Single and dual effects of different cytokinins on shoot multiplication of different apple scions

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM Publishing House, Hungary

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Key words: 6-benzylaminopurine riboside, micropropagation, kinetin, Prima, Galaxy, Jonagold

Abbreviations: 6-benzylaminopurine riboside – BAR, 6-benzylaminopurine – BA, kinetin – KIN, indole-3-butyric acid – IBA

Summary: Shoot multiplication responses of three apple scions to different concentrations of BA and BAR as single source of cytokinins and in combination with two concentrations of KIN were studied. The effects of hormones depended on genotype, type and interactions of different cytokinins. Use of BAR significantly enhanced the shoot multiplication of cv. Jonagold (6.5 shoots per explant). The multiplication rate of cv. Jonagold could not be improved by using the combination of BAR and KIN. The best proliferation was achieved by 1.0 mg l^{-1} BA combined with 1.0 mg l^{-1} KIN of cv. *Prima* (8.1) and of cv. *Galaxy* (10.4).The effect of 0.5 mg l^{-1} BA along with 1.5 mg l^{-1} KIN was similar on multiplication rate (10.9) of cv. *Galaxy*.

Introduction

Apple is one of the first woody plants that was successfully propagated *in vitro* (*Jones*, 1964). Its micropropagation has been based on the finding that BA and other factors, such as phloroglucinol, sugar source, auxin level, etc., can stimulate growth and proliferation of shoot tips (*Jones*, 1976, *Jones*, 1992, *Modgil et al.* 1999). Tissue culture procedure makes the rapid production of virus-free and uniform propagation material possible in large quantities. Although several methods have been described (*Snir & Erez* 1980, *James & Thurbon*, 1981, *Karhu*, 1995), these results are not broadly applicable because different genotypes respond in different way to *in vitro* proliferation and rooting (*Zimmerman & Broome*, 1981, *Zimmerman & Fordham*, 1985). The success of *in vitro* culture depends not only on the cultivar, but also on the origin, type and age of explant used (*Yepes & Aldwinckle*, 1994). Therefore procedures developed earlier for micropropagation of apple rootstocks could not be extended with the same success to another genotypes.

This study was undertaken to investigate the effects of 6-benzylaminopurine riboside and 6-benzylaminopurine, respectively, used as single source of cytokinins in the medium or in combination with various concentrations of kinetin on the multiplication of three apple cultivars.

Material and methods

In the experiments the following three scions were examined: *'Prima'*, *'Galaxy'*, *'Jonagold'*. Explants from field grown trees were collected, surface sterilized and inoculated on initiation medium described earlier (*Dobránszki et al.*, 2000). Shoots were subcultured and multiplied at 4-week-intervals on MS-medium (*Murashige & Skoog*, 1962) supplemented with 100 mg l^{-1} myo-inositol, 0.7% agar-agar, 3% saccharose, 0.5 mg l^{-1} BA and 0.1 mg l^{-1} IBA, to get a sufficient shoot number for multiplication experiments.

Single cytokinin-effect

In the first experiments the response of three apple cultivars to different concentration of BA and BAR in four various combinations were tested *(Table 1.)*. Shoot multiplication experiments were carried out on MS-medium *(Murashige & Skoog,* 1962) supplemented with 100 mg l^{-1} myo-inositol, 0.7% agar-agar, 3% saccharose and different combinations of plant hormones *(Table 1.)*.

Table 1 Concentration of growth regulators in the culture media used in the first experiments

Media	Concentrations of hormones (mg l^{-1})			
	BA	BAR	IBA	GA_3
Medium-1	0.5	–	0.3	0.2
Medium-2	1.0	–	0.3	0.2
Medium-3	–	0.5	0.3	0.2
Medium-4	–	1.0	0.3	0.2

Dual cytokinin-effect

In the second experiment the synergistic effect of kinetin was examined. Combinations of cytokinins was based on the results of previously experiment, thus 0.5 mg l^{-1} BA and 1.0 mg l^{-1} BAR were supplemented with KIN in the case of Jonagold, 0.5 and 1.0 mg l^{-1} BA were combined with KIN in the case of Galaxy and 1.0 mg l^{-1} BA was supplemented with KIN for cv. Prima. Two levels of kinetin were applied: 1.0 and 1.5 mg l^{-1} (combinations are presented in *Table 3)*.

All media were autoclaved for 20 min at 121 °C and 10^5 Pa. The pH of the medium was adjusted to 5.8 before autoclaving. Shoots were cut and cuttings of 40 mm each were placed on the different medium for four weeks in order to develop new shoots. Experiments were carried out in Kilner-jars (400 ml, 75 mm inside diameter and 85 mm long) and four cuttings were placed horizontally on 40 ml of medium in each jar. Cultures were grown at 22±2 °C with 16 h photoperiod provided by warm-white lamps (Tungsram) at a PPF of 105 $\mu Mols^{-1}m^{-2}$. Each treatment consisted of at least 20 replicates and experiments were repeated three-times.

Statistical analysis

After four weeks of cultures the rate of shoot multiplication were determined by counting the number of new, at least 20 mm high shoots produced per explant. The statistical analysis was made using analysis of variance followed by Tukey's test.

Results and discussion

Single cytokinin-effect

The rate of newly developed shoots depended on the type and concentration of cytokinin and on the genotype *(Table 2.)*.

In this experiment using BAR as cytokinin was favourable only for the cv. Jonagold, which showed the best shoot multiplication rate (6.5-fold) when high concentration (1.0 mg l^{-1}) of BAR was added to the medium.

Table 2 Effect of different concentration of BA and BAR on the rate of shoot multiplication (shoots per explant) after 4-week-culture.

Media	Cultivar		
	Jonagold	Prima	Galaxy
Medium-1	5.6 bc	4.9 b	9.0 bc
Medium-2	5.2 bc	5.4 b	9.5 c
Medium-3	3.2 a	2.8 a	6.5 a
Medium-4	6.5 c	3.3 a	8.0 ab

The small letters mean the homogenous groups according to Tukey's test.

On the contrary, application of BAR (regardless of its concentration) was not suitable to give high multiplication rate of cv. *Prima*. Moreover, cv. *Galaxy*, which tends to form more shoots then other cultivars, showed the most shoots in the present of BA, too. Although cv. *Prima* and *Galaxy* gave the best multiplication rate (5.4-fold and 9.5-fold, respectively) when high concentration (1.0 mg l^{-1}) of BA was applied, lower concentration (0.5 mg l^{-1}) of BA caused not lower multiplication rate in these cultivars.

Dual cytokinin-effect

In the second experiment the effect of KIN combined with either BA or BAR on the multiplication rate was tested in order to get further increasing of multiplication rate.

Our results showed that addition of KIN to the other type of cytokinin (BA or BAR) in the medium caused genotype- and concentration-depending effect on multiplication rate *(Table 3.)*.

Table 3 Effect of different combination of cytokinins on multiplication ratio

Combination and concentration of cytokinins (mg l^{-1})	Multiplication ratio (shoots per explant)		
	Jonagold	Prima	Galaxy
0.5 BA	5.6 b		9.0 a
0.5 BA + 1.0 KIN	3.3 a		9.8 ab
0.5 BA + 1.5 KIN	6.3 bc		10.9 b
1.0 BA		5.4 a	9.5 b
1.0 BA + 1.0 KIN		8.1 b	10.4 c
1.0 BA + 1.5 KIN		7.2 b	7.5 a
1.0 BAR	6.5 b		
1.0 BAR + 1.0 KIN	4.0 a		
1.0 BAR + 1.5 KIN	4.7 a		

The small letters mean the homogenous groups according to Tukey's test.

The combination of 1.0 mg l^{-1} BAR with 1.0 or 1.5 mg l^{-1} KIN caused significantly lower multiplication rate of 4.0 and 4.7 shoots per explant respectively, comparing to the 6.5-fold multiplication rate achieved on media containing 1.0 mg l^{-1} BAR alone in cv. *Jonagold*. Although lower concentration of KIN (1.0 mg l^{-1}) in addition to 0.5 mg l^{-1} BA decreased the number of newly developed shoots (3.3), the higher concentration of KIN (1.5 mg l^{-1}) increased the multiplication rate compared to the control treatment when 0.5 mg l^{-1} BA was added alone to the medium.

Finally, the best multiplication rate (6.5) was reached in cv. *Jonagold* if 1.0 mg l^{-1} BAR was applied alone. KIN was not able to increase the number of newly developed shoots in combination either with BA or with BAR.

On the contrary, the dual effect of cytokinins led to a significant increasing of multiplication rate in cv. Prima. This cultivar showed the best multiplication rate (5.4) when 1.0 mg l^{-1} BA was added to the medium in the first experiment *(Medium-2, Table 2.)*. The number of newly developed shoots could be significantly increased to 8.1 and 7.2, respectively, if 1.0 or 1.5 mg l^{-1} KIN was added to the medium in addition to 1.0 mg l^{-1} BA.

Further improving could be achieved by addition of KIN in the case of Galaxy, although the effect of KIN depended on its concentration and on the concentration of BA. The multiplication rate significantly increased when 0.5 mg l^{-1} BA was combined with high (1.5 mg l^{-1}) concentration of KIN and also when 1.0 mg l^{-1} BA was added to low (1.0 mg l^{-1}) concentration of KIN. The high concentration of BA (1.0 mg l^{-1}) and KIN (1.5 mg l^{-1}) applied together significantly decreased the multiplication rate (7.5). The best multiplication ratio (10.9) was achieved on the medium containing 0.5 mg l^{-1} BA and 1.5 mg l^{-1} KIN. However, the result reached on the medium contained 1.0 mg l^{-1} BA and 1.0 mg l^{-1} KIN was not significantly different (10.4) from the result of medium mentioned above.

Abbot & Whiteley (1976) reported earlier that KIN used in concentrations between 0.5 mg l^{-1} and 1.0 mg l^{-1} could induce satisfactory proliferation. *Modgil et al.* (1999) reported that the combination of BA and KIN was better for shoot multiplication than BA alone regarding the cultivar *Tydeman's Early Worchester*. In the present work the effect of KIN depended on genotype and on the concentration of cytokinins applied in the medium. In combination with BA, the KIN could increase the multiplication rate in all the examined genotypes: in cv. *Jonagold* from 5.6 up to 6.3, in cv. *Prima* from 5.4 up to 8.1 and in cv. *Galaxy* from 9.0 up to 10.9. However, in cv. *Jonagold* the best multiplication was achieved if high concentration (1.0 mg l^{-1}) of BAR was applied as single source of cytokinin and by the use of KIN it could not be exceeded.

In conclusion, the effects of cytokinins on *in vitro* proliferation of apple is genotype-depending. The substitution of BA with BAR could induce better proliferation in some genotypes as reported earlier *(Dobránszki et al.*, 2000) in the case of rootstocks JTE-H and MM 106. In some genotypes, but not in all, the multiplication rate could be enhanced by using BA and KIN in combination.

Acknowledges

The authors wish to say thank you to Bohács Jánosné for her technical assistance. This work was supported by OTKA (Project No. T-030103).

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