In vitro shoot proliferation of sweet cherry cultivars Karešova and Rivan

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ABSTRACT: The objective of this study was to investigate the possibility of optimizing routine tissue culture methods to proliferate two sweet cherry cultivars Karešova and Rivan. Shoot tips of two genotypes were successfully established *in vitro*. Six proliferation MS media containing 1, 2 and 4 mg/l BAP (6-benzylaminopurine), 0.5 and 1 mg/l TDZ (thidiazuron) or 10 mg/l 2iP (6-(γ,γ-dimethylallylamino)purine) were tested. The highest proliferation rate (3.0 ± 0.1) was obtained for Rivan on MS medium containing 2 mg/l BAP. In the case of cultivar Karešova, any of the cytokinins tested did not promote satisfactory proliferation. The highest proliferation rate (1.6) achieved on MS medium with 2 mg/l 2iP is not sufficient for a larger scale *in vitro* shoot production. It was proved that different genotypes of sweet cherry do not respond in the same way during proliferation *in vitro*. Future research and testing of other media and plant growth regulators will be carried out.

Keywords: *Prunus avium*; micropropagation; culture initiation; multiplication; cytokinins

Sweet cherry (*Prunus avium* L.) is an economically important fruit species in climatic conditions of Central Europe. In 2007, there were 932 ha of commercial sweet cherry plantations in the Czech Republic (Buchtová 2007).

In order to overcome difficulties in vegetative propagation of sweet cherry cultivars and clones, methods of micropropagation were developed in past years (Hammatt 1999). These methods are based on stimulation of axillary branching or adventitious shoot formation by addition of cytokinins to the culture medium. Micropropagation systems for *Prunus avium* were mostly developed and tested for genotypes aimed at forestry and timber industry (Hammatt, Grant 1997; Hammatt 1999; Mandegaran et al. 1999; Ďurkovič 2006) or for production of rootstocks (Janečková 1985; Muna et al. 1999; Erbenová et al. 2001). Nevertheless, it was proved that different genotypes of sweet cherry do not respond in the same way during establishment and proliferation *in vitro* (Snir 1982; Erbenová et al. 2001). Despite increasing numbers of reports of successful micropropagation, more research is still needed in this area.

The objective of this study was to investigate the possibility of optimizing routine tissue culture methods to proliferate two sweet cherry cultivars Karešova and Rivan. These two cultivars are widely grown in commercial plantations and private gardens in the Czech Republic. The resulting plant material will be used for experiments with *in vitro* thermotherapy. High temperature (up to 39°C) is a stress factor resulting in considerable decline of *in vitro* plants during therapy (Spiegel et al. 1995). Due to this high mortality rate there is a demand for a large number of *in vitro* plants in good growth condition with well-developed leaves and actively growing shoot tips. *In vitro* proliferation, which can be carried out throughout the year, could provide an alternative, rapid means to produce more clonal material of sweet cherry in a shorter period of time (Hammatt, Grant 1997).

MATERIAL AND METHODS

Twenty-five actively growing shoot tips (5 to 10 mm in length) were cut from shoots of each sweet cherry cultivar (Karešova, Rivan) sprouting in laboratory conditions. The donor shoots were obtained in March from mature trees growing in field collections of Research and Breeding Institute of Pomology Holovousy Ltd. Excised shoot tips were surface disinfested with a

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Table 1. Surface sterilization of sweet cherry genotypes by 0.15% $HgCl_2$

Genotype	Contaminated explants		Uncontaminated explants which did not develop shoots		Uncontaminated explants which developed shoots	
	No.	(%)	No.	(%)	No.	(%)
Karešova	3	12	11	44	11	44
Rivan	2	8	13	52	10	40

0.15% solution of mercuric chloride for 1 min. Under a laminar flow hood, they were rinsed with sterile distilled water. Following sterilization, the tips were cultured in 100 ml Erlenmayer flasks, each with 25 ml of MS (Murashige, Skoog 1962) medium (5 shoots per flask). Contamination rate of the explants after sterilization was analyzed. Uncontaminated shoots established on MS medium were transferred after one month to fresh proliferation medium.

All initiation and proliferation media contained 7.0 g/l Difco agar. The pH of the media was adjusted to 5.8 before autoclaving. Culture condition was a 16-h photoperiod provided with cool-white fluorescent tubular lamps at 22 ± 1°C.

Six proliferation MS media containing 1, 2 and 4 mg/l BAP (6-benzylaminopurine), 0.5 and 1 mg/l TDZ (thidiazuron) or 10 mg/l 2iP (6-(γ,γ-dimethylallylamino)purine) were tested. In the experiments with proliferation, 5 single shoot tips (10 mm long) were cultured in 100 ml Erlenmayer flasks, each with 25 ml of MS medium. For the two cultivars, the effect of three growth regulators in different concentrations on proliferation rate, callus formation and shoot morphology is shown. Proliferation rate was defined as the number of newly formed shoots (> 10 mm) per initial shoot tip after four weeks of culture. The shoot formation was recorded between the tenth and fifteenth subculture. In all experiments, 25 shoot tips were used. Each experiment was repeated four times. Data from four independent experiments were pooled and expressed as the mean. Treatment means were compared with the standard error (SE) of the mean.

RESULTS

Results of sterilization procedures and development of shoots from initial explants are stated in Table 1. The sterilization procedures were successful and bacterial and fungal contaminations were infrequent. Out of the 50 explants of both genotypes taken, only two explants of cultivar Rivan and three explants of cultivar Karešova were contaminated with fungal infection after the first four weeks of culture. These explants were later discarded. Out of the other 45 uncontaminated explants, only 11 explants of Karešova and 10 explants of Rivan developed shoots.

The number of newly formed shoots varied with the genotype and concentration of different plant hormones (Table 2). In the case of cultivar Rivan, shoot proliferation was influenced by cytokinin type and concentration. BAP in concentrations 1 and 2 mg/l was superior to TDZ and 2iP in ability to induce the production of new shoots. Thus the highest proliferation rate (3.0 ± 0.1) was obtained on MS medium containing 2 mg/l BAP. On the contrary, the lowest proliferation rate (1.2 ± 0.0) was noted for Rivan on MS medium containing 10 mg/l 2iP.

For cultivar Karešova, any of the five media containing BAP or TDZ did not promote markedly *in vitro* shoot formation and the number of newly formed shoots was thus very low (from 1.3 to 1.4). Karešova proliferated best on MS medium containing 10 mg/l 2iP. But proliferation rate (1.6 ± 0.1) achieved in the variant with 10 mg/l 2iP was distinctively lower than proliferation rates of cultivar Rivan on MS media with 1 or 2 mg/l BAP.

In all experiments with two selected sweet cherry cultivars, any physiological disorders or morphological abnormalities such as hyperhydricity, excessive callus formation or production of abnormally narrow leaves were not observed during *in vitro* shoot proliferation stage.

Table 2. Effect of growth regulators on proliferation rates of sweet cherry genotypes

Growth regulator (mg/l)	Mean proliferation rate ± SE	
	Karešova	Rivan
BAP		
1	1.3 ± 0.1	2.6 ± 0.2
2	1.3 ± 0.1	3.0 ± 0.1
4	1.4 ± 0.1	1.7 ± 0.1
TDZ		
0.5	1.3 ± 0.1	2.2 ± 0.1
1	1.3 ± 0.1	1.5 ± 0.1
2iP		
10	1.6 ± 0.1	1.2 ± 0.0

DISCUSSION

In the present study 45 of 50 explants obtained after disinfection were free of bacterial and fungal contamination. Contaminations were infrequent and mercuric chloride proved to be effective against bacterial and fungal microflora in field collections of sweet cherry. On the contrary, 44% (Karešova) and 52% (Rivan) of initial explants did not develop shoots after the sterilization procedure. It was probably due to the toxicity of used concentration of mercuric chloride to plant tissues of two selected sweet cherry cultivars. It was previously reported that mercuric chloride is a good disinfectant, but could be toxic to plant tissues of susceptible species and genotypes (Muna et al. 1999; Sedlák, Paprštein 2007).

In our studies, sweet cherry cultivar Rivan was micropropagated successfully using shoot culture medium with BAP. Addition of growth regulator BAP was essential for adventitious shoot initiation and development. It was previously reported that BAP could be used successfully to induce shoot multiplication in *Prunus* spp. (Muna et al. 1999; Pruski et al. 2000). Saponari et al. (1999) successfully micropropagated *Prunus mahaleb* rootstocks on medium supplemented with 1 mg/l BAP. Plant growth regulator 2iP proved to be unsuitable for the proliferation of sweet cherry cultivar Rivan.

In the case of cultivar Karešova, any of the cytokinins tested did not promote satisfactory proliferation. The highest proliferation rate (1.6) achieved on MS medium with 2 mg/l 2iP can be sufficient for *in vitro* culture establishment and maintenance, but is not satisfactory for larger-scale *in vitro* shoot production. Future research and testing of other media and plant growth regulators is thus needed.

It was proved that different genotypes of sweet cherry do not respond in the same way during proliferation *in vitro*. The observed differences in proliferation rates of two sweet cherry cultivars could result from various auxin and cytokinin metabolism of plant tissue. *In vitro* proliferation could be also affected by presence of PDV (*Prune dwarf virus*) in latent form in donor mature trees of both cultivars. Previous reports (Saponari et al. 1999) suggested that the presence of PDV adversely affects shoot proliferation of *Prunus mahaleb*.

In our experiments with two selected sweet cherry cultivars, no morphological abnormalities were observed during *in vitro* shoot proliferation stage. By contrast, most other authors (Muna et al. 1999; Pruski et al. 2000; Ďurkovič 2006) working with different *Prunus* species reported small curly shoots or poor leaf expansion on media with higher concentration of BAP.

References

BUCHTOVÁ I., 2007. Produkční ovocné sady v ČR. In: Situační a výhledová zpráva ovoce. Praha, Ministerstvo zemědělství: 46.

ĎURKOVIČ J., 2006. Rapid micropropagation of mature wild cherry. Biologia Plantarum, *50*: 733–736.

ERBENOVÁ M., PAPRŠTEIN F., SEDLÁK J., 2001. *In vitro* propagation of dwarfed rootstocks for sweet cherry. Acta Horticulturae, *560*: 477–480.

HAMMATT N., 1999. Delayed flowering and reduced branching in micropropagated mature wild cherry (*Prunus avium* L.) compared with rooted cuttings and seedlings. Plant Cell Reports, *18*: 478–484.

HAMMATT N., GRANT N.J., 1997. Micropropagation of mature British wild cherry. Plant Cell, Tissue and Organ Culture, *47*: 103–110.

JANEČKOVÁ M., 1985. Indukce tvorby kořenů u vybraného vegetativně množitelného klonu třešně ptáčnice (*Cerasus avium* /L./ Moench) v kultuře *in vitro*. Sborník ÚVTIZ, Zahradnictví, *12*: 165–168.

MANDEGARAN Z., ROBERTS A.V., HAMMATT N., 1999. The ability of *Prunus avium* × *P. pseudocerasus* Colt to form somatic embryos *in vitro* contrasts with the recalcitrance of *P. avium*. Plant Cell, Tissue and Organ Culture, *59*: 57–63.

MUNA A.S., AHMAD A.K., MAHMOUD K., ABDULRAHMAN K., 1999. *In vitro* propagation of a semi-dwarfing cherry rootstock. Plant Cell, Tissue and Organ Culture, *59*: 203–208.

MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, *15*: 473–497.

PRUSKI K.W., LEWIS T., ASTATKIE T., NOWAK J., 2000. Micropropagation of Chokecherry and Pincherry cultivars. Plant Cell, Tissue and Organ Culture, *63*: 93–100.

SAPONARI M., BOTTALICO G., SAVINO G., 1999. *In vitro* propagation of *Prunus mahaleb* and its sanitation from Prune dwarf virus. Advances in Horticultural Science, *13*: 56–60.

SEDLÁK J., PAPRŠTEIN F., 2007. *In vitro* propagation of blue honeysuckle. Horticultural Science, *34*: 129–131.

SPIEGEL S., STEIN A., TAM Y., 1995. *In vitro* thermotherapy of rosaceous fruit trees. Acta Horticulturae, *386*: 419–420.

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In vitro množení odrůd třešně Karešova a Rivan

ABSTRAKT: Cílem práce bylo studium možnosti množení dvou odrůd třešně Karešova a Rivan v *in vitro* kultuře. Růstové vrcholy obou genotypů byly úspěšně zavedeny do *in vitro* kultury. Bylo zkoumáno šest multiplikačních MS médií s obsahem 1, 2 a 4 mg/l BAP (6-benzylaminopurin), 0,5 a 1 mg/l TDZ (thidiazuron) nebo 10 mg/l 2iP (6-(γ,γ-dimethylallylamino)purin). Nejvyšší multiplikační koeficient (3,0 ± 0,1) byl obdržen pro odrůdu Rivan na MS médiu s obsahem 2 mg/l BAP. V případě odrůdy Karešova nevyvolal žádný z použitých cytokininů dostatečně vysokou multiplikaci. Nejvyšší dosažený multiplikační koeficient (1,6) z varianty MS média se 2 mg/l 2iP je příliš nízký pro produkci *in vitro* výhonů ve větším měřítku. Bylo potvrzeno, že různé genotypy třešně reagují odlišně v průběhu růstu a množení v *in vitro* kultuře. Z tohoto důvodu bude prováděno další testování růstových médií a regulátorů růstu.

Klíčová slova: *Prunus avium*; mikropropagace; ustavení kultury; multiplikace; cytokininy

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