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In Vitro Propagation of the PR 204/84 Peach Rootstock (*Prunus persica* x *P. amygdalus*): the Effect of BAP, GA_3, and Activated Charcoal on Shoot Elongation

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Summary

PR 204/84 rootstock (peach x almond) is a promising rootstock for peach trees as an alternative to GF 677. The present study was the first attempt to micropropagate this genotype. Two experiments were conducted. In the first experiment, two benzylaminopurine (BAP) concentrations (0.08, 0.28 μM) were employed in combination with four gibberellic acid (GA_3) concentrations (0, 0.028, 0.28, 2.8 μM). It was observed, that although there is not a clear statistical difference between 0.08 μM BAP plus 0.028 μM GA_3 and 0.28 μM BAP plus 0 μM GA_3 in terms of shoot length, the first treatment was the most appropriate because the shoots possessed better macroscopic appearance. In the second experiment, two GA_3 concentrations (0.028, 0.28 μM) were combined with four concentrations of activated charcoal (AC) (0, 4.2, 8.4, 20.8 mM). BAP was fixed at 0.08 μM. It was observed that the treatments 0.028 μM GA_3 plus 20.8 mM AC and 0.28 μM GA_3 plus 8.4 mM AC in the medium during the elongation stage were not statistically different in terms of shoot length. However, inclusion of AC in the medium resulted in an improved overall macroscopic appearance of leaves and shoots in comparison to 0 mM AC.

Zusammenfassung

***In vitro*-Vermehrung der Pfirsichunterlage PR 204/84 (*Prunus persica* x *P. amygdalus*): Wirkung von BAP, GA_3 und Aktivkohle auf das Sprosswachstum.** Die Unterlage PR 204/84 (Pfirsich x Mandel) ist eine vielversprechende Pfirsichunterlage und eine Alternative zu GF 677. Die vorliegende Untersuchung ist der erste Versuch der *in vitro*-Vermehrung dieses Genotyps. Zwei Experimente wurden durchgeführt. Im ersten wurden zwei Benzylaminopurin- (BAP-) (0.08, 0.28 μM) mit vier Gibberellinsäure-(GA_3-)Konzentrationen (0, 0.028, 0.28, 2.8 μM) kombiniert. Obwohl keine statistischen Unterschiede zwischen den Kombinationen 0.08 μM BAP plus 0.028 μM GA3 sowie 0.28 μM BAP plus 0 μM GA_3 hinsichtlich Sprosslänge nachweisbar waren, erwies sich die erstgenannte Kombination als geeigneter, da das makroskopische Erscheinungsbild der Sprosse besser war. In einem zweiten Experiment wurden zwei GA_3-(0.028, 0.28 μM) mit vier Aktivkohle- (AC-)Konzentrationen kombiniert. BAP blieb konstant bei 0.08 μM. Dabei zeigte sich, dass die Kombinationen 0.028 μM GA_3 plus 20.8 mM AC sowie 0.28 μM GA_3 plus 8.4 mM AC im Medium während der Sprossstreckungsphase keinen statistisch nachweisbaren Einfluss hinsichtlich der Sprosslänge hatten. Allerdings verbesserte die Zugabe von Aktivkohle das makroskopische Erscheinungsbild von Blättern uns Sprossen.

Key words. benzylaminopurine – *Prunus* micropropagation – shoot proliferation.

Introduction

The PR 204/84 peach rootstock is a hybrid between peach and almond and was selected in the Pomology Institute of Greece. It is an alternative rootstock to GF 677 (peach x almond). It is adapted well to areas with low soil fertility, arid or semi arid climates, as well as in calcareous soils and replant sites. Furthermore, PR 204/84 has greater resistance to *Phytopthora*, *Verticillium dahliae* and *Agrobacterium tumefaciens* than GF 677 rootstock. However, vegetative propagation by hardwood cuttings resulted in relatively low percentage of success and it is more difficult to root than GF 677 (TSIPOURIDIS 2003). Therefore, there is a need to evaluate the micropropagation performance of PR 204/84 rootstock. Problems relevant to small shoot length at the end of the multiplication stage have been encountered in *in vitro* studies of *Prunus* species (CHENG 1979; ZUCCHERELLI 1979). At the elongation stage, BAP is generally reduced in concentration, or even omitted from the medium. GA_3 is often added to the elongation medium alone, or in combination with AC at various concentrations depending on the species (GEORGE 1996).

Thus, the objective of the present research was to study the effects of BAP, GA_3, and AC on shoot elongation stage of the PR 204/84 rootstock shoots *in vitro*.

Materials and Methods

First experiment

Shoot clumps obtained after a proliferating subculture on a MURASHIGE and SKOOG (MS) (1962) medium supplemented with 4 μM BAP+0.05 μM NAA (for 3 weeks) were divided into shoot clusters consisting of 4 axillary shoots with 4 leaves and finally selected for the experiment. Shoot clusters were used to determine the effects of GA_3 in combination with BAP in the medium on shoot elongation. Two BAP concentrations (0.08, 0.28 μM) were employed in combination with four GA_3 concentrations (0, 0.028, 0.28, 2.8 μM). In each trial, 0.5 l glass jars (4 shoot clusters per jar) containing 120 ml of MS medium supplemented with the above BAP and GA_3 concentrations at all possible combinations, were employed. The pH of the media was adjusted to 5.8 prior to autoclaving at 121 °C for 15 min. The jars were maintained in the growth room at 22±1 °C and 16 h cool white fluorescent light supplied (45μM m^{-2} s^{-1}, 400–700 nm) per day. Measurements of shoot length were taken after 2 weeks. Furthermore, observations were taken relevant to the general appearance of shoots and several characteristics such as chlorosis, tip necrosis, and stimulation of axillary shoots. A total of 8 treatments were employed with 20 replications (shoot clusters) per treatment. The experiment was repeated twice, and the reported data are the means of the two experiments. The statistical design employed was the randomized complete block one. Differences between means were evaluated using LSD test at P≤0.05. Furthermore, standard error of means was calculated.

Second experiment

Shoot clumps obtained after a proliferating subculture on MS medium supplemented with 4 μM BAP+0.05 μM NAA (for 3 weeks) were divided into shoot clusters consisting of 4 axillary shoots with 4 leaves. Two GA_3 concentrations (0.028, 0.28 μM) were selected from the first experiment. BAP concentration was kept consistent at 0.08 μM. The experiment was a factorial design combining two GA_3 concentrations (0.028, 0.28 μM) with four concentrations of AC (0, 4.2, 8.4, 20.8 mM). Twenty replicates (shoot clusters) per treatment were used with four replicates per glass jar. Activated charcoal was added into the medium prior to autoclaving at 121 °C for 15 min. After two weeks in culture, measurements of shoot length were performed. Furthermore, observations were taken relevant to the macroscopic appearance of shoots. Culture conditions and statistical analysis were similar to the first experiment.

Results and Discussion

First experiment

Inclusion of 0.08 μM BAP plus 0.028 μM GA_3 in the culture medium resulted in the formation of longer shoots in comparison to the other treatments containing 0.08 μM BAP (Table 1). In spite of the shortest stems, shoot clusters grown on media containing 0.08 μM BAP plus 0.28 μM GA_3 produced shoots with good characteristics. On the other hand, the presence of 2.8 μM GA_3 had a negative effect in comparison to 0.028 and 0.28 μM GA_3 on shoot quality by causing the cultures to decline. Thus, there is a benefit in terms of shoot length and overall appearance of shoots from using 0.028 μM GA_3 in the presence of 0.08 μM BAP in the culture medium. In order to elongate short shoots (attributed to an excess of BAP) during the proliferation phase of 'Mr.S. 2/5' plum rootstock, LORETI et al. (1982) placed the explants onto a new medium supplemented with 0.4 μM BAP and 1.4 μM GA_3. DIMASSI-THERIOU (1989) reported for GF 677 peach rootstock grown on MS culture medium, that a high concentration of GA_3 (2.8 μM) in combination with 4 μM BAP promoted maximum shoot length with good quality of shoots. LORETI et al. (1990) placed shoots of the peach rootstock PSB2 onto a medium containing reduced BAP (0.8 μM) and 0.28 μM GA_3, in order to promote maximum shoot length. Shoot formation of explants cultured on media containing 0.28 μM BAP was widely indepentend on GA_3 con-

Table 1. The effect of BAP and GA_3 concentration of the culture medium on shoot length of PR 204/84 cultures *in vitro*. (n=20, ±SE).

BAP (μM)	GA_3 (μM)	Shoot length (mm)
0.08	0	19.7±0.6
	0.028	24.8±1.2
	0.28	15.9±0.5
	2.8	17.4±0.5
0.28	0	22.5±1.2
	0.028	19.3±0.6
	0.28	21.1±1.2
	2.8	23.1±1.4
	$LSD_{0.05}$	2.2

Table 2. The effect of GA_3 and AC concentration of the culture medium on shoot length of PR 204/84 cultures *in vitro*. (0.08 μM BAP in all media). (n=20, ±SE).

GA_3 (μM)	AC (mM)	Shoot length (mm)
0.028	0	24.4±1.1
	4.2	25.8±1.1
	8.4	23.7±1.0
	20.8	24.5±1.1
0.28	0	15.6±0.8
	4.2	23.6±1.2
	8.4	26.8±1.3
	20.8	23.9±1.2
	$LSD_{0.05}$	2.4

Table 3. Analysis of variance of the two experiments.

	Source	Df	Mean Square	F
1st experiment	BAP	1	33.62	21.77***
	GA_3	3	18.19	11.78***
	BAP x GA_3	3	53.87	34.88***
	Error	32	1.54	
2nd experiment	GA_3	1	45.79	7.597**
	AC	3	49.36	8.189***
	GA_3 x AC	3	62.93	10.440***
	Error	32	6.03	

, *: Significant at P≤0.01 or P≤0.001, respectively.

centration (Table 1). But the shoots produced slightly chlorotic leaves and abscission of some leaves near the base of the explants was recorded especially at high GA_3 concentrations (0.28, 2.8 μM). In addition, the tips of the shoots were stressed (apical chlorosis, and dieback) and some showed symptoms of vitrification. Therefore, when shoots are placed for growth on the elongation medium, BAP concentration should be kept low. GEORGE (1996) reported at this phase of *in vitro* propagation, that if cytokinin concentration is high, the produced shoots become hyperhydric and leaves of some species have unusual shape.

An analysis of variance revealed that there is a significant effect of BAP (P≤0.001), GA_3 (P≤0.001), and BAP x GA_3 (P≤0.001) on shoot elongation (Table 3).

In conclusion, although there is not a clear statistical difference between 0.08 μM BAP plus 0.028 μM GA_3 and 0.28 μM BAP plus 0 μM GA_3 in terms of shoot length, the first treatment was the most appropriate, because the produced shoots possessed better macroscopic appearance.

Second experiment

Shoot clusters cultured on media supplemented with 0.08 μM BAP plus 0.028 μM GA_3 plus 4.2 mM AC produced the most elongated shoots per explant but this treatment was not significantly different in comparison to the other AC treatments (Table 2). When 0.028 μM GA_3 was present in media for shoot elongation, the stimulation of axillary shoots was inhibited, and the cultures produced elongated shoots with good characteristics. Explants placed into a medium supplemented with 0.028 μM GA_3 and 20.8 mM AC showed an improvement of shoot macroscopic appearance (vigorous shoots with dark green coloured leaves) in comparison to the other AC concentrations. GA_3 is mostly used to elongate and develop shoots at the elongation stage, but it should be applied with caution, because it may prevent the shoots from rooting (GEORGE 1996). AC has the ability to modify medium composition, and thereby, in some circumstances, to improve or regulate plant growth *in vitro* (GEORGE 1996).

The addition of AC to the medium in combination with 0.28 μM GA_3 plus 0.08 μM BAP promoted shoot elongation, at all AC concentrations tested in comparison to 0 mM AC. In the absence of AC in the medium (control), the elongation rate decreased, and 0.28 μM GA_3 plus 0.08 μM BAP were not suited to promote high elongation of shoots (Table 2). Certainly, the best treatment 0.08 μM BAP plus 0.28 μM GA_3 plus 8.4 mM AC is not significantly different in terms of shoot length from 0.08 μM BAP plus 0.028 μM GA_3 plus 0 mM AC. However, inclusion of AC in the medium resulted in an improved overall macroscopic appearance of leaves and shoots in comparison to 0 mM AC. JONA and VIGLIOCCO (1985) added 1.4-2.8 μM GA_3 and 166 mM AC to the medium to elongate shoots of *P. persica*. They showed that AC overcame the negative effects of BAP previously used in a shoot proliferation medium. ZUCHERELLI (1979) placed shoot clusters of the peach rootstock GF 677 in a medium for elongation containing 1.4 μM GA_3 and 8.4 mM AC. The same worker reported that AC was capable to inhibit the action of BAP, and even prevents the degeneration of shoot cultures.

An analysis of variance revealed that there is a significant effect of GA_3 (P≤0.01), AC (P≤0.001) and GA_3 x AC (P≤0.001) on shoot elongation (Table 3).

In conclusion, inclusion of 0.08 μM BAP plus 0.28 μM GA_3 plus 8.4 mM AC in the culture medium promoted longer shoots with good macroscopic characteristics.

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