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The Effects of Different Cytokinin Types and Their Concentration on *in Vitro* Growth of Apricot (*Prunus armeniaca* L.) Shoots

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ABSTRACT

The objective of this study was to assess the effects of different cytokinin types [(6-benzylaminopurine (BAP), thidiazuron (TDZ) and meta-topolin (mT)] and their concentration on *in vitro* growth of apricot shoots. Sufficiently developed shoots were transferred to different multiplication media, which differed regarding the presence of cytokinin and its concentration. The subcultivation period lasted nine weeks. Analysis of covariance (ANCOVA) was performed to investigate the influence of plant growth regulators and their concentration on the final shoot weight of obtained tissue per regenerating explant. The results showed that all three cytokinins had a positive effect on shoot growth. There was a significant difference between used concentrations in the case of TDZ and BAP, while in the case of mT, the concentration did not significantly affect shoots' regeneration. In our study, the most significant increase in shoots weight was obtained on medium supplemented with 1.0 mg/L BAP.

Key words: *Prunus armeniaca*, micropropagation, *in vitro* propagation, BAP, TDZ, mT

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is one of the most important stone fruit crops and has been cultivated for centuries as a fruit crop and ornamental plant in many countries. Usually, it is propagated by vegetative cuttings that may be infected with pathogens. One of the most important and frequently present pathogens is the Plum pox virus (PPV), the agent responsible for the Sharka disease. The natural host range of this virus is restricted to *Prunus* spp. (stone fruits and ornamental trees). Efforts to eliminate pathogens from infected accessions are an important part of germplasm quarantine and exchange programs (Cheong Eun & An, 2015). *In vitro* tissue culture with or without thermotherapy and/or chemotherapy is an efficient method to obtain pathogen-free plants. Therefore, the optimal protocol for *in vitro* propagation of apricot must be developed. Wang et al. (2013) show that optimal protocol depends greatly on genotype, so the media's optimisation needs to be done for different germplasms.

Less information is known about apricot micropropagation compared to other stone fruit plants. Some research suggests a very low ability to form axillary shoots in apricots *in vitro* culture (Kataeva & Kramarenko, 1989; Skirvin et al., 1986) or an almost complete absence of axillary shoot formation (Snir, 1984).

In the micropropagation of apricots, the first stage (disinfection of buds and their induction) can be problematic. The success of induction itself, of course, depends to a large extent on the cultivar, the age of the tree from which we take the buds, and the environment in which the tree grows (Cheong Eun & An, 2015; Wang et al., 2013).

The type and concentration of PGR (plant growth regulators) used in micropropagation vary widely, depending on the species that are being studied, and they have to be optimized for each genotype. Cytokinins are an essential class of growth regulators that, along with auxins, control

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numerous physiological and plant development processes. The choice of cytokinin is one of the most critical factors in developing of protocols for the *in vitro* culture of plants (San José et al., 2021).

BAP (6-benzylaminopurine) is one of the most frequently used cytokinin due to its efficiency and relatively low cost. However, using this cytokinin in micropropagation procedures can produce physiological disorders such as hyperhydricity and apical necrosis. The discovery of a new group of aromatic cytokinins, the topolins, has led to new possibilities for their use in the micropropagation of numerous species (Zaytseva et al., 2021). In the last few years, the use of meta-topolin (mT) and its derivatives has increased rapidly, especially in initiating new cultures, optimizing protocols, and inhibiting the negative features of some other cytokinins (San José et al., 2021). Meta-topoline (mT) is a cytokinin that can be used as an alternative to the cytokinin benzyladenine (BA); after the acclimatization process, the plants develop roots better structured and more abundant than those grown in the medium with an added BA (Werbrouck et al., 1996). Gentile et al. (2014) demonstrated that meta-topolin positively affects shoot growth and quality of shoots and reduces the proportion of plants with hyperhydricity symptoms. Thidiazuron (TDZ) is an aromatic natural cytokinin and is one of the most active cytokinin-like substances for woody plant tissue culture. It enables efficient micropropagation of many woody species. Low plant regulator concentrations (<1 μM) can induce more significant axillary proliferation than many other cytokinins. A concentration higher than 1 μM, can stimulate the formation of callus, adventitious shoots or somatic embryos (Huetteman & Preece, 1993).

This study evaluates the effects of three cytokinins (BAP, TDZ and mT) and their concentration on **in vitro** growth of a local apricot genotype named Pišečka marelica.

MATERIAL AND METHODS

Plant material

The experiment was carried out in the Plant Tissue Culture Laboratory at Faculty of Agriculture and Life Sciences (FKBV), University of Maribor. Shoots were collected on March 22, 2017, from an adult apricot tree in the Slovenian plant gene bank of stone fruit trees at Pohorski dvor, FKBV, Hoče, NE Slovenia and transferred to the laboratory. The accession included in this study was a local cultivar named Pišečka marelica having the accession number 6339. Buds were used as explants and sterilised as described by Šenveter (2018). For this study, previously sterilised and developed shoots were used.

Shoot proliferation

Developed shoots approximately 1–1.5 cm long (Figure 1) were placed on the medium, which contained the following substances: Woody plant medium (WPM) (Lloyd & McCown, 1980) mineral salts including vitamins (Duchefa, Haarlem, The Netherlands) supplemented with 13 g/L sucrose, 11 g/L D-sorbitol, 0.1 mg/L biotin, 0.01 g/L folic acid, 0.1 mg/L riboflavin and solidified with 6.0 g/L agar (Plant Agar, Duchefa, Haarlem, The Netherlands). The pH was adjusted to 5.7 before adding gelling agent and autoclaving. Seven different media were prepared according to plant growth regulators: one without plant regulators (control) and six containing 0.01 mg/L 1-naphthaleneacetic acid (NAA) and three different cytokinins: 6-benzylaminopurine (BAP), thidiazuron (TDZ), and meta-topolin (mT) each at two concentrations; 0.5 mg/L and 1.0 mg/L. We set 30 shoots on each of seven media, each shoot in its own "baby jar". Each shoot was weighted before planting, and its initial weight was recorded. The shoots were moved to a freshly prepared medium every three weeks during the experiment. Cultures were maintained in a growth chamber at 23 °C with a 15 hours long photoperiod (15,000 Lux). After nine weeks of cultivation, the fresh weight of newly formed shoots (final weight) was assessed. Shoots were also observed for hyperhydricity.

Statistical analysis

The experiment was conducted as a completely random design, with 30 explants per treatment. A univariate (control) and two–way between–groups univariate analysis of covariance (ANCOVA) was performed to investigate the influence of plant growth regulator and its concentration

Figure 1: Regeneration of shoots of apricot (*P. armeniaca*, Pišečka marelica): shoot developed after disinfection and induction of bud (a); shoots used as an explants in experiment (b); shoots obtained on medium supplemented with meta-topolin (c)

Table 1: *F* statistics of ANCOVA on the effects of PGR, the used concentration, and their interaction on shoot regeneration of apricot explants expressed by final fresh shoot weight (g)

Factor	Shoot final weight (g)
Covariate (initial shoot weight)	7.934**
PGR	24.340***
Concentration (C)	8.122**
PGR × C	4.666*
	Mean[a] ± SEM
PGR	
BAP	1.92 ± 0.153 a
TDZ	1.97 ± 0.178 a
mT	0.55 ± 0.161 b
Concentration	
0.5 mg/L	1.22 ± 0.125 b
1.0 mg/L	1.74 ± 0.125 a

*, **, *** corresponding to P < 0.05, 0.01 and 0.001, respectively
[a] Covariates appearing in the model are evaluated at the 0.0412
a–b Different letters indicate significant differences among treatments (Pairwise comparison, Bonferroni adjustment)

on the final shoot weight measurements. The initial shoot weight was entered as a covariate, and its effect was controlled in the analysis. In order to run the analysis under optimal conditions, we attempted to detect any deviation from ANCOVA assumptions that might distort the tests of statistical significance (Field, 2013). Statistical significance was indicated by a P-value < 0.05. Results are presented as estimated means ± standard error of the mean (SEM). A Bonferroni adjustment setting was applied to assess the between-subject effect analysis. All analyses were carried out using the IBM Statistics software (Version 25).

RESULTS AND DISCUSSION

It is well known that plant growth regulators (PGR) greatly influence shoot regeneration. TDZ and BAP are the most used cytokinins in *Prunus* regeneration studies, while kinetin, isopentenyl adenine (2iP), and mT have been used less (Ruzic & Vujović, 2008). Results about the effectiveness of PGR on regeneration and shoot development of *Prunus* are contradictory. Often TDZ has been reported to be more effective than BAP (Bhagwat & David Lane, 2004; Canli & Tian, 2008; Espinosa et al., 2006; Hammatt & Grant, 1998; Matt & Jehle, 2005; Pérez-Tornero & Burgos, 2000), but in other reports (Ruzic & Vujović, 2008; Tang et al., 2002) BAP has been found more effective than TDZ. These findings are in accordance with our study, where BAP shows the highest regeneration and gained the highest final weight of shoots. Different species and genotypes used in conducted experiments can explain contradictory results.

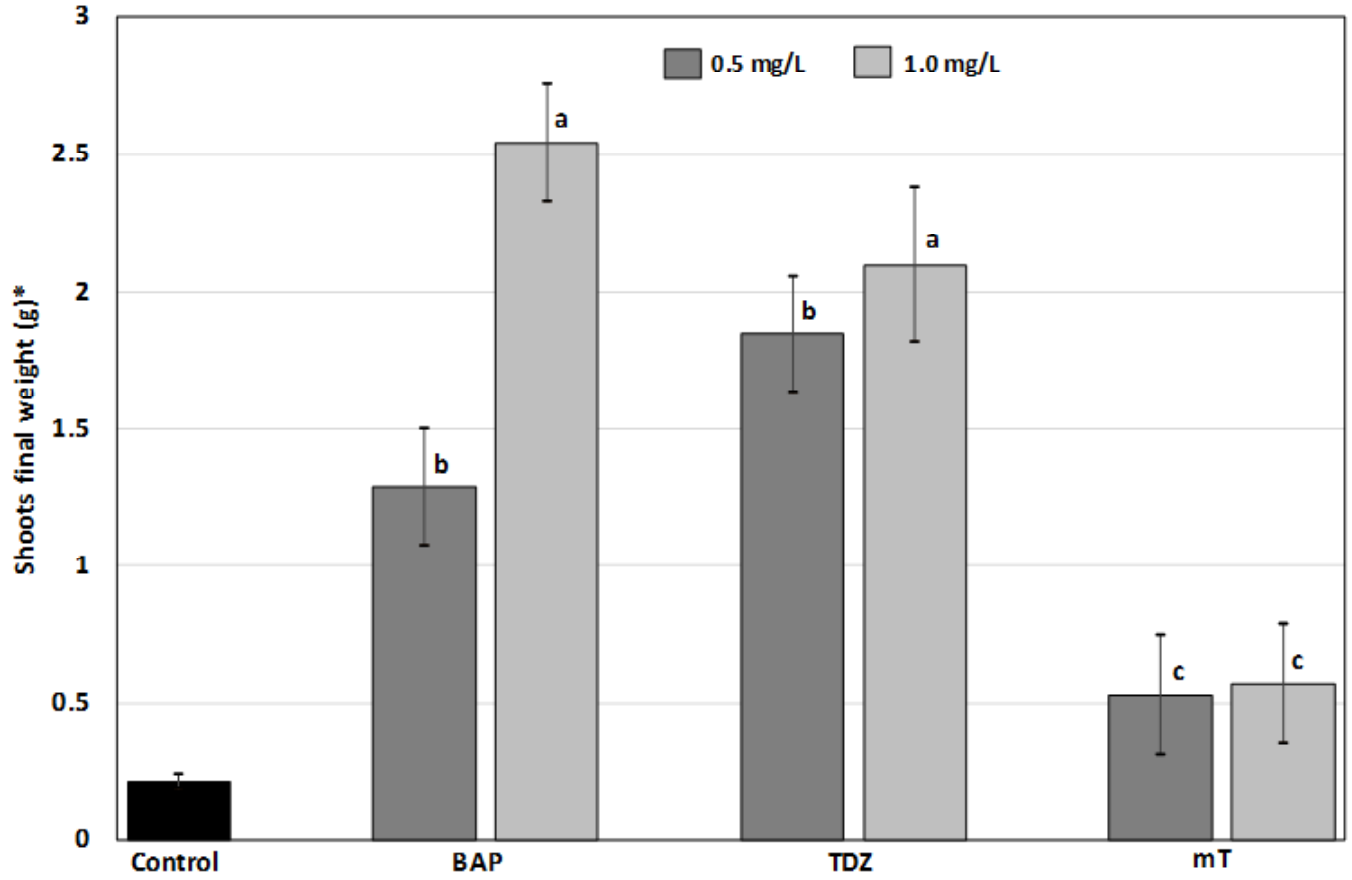

Figure 2: Effect of various concentration of BAP, TDZ and mT on shoot proliferation of *P. armeniaca,* Pišečka marelica explants

* Estimated means (ANCOVA)
a–b Different letters (± SEM) indicate significant differences among treatments (Pairwise comparison, Bonferroni adjustment)

The ANCOVA results demonstrated that the PGR effect is the main factor influencing the shoot regeneration of apricot explants (Table 1). On average, BAP and TDZ resulted in statistically higher final shoot weight (0.92 and 0.97 g, respectively) than mT. The two-way interaction between observed factors was also detected (Table 1) and shown in Figure 2. The most effective in shoot regeneration was BAP (2.54 g) and TDZ (2.10 g) in higher – 1.0 mg/L concentration, and the least effective cytokinin was mT regardless of concentration (0.53 and 0.57 g). Interestingly, we noticed that the final shoot weight was for 13.7% higher using 1.0 mg/L of TDZ. Results are contrary to those of Ning et al. (2007), who reported that TDZ at lower concentrations promotes and at higher concentrations usually inhibits shoot regeneration.

Our data show that exogenous PGR is essential for shoot regeneration of apricot explants. Regeneration of apricot shoots cultured on plant growth regulators-free medium (control) was much lower (0.21 g) than explants cultured on a medium containing BAP, TDZ or mT (Figure 2). Nas et al. (2010) report that regeneration rations of *P. microcarpa* cotyledons explants cultured on PGR-free medium (control) were lower than explants cultured on a medium containing BAP, TDZ or mT, which is in accordance with our results.

Even meta-topolin was the least effective cytokinin in our study, the developed shoots were healthier (Figure 1), and the number of hyperhydric explants was reduced (no hyperhidricity observed) in comparison with BAP (35.3% hypehydricity) and TDZ (60% hyperhidricity) (data not shown). These data agree with Gentile et al. (2014), who obtained a better control of hypehidricity by using mT in **Prunus** rootstocks than BAP and TDZ.

CONCLUSION

To our knowledge, this is the first report of cytokinin effect on shoot proliferation of apricot genotype named Pišečka marelica. The results presented could be used for micropropagation protocol for apricot multiplication *in vitro*. In our study, the best regeneration was obtained on medium supplemented with BAP in concentration of 1.0 mg/L in combination with 0.01 mg/L NAA.

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Vpliv različnih tipov citokininov in njihova koncentracija na *in vitro* rast poganjkov marelice (*Prunus armeniaca* L.)

IZVLEČEK

Cilj te raziskave je bil oceniti vpliv različnih tipov citokininov [(6-benzilaminopurin (BAP), tidiazuron (TDZ) in meta-topolin (mT)] in njihove koncentracije na *in vitro* rast poganjkov marelice. Dobro razvite poganjke smo prenesli na različna razmnoževalna gojišča, ki so se razlikovala glede na dodan citokinin in njegovo koncentracijo. Poskus je trajal devet tednov. Vpliv dodanih rastlinskih rastnih regulatorjev in njihove koncentracije na končno maso pridobljenih poganjkov smo preverili z analizo kovariance (ANCOVA). Rezultati so pokazali, da imajo vsi trije v raziskavi uporabljeni citokinini pozitiven učinek na rast poganjkov. Pri TDZ in BAP je bila statistično značilna razlika pri različnih koncentracijah, medtem ko pri mT koncentracija ni statistično značilno vplivala na rast poganjkov. Glede na naše rezultate, smo največji prirast mase dobili na gojišču z dodanim BAP v koncentraciji 1,0 mg/L.

Ključne besede: *Prunus armeniaca*, mikropropagacija, razmnoževanje *in vitro*, BAP, TDZ, mT