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Research Article

The Effects of IBA and BAP on *In Vitro* Shoot Production of Almond (*Amygdalus communis* L.)

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Abstract: In this study the possibilities of *in vitro* vegatitive propagation of almond (*Amygdalus communis* L.) cv. Texas and cv. Nonpareil by shoot-tip culture were investigated. Different levels of IBA (0.0, 0.1 and 0.5 mg/l) and BAP (0.0, 0.5, 1.0, 2.0 and 3.0 mg/l) were tested by observing shoot development and growth during three successive stages, namely initiation, transplantation and multiplication. During the initiation stage, hormone-free medium or medium with low IBA (0.1 mg/l) only seemed favourable for shoot growth. During both the transplantation and multiplication stages, the combination of 0.1 mg/l IBA and 1.0 mg/l BAP was found to be the most effective in terms of new shoot production and shoot growth rate. In general, BAP was shown to be essential for shoot development during the last two stages but high levels (2.0 or 3.0 mg/l BAP) caused vitrification and callus formation which subsequently reduced the viability of the shoots.

Key Words: Almond, tissue culture, micropropagation, shoot-tip culture.

IBA ve BAP'nin Bademde (*Amygdalus communis* L.) *İn vitro* Sürgün Verimine Etkileri

Özet: Bu araştırmada, Texas ve Nonpareil badem (*Amygdalus communis* L.) çeşitlerinin sürgün ucu kültürü ile *in vitro* vejetatif çoğaltma olanakları araştırılmıştır. Bu amaçla farklı IBA ve BAP düzeyleri, takip eden üç farklı kültür aşamasında (ilk dikim, şaşırtma ve çoğaltma) ayrı ayrı test edilmiş ve sonuçlar sürgün gelişmesi için hormon içermeyen veya sadece düşük düzeyde IBA (0.1 mg/l) içeren ortamların daha uygun olduğu belirlenmiştir. Hem şaşırtma hem de çoğaltma aşamasında ise, 0.1 mg/l IBA ile 1.0 mg/l BAP kombinasyonu sürgün verimi ve gelişmesi bakımından en iyi sonuçları vermiştir. Genel olarak, sürgün oluşumu için her iki aşamada da BAP'nin gerekli olduğu tesbit edilmiş fakat yüksek düzeylerde (2.0 veya 3.0 mg/l BAP) kullanıldığı zaman camlaşmaya ve sürgünlerin canlılığının azalmasına yol açan kallus oluşumuna neden olduğu görülmüştür.

Anahtar Sözcükler: Badem, doku kültürü, mikroçoğaltım, sürgün-ucu kültürü

Introduction

The almond (*Amygdalus communis* L. [syn. *Prunus amygdalus* Batsch]) is an important fruit species in Turkey, mainly grown along the coast of the Aegean and Mediterranean regions. Due to its heterozygous nature, clonal propagation of almond through tissue culture is critical for obtaining uniform material for both breeding and production purposes (1). With the aim of developing a reliable propagation protocol of almond, we have previously studied the effects of different sucrose, agar and pH levels on *in vitro* shoot production (2). In this study, we examined the effects of plant growth regulators on shoot development using different concentrations and combinations of IBA and BAP and the results were evaluated with regard to findings of the previous study (2).

Materials and Methods

Explant preparation, sterilisation, incubation and data evaluation were carried out as described previously (2). Murashige and Skoog medium (3) was supplemented with several concentrations of IBA (0.0, 0.1 and 0.5 mg/l) and BAP (0.0, 0.5, 1.0, 2.0 and 3.0 mg/l). Based on the results obtained from our previous work (2), sucrose was used at 5% during the first two stages but reduced to 3% during the last stage, agar at 0.5% during the first stage but then increased to 0.7% during the last two stages and with a pH of 5.5 during all stages.

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Results

Initiation Stage

No new shoot proliferation was observed during this stage and the explants were, therefore, evaluated for their growth rates only. The results clearly indicated that the medium with no plant growth regulators or with low (0.1 mg/l) IBA only was greatly favoured for shoot growth in both cultivars (Table 1). As BAP and IBA levels increased, the length vigour of the shoots decreased significantly. The shoot growth rate was lowest with the combination of the highest BAP (3.0 mg/l) and IBA (0.5 mg/l) concentrations. On the other hand, an extensive amount of callus developed when 1.0 mg/l IBA was used in the culture medium, which then caused the shoots to become inviable during the subcultures. Therefore, 1.0 mg/l IBA was excluded from the subsequent experiments during the transplantation and multiplications stages.

Transplantation Stage

It is clear from the data in Table 2 that BAP was essential for shoot production in both cultivars since no shoot development occurred on media without BAP. It appears that shoot production had a tendency to increase as BAP concentrations increased up to 2.0 mg/l but then decreased considerably at the highest BAP level (3.0 mg/l) when the medium contained no IBA, the highest number of shoots produced being 5.65 and 8.25 in cv. Texas and cv. Nonpareil, respectively. When IBA was added to the medium at a concentration of 0.1 or 0.5, the lower BAP concentrations (i.e., 0.5 and 1.0 mg/l) became more favourable for shoot production (Table 2). Shoot production was seriously reduced by combinations of high BAP (2.0 or 3.0 mg/l) and IBA (0.5 mg/l) concentrations in both cultivars.

As for shoot growth rates, the best results were obtained when low IBA (0.1 mg/l) and relatively low BAP (1.0 mg/l) concentrations were combained in the culture medium (Table 2). Shorter shoots developed at higher concentrations, i.e. at 0.5 mg/l IBA and 2.0 or 3.0 mg/l BAP combinations, which was consistent with the results obtained for mean shoot production per explant during this stage. A similar pattern was also observed in the colour of the shoots developed, 0.0 or 0.1 mg/l IBA with 1.0 mg/l BAP produced green and healthy shoots whereas those shoots developed at higher concentrations (0.5 mg/l IBA and 2.0 or 3.0 mg/l BAP) had a greenish-yellow colour. In addition, small amounts of greenish-white callus were observed at the base of the shoots with these combinations, and no vitrification occurred during this stage.

		BAP (mg/l)				
Cultivar	IBA (mg/l)	0.0	0.5	1.0	2.0	3.0
Texas	0.0	3.85^{a}	2.83^{b}	2.50^{b}	1.54^{c}	1.13^{c}
	0.1	3.50^{a}	2.58^{b}	2.25^{b}	1.25^{c}	1.20^{c}
	0.5	2.66^{b}	1.34^{c}	1.17^{c}	1.02^{c}	1.00^{c}
Nonpareil	0.0	3.80^{a}	2.86^{b}	2.69^{b}	1.62^{c}	1.18^{c}
	0.1	3.58^{a}	2.60^{b}	2.50^{b}	1.22^{c}	1.06^{c}
	0.5	2.70^{b}	1.57^{c}	1.41^{c}	1.15^{c}	1.05^{c}

Table 1. Mean shoot growth rate per explant during the initiation stage. Explants were cultured on MS medium containing several concentrations of IBA and BAP, 5% sucrose, 0.5% agar and pH 5.5 for three weeks. Values with the same letter are not significantly different at p=0.01.

Table 2. Mean shoot number (N) and mean shoot growth rate (GR) during the transplantation stage. Explants were subcultured on MS medium containing several concentrations of IBA and BAP, 5% sucrose, 0.7% agar and pH 5.5 for three weeks. Values with the same letter are not significantly different at p=0.01.

		BAP (mg/l)									
	IBA	0.0		0.5		1.0		2.0		3.0	
Cultivars	(mg/l)	N	GR	N	GR	N	GR	N	GR	N	GR
Texas	0.0	0.00	0.00	2.16^{de}	2.00^{b}	5.41^{ab}	1.47^{b}	5.65^{a}	1.50^{bc}	4.58^{ab}	1.35^{c}
	0.1	0.00	0.00	5.50^{ab}	1.29^{c}	4.91^{ab}	2.60^{a}	3.16^{cd}	1.45^{bc}	1.91^{de}	1.10^{c}
	0.5	0.00	0.00	5.08^{ab}	1.18^{c}	4.04^{bc}	1.43^{bc}	2.05^{de}	1.08^{c}	1.58^{e}	1.06^{c}
Nonpareil	0.0	0.00	0.00	4.00^{c}	2.50^{ab}	5.43^{bc}	2.33^{bc}	8.25^{a}	1.68^{cd}	6.33^{b}	1.14^{d}
	0.1	0.00	0.00	4.75^{bc}	3.09^{a}	6.16^{b}	2.66^{ab}	2.14^{d}	1.22^{d}	4.12^{c}	1.10^{d}
	0.5	0.00	0.00	4.75^{bc}	1.77^{cd}	4.08^{c}	1.31^{d}	2.33^{d}	1.06^{d}	4.38^{bc}	1.08^{d}

Multiplication Stage

The fact that no shoot development occurred on media without BAP regardless of the presence of IBA indicated that BAP was essential for shoot development during this stage too (Table 3). When IBA was excluded from the culture medium, the increasing BAP concentrations seemed to broadly promote shoot formation in both cultivars. However, when IBA was included in the culture medium at 0.1 or 0.5 mg/l concentrations, the higher BAP levels (2.0 or 3.0 mg/l) became less effective for shoot formation, with mean shoot production significantly decreasing to 1.6-3.9 shoots per explant while it ranged from 5.52 to 7.87 shoots per explant in combinations of the same IBA levels (i.e., 0.1 or 0.5 mg/l) with lower BAP (0.5 or 1.0 mg/l) in both cultivars (Table 3). As during the previous (transplantation) stage, the medium containing high concentration of IBA (0.5 mg/l) and BAP (2.0 or 3.0 mg/l) appeared to be the least effective for shoot production in the cultivars. It should be noted that the shoot-tip explants of both cultivars behaved quite similarly during both transplantation and multiplication stages in their culture responses to IBA and BAP.

In terms of shoot growth rates, low BAP concentrations (0.5 or 1.0 mg/l) with no or low IBA concentration (0.1 mg/l) produced much longer and healthier shoots than the other combinations (Table 3). The combinations of high IBA (0.5 mg/l) and BAP (2.0 or 3.0 mg/l) also produced greenish-yellow shoots while low concentrations (0.1 mg/l IBA and 0.5 or 1.0 mg/l BAP) produced green and healthy shoots throughout the culture. In addition, combinations of high levels of IBA (0.5 mg/l) and BAP (2.0 or 3.0 mg/l) caused greenish-white callus formation at the base of the shoots. As subcultures proceeded during this stage, high BAP concentrations resulted in some degree of vitrification, and production of shorter shoots with narrow and curled leaves.

Discussion

Depending on species or cultivars, the most important part of the success obtained in the propagation of many plant materials through tissue cultures has been frequently based on the successful adjustment of the type and combination of plant growth regulators (4-6). Of these, cytokinins and auxins are of extreme importance for, respectively, shoot and root development in plant tissues (7, 8). In our experiments, the growth of shoot explants during the initiation stage was greatly favoured by the absence of IBA and BAP (i.e., hormone-free medium) but unfortunately no new shoot proliferation was observed. Auxins are known to induce cell division in plant tissues and, thus, result in a large amount of callus production in many species (9, 10). When during the initiation stage, we included IBA at 1.0 mg/l combination with BAP, it caused an increased amount of callus formation, which subsequently seriously reduced the viability of the shoot explants. This suggests that relatively low auxin and cytokinin levels should be used during the initiation stage of shoot-tip cultures, as other studies have suggested (11, 12).

During the transplantation and multiplication stages, a similar response pattern was observed in relation to the effects of varying IBA and BAP concentrations and combinations on shoot development of shoot-tip explants. It was shown that the presence of low IBA and BAP, when used in combination, increased both the number of shoots developed and their subsequent growth. In this context, the concentration of 0.1 mg/l IBA and 1.0 mg/l BAP was determined to be the best combination for both shoot production and shoot growth in both cv. Texas and cv. Nonpareil. This combination was also found to be the most suitable for *in vitro* propagation of some other almond cultivars through shoot-tip culture (13, 14).

Table 3. Mean shoot number (N) and mean shoot growth rate (GR) during the transplantation stage. Explants were subcultured on MS medium containing several concentrations of IBA and BAP, 3% sucrose, 0.7% agar and pH 5.5 for 12 weeks ihvolving four subcultures. Values with the same letter are not significantly different at p=0.01.

		BAP (mg/l)									
	IBA	0.0		0.5		1.0		2.0		3.0	
Cultivars	(mg/l)	N	GR	N	GR	N	GR	N	GR	N	GR
Texas	0.0	0.00	0.00	3.02^{b}	1.41^{bc}	6.67^{a}	3.05^{a}	6.03^{a}	1.70^{bc}	5.83^{a}	1.20^{bc}
	0.1	0.00	0.00	5.52^{a}	1.70^{bc}	6.43^{a}	2.85^{a}	3.90^{b}	1.58^{bc}	2.72^{c}	1.72^{bc}
	0.5	0.00	0.00	6.44^{a}	1.79^{b}	5.45^{a}	1.22^{bc}	1.60^{d}	1.06^{c}	1.88^{cd}	1.22^{bc}
Nonpareil	0.0	0.00	0.00	4.02^{d}	2.81^{ab}	7.62^{ab}	3.40^{a}	6.77^{bc}	1.31^{cd}	6.41^{c}	1.22^{de}
	0.1	0.00	0.00	6.35^{c}	1.41^{cd}	7.87^{a}	2.64^{ab}	2.64^{ef}	1.14^{de}	3.22^{de}	1.20^{de}
	0.5	0.00	0.00	6.19^{c}	2.14^{bc}	5.61^{c}	1.20^{de}	1.93^{f}	1.25^{de}	2.04^{f}	1.10^{e}

High BAP levels (2.0 or 3.0 mg/l) caused the shoots to turn greenish-yellow with some vitrification in our explants. However, when low BAP (0.5 or 1.0 mg/l) was used, the shoots remained green and healthy, and no vitrification occurred. This may indicate that vitrification can be reduced by lowering BAP concentration in the culture medium as it has already been reported that high BAP levels caused vitrification in several plant species (15, 16).

With regard to our previous work (2), the optimal levels of sucrose, agar, pH, IBA and BAP on *in vitro* shoot regeneration from shoot-tip explants in almond cv. Texas and Nonpareil are summarised in Table 4. It outlines that a high sucrose level (5-6%) should be used during the first and second stages of the culture but it should be reduced to a lower agar level (3-4%) during the final stage. As for agar, unlike sucrose, the first stage needs a low agar level (0.5%) while the second and third stages require an increase up to 0.7% The pH optimum seems to be the most consistent, with all stages producing their highest yield at pH 5.5. For plant growth regulators, 0.1 mg/l IBA in combination with 1.0 mg/l BAP seems to be optimal for all three culture stages. Our results demonstrated that 5,000-20,000 new shoots can be produced from a single shoot in cv. Texas and cv. Nonpareil at the end of experiments lasting 18 weeks involving several subcultures. However, our preliminary attempts to root these shoots proved difficult. Therefore, a reliable protocol for *in vitro* propagation of almond can only be achieved if this problem is overcome in a reproducible manner, which would certainly need further studies.

Stage	Sucrose (%)	Agar (%)	pH	IBA (mg/l)	BAP (mg/l)
Initiation	5-6	0.5	5.5	0.1	1.0
Transplantation	5-6	0.7	5.5	0.1	1.0
Multiplication	3-4	0.7	5.5	0.1	1.0

Table 4. The overall summary of the results showing the optimum sucrose, agar, pH, IBA and BAP levels for *in vitro* shotts development and growth during three successive culture stages in almond (Prunus amygdalus Batsch) cv. Texas and cv. Nonpareil.

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