidiazuron (TDZ); Dejampour et al. (2011) utilized a modified Driver and Kuniyaki Walnut (DKW) medium supplemented with 0.5 mg/L BAP; Sharifmoghaddam et al. (2011) used MS medium enriched with BAP and indole-3-butyric acid (IBA); Aghaye and Yadollahi (2012) experimented with MS medium containing various concentrations of BAP; Pakyürek and Hepaksoy (2019) used MS medium containing BAP, IBA, and gibberellic acid (GA_3) in different concentrations; Abbasi et al. (2019) utilized MS medium supplemented with BAP and TDZ; Ritterbusch et al. (2020) applied MS medium with different levels of BAP and Kin; and Borkheyli et al. (2021) employed MS, Woody Plant Medium (WPM), and DKW media with different BAP concentrations.

Genotypes obtained through selection studies need to be preserved and propagated. Plant tissue culture techniques provide an opportunity for propagating selected plants. This study aimed to investigate the growth performance of genotypes obtained through selection breeding under *in vitro* conditions.

MATERIALS AND METHODS

Plant Materıal

The plant materials used in this study included genotypes obtained through selection breeding from the Niğde and Mersin-Mut regions (Küden et al., 2023), as well as the previously selected local genotype 48-6 (Dokuzoğuz & Gülcan, 1973). The 48-6 genotype was obtained from the Alata Horticultural Research Institute. Eight genotypes were examined for their growth performance under *in vitro* conditions, including two from Niğde, five from the Mersin-Mut region, and the 48-6 genotype. Information regarding these genotypes is presented in Table 1.

Table 1. Names of the genotypes and the provinces where they were selected.

No	Name of genotype	Province
1	33-B-15	Mersin-Mut
2	33-B-32	Mersin-Mut
3	33-B-44	Mersin-Mut
4	33-B-54	Mersin-Mut
5	33-B-66	Mersin-Mut
6	51-B-02	Niğde
7	51-B-16	Niğde
8	48-6	Local genotype

Establishment of Experiment

Microshoots of eight genotypes were cultured in full NRM (Nas & Read, 2004) and ½ NRM media supplemented with different concentrations of IBA (0, 1, and 2 mg/L). The pH of the media was adjusted to 5.7. The media were poured into culture containers and sterilized in an autoclave at 121°C and 1.05 atm pressure. The plants were kept in darkness for the first week and then cultured under a 16-hour light/8-hour dark photoperiod. The plants remained in the culture medium for 8 weeks, and the temperature of the growth chamber was maintained at 25°C.

The experiment was designed according to a factorial arrangement in a completely randomized design (CRD) with three replications. In each treatment, 10 plants were planted. Data on plant height (cm**)** and number of leaves (count) were analyzed. The obtained data were subjected to variance analysis, and differences between significant means were determined using the LSD test. Statistical analyses were performed using JMP 5.01 software.

RESULTS AND DISCUSSION

According to the statistical analysis results, the interactions between genotypes × medium strength and genotypes × medium strength × IBA concentrations were significant regarding plant height ($P \leq 0.01$). Data on plant height (cm) and number of leaves (count) under different concentrations of IBA (0, 1, and 2 mg/L) in full NRM and ½ NRM media are presented in Table 2.

When genotype means were examined, the 48-6 genotype was identified as the most successful, with a plant height of 2.04 cm, while the 51-B-16 genotype had the shortest plant height at 0.69 cm. Regarding medium strength, plants in the ½ NRM medium reached a greater plant height (1.45 cm) than those in the full NRM medium. Differences between IBA concentrations showed that the most extended plant height was obtained in a

medium without plant growth regulators. According to the statistical analysis results, plant height ranged from 0.60 cm to 2.80 cm. The longest plant height (2.80 cm) was observed in the 48-6 genotype in ½ NRM medium supplemented with 2 mg/L IBA. In comparison, the shortest (0.60 cm) was recorded in the 51-B-16 genotype in full NRM medium supplemented with 2 mg/L IBA.

Regarding leaf number, significant differences were found between genotypes ($P \leq 0.01$). When genotype means were examined, the number of leaves was obtained in the 33-B-44 genotype (12.26 leaves), while the 33-B-54 genotype had the lowest number (6.71 leaves). Regarding medium strength, full NRM resulted in a higher number of leaves (10.20) than ½ NRM. However, differences between IBA concentrations and genotype × medium strength × IBA concentration interactions were not statistically significant. The 0, 1, and 2 mg/L IBA concentrations were in the same statistical group. According to statistical analysis results, the number of leaves ranged from 6 to 14.70. The highest number of leaves (14.70) was recorded in the 33-B-44 genotype in full NRM medium without plant growth regulators. In contrast, the lowest (6 leaves) was observed in the 33-B-54 genotype in ½ NRM medium without plant growth regulators.

Sevgin (2010) aimed to optimize the rooting stage in *in vitro* micropropagation of difficult-to-root almond cultivars **(**GF-677, Nonpareil, Ferragnes, and Tuano). As a result of the study, rooting rates in full-strength and ½-strength NRM media were similar**.** No rooting was obtained in the Ferragnes and Nonpareil almond cultivars. The best rooting was observed in the GF-677 rootstock**,** with a 64% rooting rate in the presence of 1 mg/L IBA**.** Nas et al. (2010) investigated the *in vitro* regeneration ability of *Prunus microcarpa* subsp. tortusa using various explants (root, cotyledon, and hypocotyl segments) and cytokinins (BAP), meta-topolin (mT), and TDZ). They cultured the micro shoots obtained from the proliferation medium in ½-strength NRM medium containing different concentrations of IBA. Over 70% of the microshoots were rooted within two weeks, and each shoot developed an average of three roots. In this study, rooting was observed in the 33-B-54 genotype in a ½ NRM medium supplemented with 2 mg/L IBA. However, the average root number was 0.2, which was very low. Since the rooting rate was insufficient, this data was not analyzed statistically. This result may be attributed to the genotype effect. Photographs showing the development of the 48-6 genotype in ½-strength NRM medium supplemented with 2 mg/L IBA and in full-strength NRM medium supplemented with 2 mg/L IBA are presented in Figure 1. Images of the 51-B-16 and 33-B-66 genotypes are shown in Figure 2.

2 mg/L IBA-Supplemented ½ NRM

2 mg/L IBA-Supplemented Full NRM

Figure 1. Image of the development of the 48-6 genotype.

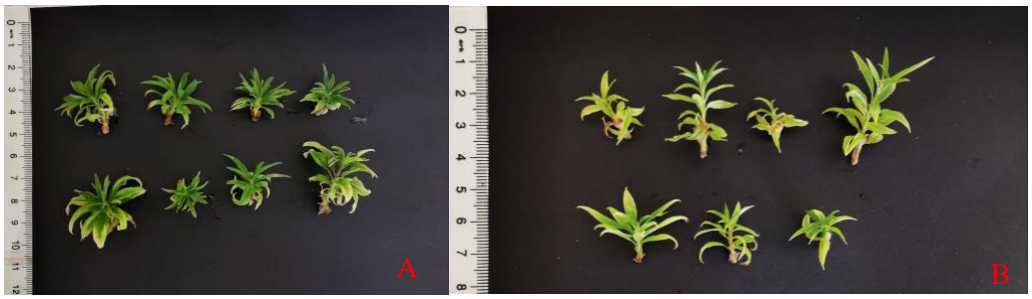

Figure 2. 51-B-16 Genotype in ½ NRM Supplemented with 2 mg/L IBA (A) and 33-B-66 Genotype in Hormone-Free Full NRM (B)

Table 2. Effects of different IBA concentrations on plant height (cm) and number of leaves in full NRM and ½ NRM media.

Genotype	Medium Strength	IBA Concentration	Plant height (cm)	Leaf Number	Plant Height Genotype Avg.	Leaf Number Genotype Avg.
33-B-15	NRM	0	1.54^{f-l}	9.80^{e-o}	1.33^{c}	
		1	1.40^{h-n}	8.80^{h-q}		
		2	0.97^{o-u}	7.90^{m-s}		
	½ NRM	0	$1.35^{ı-o}$	10.10^{e-n}		10.05^{cd}
		1	$1.35^{ı-o}$	11^{c-j}		
		2	$1.38^{ı-n}$	12.70^{a-d}		
33-B-32	NRM	0	1.12^{m-s}	10.70^{e-k}	1.42^{c}	
		1	$1.16l^{m-r}$	12.20^{b-e}		11^{bc}
		2	0.78^{r-u}	11.20^{e-h}		
	½ NRM	0	$1.75^{d-ı}$	10.30^{d-m}		
		1	$1.75^{d-ı}$	10.50^{d-l}		
		2	1.98^{b-e}	$11.10^{e-ı}$		
33-B-44	NRM	0	1.82^{d-g}	14.70^{a}	1.59^{b}	
		1	1.33^{j-o}	13.10^{a-c}		
		2	1.17^{l-r}	10.80^{e-k}		12.26^{a}
	½ NRM	0	$1.75^{d-ı}$	11.70^{e-f}		
		1	1.71^{d-j}	11.20^{e-h}		
		2	1.79^{d-h}	12.10^{e-e}		
33-B-54	NRM	0	0.86^{p-u}	6.90^{q-s}	0.90^{e}	
		1	0.84^{q-u}	6.90^{g-s}		
		2	0.74^{s-u}	7.70^{n-s}		
	½ NRM	0	$1.35^{ı-o}$	6^{s}		6.71^{f}
		1	0.90^{p-u}	6.60^{q-s}		
		2	0.75^{s-u}	6.20^{rs}		
33-B-66	NRM	0	2.07^{b-d}	11.70^{e-f}	1.68^{b}	
		1	1.44^{g-m}	11.90^{e-e}		
		2	1.20^{k-q}	11.30^{e-g}		
	½ NRM	0	1.85c-f	8.20^{l-s}		10.07^{cd}
		1	$1.75^{d-ı}$	8.60^{j-r}		
		2	1.81^{d-g}	$8.75^{ı-q}$		
51-B-02	NRM	0	1.25^{k-p}	14.55^{ab}	1.07^{d}	
		1	1.20^{k-q}	8.50^{k-r}		
		2	$1.35^{ı-o}$	10.70^{e-k}		9.37^{d}
	½ NRM	0	1.01^{n-t}	7.20^{p-s}		
		1	0.89^{p-u}	7.60^{o-s}		
		2	0.75^{s-u}	7.70^{n-s}		
51-B-16	NRM	0	0.67^{tu}	8.20^{l-s}	0.69^{f}	
		1	0.78^{r-u}	9^{g-q}		
		2	0.60^{u}	7.20^{p-s}		
	½ NRM	0	0.73^{s-u}	7.60^{o-s}		7.73^{e}
		1	0.68^{tu}	7.20^{p-s}		
		2	0.70^{tu}	7.20^{p-s}		
48-6	NRM	0	2.24^{bc}	11.40^{e-g}	2.04^{a}	
		1	1.58^{e-k}	10.40^{d-l}		
		2	1.50^{f-m}	9.40^{f-p}		
	½ NRM	0	1.85^{c-f}	12.20^{b-e}		11.36^{ab}
		1	2.30^{b}	12.10^{e-e}		
		2	2.80^{a}	12.70^{a-d}		
	IBA Concentration Avg.	0	1.45^{a}	10.07^{a}		
		1	1.31^{b}	9.72^{a}		
		2	1.26^{b}	9.66^{a}		
	Medium Strength Avg.	NRM	1.23^{b}	10.20^{a}		
		½ NRM	1.45^{a}	9.43^{b}		

Plant length: $Lsd_{Genotype}$:0.083 $Lsd_{Genotype * Nutrient Medium *IBA dose}$: 0.20 $Lsd_{number of leaves}$: 1.23
Number of Leaves: $Lsd_{Genotype}$:1.23 $Lsd_{Genotype * Nutrient Medium *IBA dose}$: 0.20

CONCLUSION

Almond is an economically important fruit. Since genotypes obtained through breeding are new individuals, they are valuable plant materials. Therefore, they must be preserved and propagated. In this regard, tissue culture techniques are effectively utilized. The micropropagation and rooting of selected genotypes through tissue culture techniques are particularly crucial. As these genotypes may possess characteristics of new rootstocks and cultivars, every finding obtained is of great significance.

Since almond is classified among the recalcitrant fruit species that respond poorly to tissue culture studies, the results of this study are valuable. This study investigated the development of almond genotypes selected from different regions under tissue culture conditions. As a result of the study, rooting was observed in the 33-B-54 genotype in a ½ NRM medium supplemented with 2 mg/L IBA. However, the average number of roots was 0.2, indicating insufficient rooting. Modifying nutrient media, developing new nutrient formulations, and using plant growth regulators at different concentrations and combinations to enhance growing performance under in vitro conditions is recommended.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that there is no actual, potential or perceived conflict of interest in this research article.

Author contribution

S.G, M.H.E, D.D, Y.A.K: Study data analysis, conceptualization and design of the research; S.G: Wrote the original draft; Y.A.K, D.D: Editing and preparation of the manuscript. All authors have read and approved the manuscript.

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