Post-effects of cytokinins and auxin levels of proliferation media on rooting ability of *in vitro* apple shoots (*Malus domestica* Borkh.) 'Red Fuji'

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

Summary: Rooting ability of in vitro apple shoots of 'Red Fuji' grown on proliferation media with different hormone content were tested at three IBA levels in root induction media. Rooting percentage could be slightly increased with an increase in IBA concentration in proliferation media. The highest IBA concentration (3.0 mg l^{-1}) in root induction media showed strong inhibitory effect on rooting capacity of in vitro shoots. The highest rooting percentage (95%) could be achieved by shoots grown on proliferation media containing TOP or BA+KIN as cytokinins before rooting.

Introduction

Rooting ability of *in vitro* apple shoots can be influenced by genotype *(Harbage & Stimart* 1996 a, b, *Zhou* et al. 1992, De *Paoli & Battistini* 1999), by number of subcultures *(Noiton* et al., 1992, *Sriskandarajah* et al., 1982) and by the age of *in vitro* shoots *(Karhu & Ulvinen,* 1995). Environmental conditions during last proliferation phase can also play important role in rooting capacity of *in vitro* shoots. *Sriskandarajah* et al. (1982) could increase rooting ability of the apple 'Delicious' shoots by altering of photoperiod and light intensity. *Webster & Jones* (1989) proved the favourable effect of shoot-etiolation on rooting. Moreover, presence of phloroglucinol in the last proliferation media can increase the rooting capacity of *in vitro* shoots *(Webster & Jones,* 1989, *James & Thurbon,* 1981). Type of cytokinin and its concentration in proliferation media can affect the rooting features of *in vitro* apple shoots *(Webster & Jones* 1991, van *Nieuwkerk* et al. 1986, *Marin* et al. 1993) or in other species too *(Jámbor-Benczúr & Márta-Riffer,* 1990). *Werbrouck* et al. (1996) compared the effect of benzyladenin and a newly isolated cytokinin named meta-topolin (TOP) on *in vitro* shoot and root production in the case of *Spathiphyllum floribundum* and found TOP to be more favourable than BA in rooting and acclimation. *Kubalákova & Strnad* (1992) also tested it in their experiments with sugar beet and described TOP as highly active cytokinin, which did not show any inhibitory carry-over effect on rooting even if used in high concentration.

In this study we examined the rooting ability of *in vitro* apple shoots grown on media with different levels of auxin and different type of cytokinins at three different levels of indole-3-butyric acid in root induction media.

Material and method

Plant material

In vitro cultures of 'Red Fuji' were established as reported earlier *(Dobránszki* et al., 2000). Media for shoot

proliferation (PM) contained *Murashige-Skoog* (1962) salts and vitamins, supplemented with 100 mg l^{-1} myo-inozitol and 30.0 g l^{-1} sucrose. The media was solidified by 7.0 g l^{-1} agar-agar and pH was adjusted to 5.7 before autoclaving. Shoots were collected from different media: four cytokinin contents were combined with two levels of IBA for proliferation of *in vitro* shoots before rooting experiments *(Table 1)*. All media were supplemented with 0.2 mg l^{-1} GA_3. The cultures were grown at 22±2 °C with 16 h photoperiod provided by warm-white lamps (Tungsram) at PPF of 105 µmol s^{-1} m^{-2} for four weeks.

Table 1 Cytokinin and auxin content of proliferation media (PM)

Media	Cytokinin		Auxin (IBA)
	type	mg l^{-1}	mg l^{-1}
M1	BA	1.0	0.1
M2	BA	1.0	0.3
M3	BAR	1.0	0.1
M4	BAR	1.0	0.3
M5	BA+KIN	1.0+1.5	0.1
M6	BA+KIN	1.0+1.5	0.3
M7	TOP	1.0	0.1
M8	TOP	1.0	0.3

Rooting experiments

Five shoots per baby jar were placed vertically on 30 ml root induction media (RIM), which contained MS salts at half strength, 100 mg l^{-1} myo-inozitol, 0.5 mg l^{-1} vitamin B_1, 20.0 g l^{-1} sucrose, and 7.0 g l^{-1} agar-agar. Three IBA levels were used: 1.0, 2.0 and 3.0 mg l^{-1}. The pH was adjusted to 5.5 prior to autoclaving. Cultures were incubated at 26 °C in total darkness for a week before transferring to hormone-free root elongation medium, which contained MS salts at half strength supplemented with 50 mg l^{-1} myo-inozitol, 30.0 g l^{-1} sucrose, 2.0 ml l^{-1} Wuxal and 7.0 g l^{-1} agar-agar. Environmental conditions were the same as for proliferation. Rooting percentage, number of roots per rooted shoot and the length of roots were observed after two weeks. All shoots were scored for callus development according to the following scale: 0: no callus, 1: weak callus, 2: medium and 3: abundant callus development observed. The statistical analysis was made by analysis of variance followed by Tukey's test, by using of SPSS 7.5 for Windows program. Percentage data were transformed to arcsine values prior to analysis. The results are presented in a non-transformed format.

Results and discussion

Interaction between auxin content of proliferation and that of root induction media affected the rooting ability of shoots. The rooting capacity of *in vitro* shoots slightly increased with an increase in the IBA concentration from 0.1 mg l^{-1} to 0.3 mg l^{-1} in PM, when lower IBA levels (1.0 and 2.0 mg l^{-1}) were used in root induction media *(Figure 1)*. When BA+KIN were used as cytokinins, differences in rooting rate caused by IBA level in PM proved to be statistically significant: rooting percentage increased from

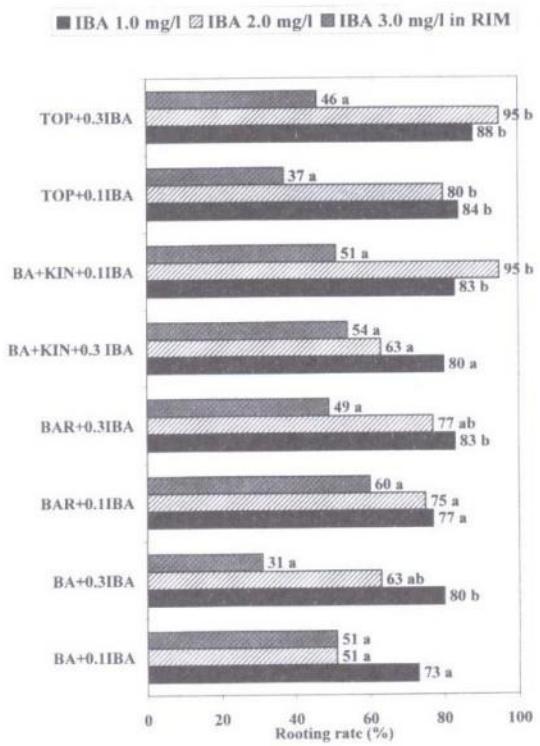

Figure 1 Rooting ability of in vitro shoots grown on different proliferation media. The same letters within the same PM group indicate statistically homogenous groups (Tukey-groups)

63 to 95% at medium IBA level in RIM (2.0 mg l^{-1}). *Similarly, Webster & Jones* (1989) also found that presence of phloroglucinol, which proved to be synergistic with auxin *(James & Thurbon,* 1981) in proliferation media enhanced the rooting ability of *in vitro* shoots of M. 9 rootstock of apples.

In contrast, when RIM contained 3.0 mg l^{-1} IBA, the rooting percentage of microcuttings from PM with higher IBA concentration was decreased except for the case when TOP was used as cytokinin. In our experiments shoots from proliferation media (PM) with different IBA content may have different level of endogenous auxin, which can modify the requirements of shoots for exogenous auxin *(Alvarez* et al., 1988). These results suggest that cultivars of susceptibility for exogenous auxin during root induction phase should be grown on proliferation media containing increased auxin level before rooting at a lower auxin concentration.

In general, the rooting percentage decreased as IBA concentration in RIM increased, except for shoots grown on PM with TOP+0.3 mg l^{-1} IBA and BA+KIN+0.3 mg l^{-1} IBA. In these cases the 2.0 mg l^{-1} IBA in RIM proved to be optimal concentration resulting the highest rooting rates in our experiments (95%). Differences, which proved to be statistically significant are indicated in *Figure 1*.

James & Thurbon (1979) described inhibitory effect of auxin on shoots explored to auxin for long time: abundant callus formed and fewer roots developed. *Modgil* et al. (1999) also found an inhibitory effect of high concentration

of auxin on rooting and *Isutsa* et al. (1998) reported that high IBA level in RIM (3.0 mg l^{-1}) resulted in strong callus formation and abnormal roots. In this study we found differences in callus development not only related to auxin levels of RIM also related to type of cytokinin applied in PM *(Table 2)*. Surprisingly, when BAR (M3 and M4) were used as cytokinin the strongest callus development was observed at the lowest IBA content of RIM: in these cases many shoots did not form either roots or callus at the highest IBA level in RIM (including many zero values in the average). Similar phenomena were observed on RIM with 2.0 mg l^{-1} IBA when BA was used as cytokinin (M1 and M2). When TOP and BA+KIN were applied in PM slight increasing in callus development could be observed as IBA increased in RIM, although it is proved statistically significant only in the case when shoots were grown on media with TOP+0.1 mg l^{-1} IBA. When different cytokinins are compared, shoots grown on media with BAR seemed to induce forming abundant callus.

Table 2 Effect of hormone content of PM and RIM on callus development

Hormone content of PM	Callus development		
	IBA content in RIM mg l^{-1}		
	1.0	2.0	3.0
BA + IBA 0.1 mg l^{-1} (M1)	1.90 c B	0.97 a A	1.40 b AB
BA + IBA 0.3 mg l^{-1} (M2)	1.19 a A	0.91 a A	1.17 a AB
BAR + IBA 0.1 mg l^{-1} (M3)	1.60 a B	1.35 a AB	1.23 a AB
BAR +IBA 0.3 mg l^{-1} (M4)	1.77 b B	1.66 b B	0.86 a A
BA+KIN+IBA 0.1 mg l^{-1} (M5)	1.03 a A	1.03 a A	1.29 a AB
BA+KIN+IBA 0.3 mg l^{-1} (M6)	0.93 a A	0.95 a A	1.29 a AB
TOP+IBA 0.1 mg l^{-1} (M7)	0.88 a A	1.05 ab A	1.40 b AB
TOP +IBA 0.3 mg l^{-1} (M8)	1.08 a A	1.10 a A	1.49 a B

The same small letters in rows indicate statistically homogenous groups (Tukey-groups), which mean the effects of different RIM.
The same capital letters in columns indicate statistically homogenous groups (Tukey-groups), which mean the effects of different PM.

Webster & Jones (1991) reported that BA content of proliferation media affected the rooting abilities of microcuttings but the effect depended on genotypes. Van *Nieuwkerk* et al. (1986) found that shoots grown on high concentration of TDZ had decreased rooting ability. We found that type of cytokinin in PM can also affect the rooting ability of shoots. When root induction were made by 1.0 mg l^{-1} IBA treatment the highest percentage were achieved by shoots from PM with TOP (88%) although differences were not statistically significant.

Microcuttings from PM with BA+0.1 mg l^{-1} IBA showed the lowest rooting percentage (51%) when RIM contained 2.0 mg l^{-1} IBA. Rooting ability of shoots from PM with BA+KIN+0.3 mg l^{-1} IBA and TOP+0.3 mg l^{-1} IBA were significantly the highest (both 95%).

In general rooting percentages were very low when RIM contained 3.0 mg l^{-1} IBA. In this case the relative best results (60%) could be obtained with shoots from PM with BAR+0.1 mg l^{-1} IBA.

Although *Alvarez* et al. (1988) and *Harbage & Stimart* (1996a) found that IBA content of root induction media affected significantly the number of roots. In our experiments the number of roots per rooted shoots and the length of roots were not affected by IBA concentration of RIM *(Table 3)*. However, some carry-over effect of auxin levels of PM could be detected. Higher concentration of IBA in PM tended to increase the number of roots when BA and BAR applied. In contrast, shoots from PM with higher IBA concentration seemed to form fewer roots at 1.0 and 2.0 mg l^{-1} IBA level in RIM when TOP or BA+KIN were used in PM. Differences in root number were statistically significant only in some cases.

Table 3 Effect of IBA content of PM on the number of roots per rooted shoots

IBA content in RM mg l^{-1}	Number of roots per rooted shoots	
	Hormone content of PM	
	BA + IBA 0.1 mg l^{-1} (M1)	BA + IBA 0.3 mg l^{-1}(M2)
1.0	1.7 a	2.5 a
2.0	2.4 a	2.3 a
3.0	1.2 a	3.0 b
	BAR + IBA 0.1 mg l^{-1} (M3)	BAR + IBA 0.3 mg l^{-1}(M4)
1.0	1.7 a	2.8 b
2.0	2.6 a	3.5 a
3.0	2.2 a	2.7 a
	BA+KIN+IBA 0.1 mg l^{-1} (M5)	BA+KIN+IBA 0.3 mg l^{-1}(M6)
1.0	3.0 a	2.0 a
2.0	3.7 b	1.7 a
3.0	2.0 a	2.7 a
	TOP +IBA 0.1 mg l^{-1} (M7)	TOP + IBA 0.3 mg l^{-1} (M8)
1.0	2.1 a	1.6 a
2.0	2.4 a	2.0 a
3.0	1.9 a	2.4 a

The same small letters in rows indicate statistically homogenous groups (Tukey-groups).

The length of roots was not influenced by IBA level in PM when BA and BAR applied. Higher concentration of IBA resulted in a decrease in length when TOP and BA+KIN were used, although differences were not proved to be significant in all cases *(Table 4)*.

Table 4 Effect of IBA content of PM on the length of roots

IBA content in RM mg l^{-1}	Length of roots (mm)	
	Hormone content of PM	
	BA + IBA 0.1 mg l^{-1} (M1)	BA + IBA 0.3 mg l^{-1}(M2)
1.0	11.4 a	9.8 a
2.0	15.4 a	16.1 a
3.0	12.3 a	13.5 a
	BAR + IBA 0.1 mg l^{-1} (M3)	BAR +IBA 0.3 mg l^{-1} (M4)
1.0	8.9 a	9.5 a
2.0	14.0 a	15.2 a
3.0	15.7 a	11.9 a
	BA+KIN+IBA 0.1 mg l^{-1} (M5)	BA+KIN+IBA 0.3 mg l^{-1}(M6)
1.0	15.7 a	12.1 a
2.0	16.0 b	8.1 a
3.0	16.7 a	11.3 a
	TOP+IBA 0.1 mg l^{-1} (M7)	TOP +IBA 0.3 mg l^{-1}(M8)
1.0	13.0 b	7.8 a
2.0	13.9 b	7.8 a
3.0	15.3 a	13.4 a

The same small letters in rows indicate statistically homogenous groups (Tukey-groups).

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