In vitro propagation of blue honeysuckle

J. Sedlák, F. Paprštein

Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy, Czech Republic

ABSTRACT: We have developed a rapid shoot multiplication procedure for *in vitro* propagation of blue honeysuckle (*Lonicera kamtschatica* [Sevast.] Pojark). Shoot tips of two genotypes 20/1 and Altaj were successfully established *in vitro* and micropropagated on the Murashige and Skoog (MS) based media containing different concentrations of 6-benzylaminopurine (BAP). Multiplication rates varied depending on the genotype and concentration of BAP. The highest multiplication rate was obtained for the genotype 20/1 that produced 10.5 ± 0.7 shoots (longer than 10 mm) on the MS medium containing 2 mg/l BAP. The lowest multiplication rate was obtained for Altaj producing only 1.6 ± 0.1 shoots on MS medium containing 4 mg/l BAP. Moreover, *in vitro* rooting on the modified MS medium supplemented with 2.5 mg/l indole-3-butyric acid (IBA) was reported. Rooted shoots were transferred to the greenhouse for further evaluation.

Keywords: *Lonicera*; micropropagation; culture initiation; multiplication; 6-benzylaminopurine

Lonicera kamtschatica (Sevast.) Pojark is a honeysuckle native to Northeastern Asia (Mansfeld 1986). This frost hardy species has a potential as a cultivated small fruit crop in the climatic conditions of the Czech Republic. Cultivars of *L. kamtschatica* with edible dark blue fruits have been recently introduced in the Czech Republic. Fruits of *L. kamtschatica* ripen very early in the season prior to or together with the earliest strawberries.

In vitro culture techniques offer the opportunity for *in vitro* collecting, rapid propagation and distribution of plant material. These methods are promising for production of more plant material in a shorter period of time with less labor and at lower production costs. Several micropropagation protocols have been described to improve conditions for micropropagation of different *Lonicera* species (Boonnour et al. 1988; Karhu 1997a, 2003). Unfortunately, these methods are not broadly applicable because the effectiveness of the culture medium seems to be highly genotype-specific.

In the presented paper, *in vitro* propagation was studied to provide an efficient plant production system for edible honeysuckle.

MATERIAL AND METHODS

Twenty actively growing shoot tips of two genotypes Altaj and 20/1 (5–10 mm in length) were cut after bud break from three-year-old potted plants in the greenhouse. This initial plant material was surface disinfected with a 0.15% solution of mercuric chloride for 1 min. Under a sterile laminar flow hood, the shoot tips were rinsed with sterile distilled water. Following sterilization, the tips were cultured in the Erlenmayer flasks, each with 10 ml of MS medium (Murashige, Skoog 1962) gelled with 0.8% (W/v) agar (Difco). Contamination rate and survival of the explants after sterilization were analyzed.

To determine favorable conditions for shoot initiation, cytokinin BAP (6-benzylaminopurine) was applied at 1, 2 and 4 mg/l. BAP was filter sterilized (25 mm, Acrodisc Syringe Filter 0.2 μm, Pall Gelman, USA) and added to the basal MS medium after autoclaving. The pH of the medium was adjusted to 5.7 before autoclaving. Culture condition was a 16 h photoperiod provided by cool-white fluorescent tubular lamps at 60 μmol/m^2/s and 22 ± 1°C. The number of shoots (> 10 mm) per initial shoot tip was recorded after four weeks of culture. In experiments with multiplication, 100 shoot tips were used. Treatment means were compared with the standard error (SE) of the mean.

Microcuttings (10–20 mm long), coming from proliferation medium, were rooted in the MS medium with macro- and micronutrients reduced to one third with 2.5 mg/l IBA. Although several media were evaluated for induction of roots, only results from medium

Supported by the Ministry of Agriculture of the Czech Republic, Project No. NAZV QF3223.

Table 1. Surface sterilization with 0.15% $HgCl_2$

Genotype	Contaminated explants		Uncontaminated explants that did not develop shoots		Uncontaminated explants that developed shoots	
	number	(%)	number	(%)	number	(%)
20/1	0	0	2	10	18	90
Altaj	1	5	10	50	9	45

that showed maximal root induction are presented in this report. Culture conditions during root initiation and root growth were the same as during shoot culture. Hundred microcuttings were used for this treatment. Numbers of rooted shoots were recorded four weeks after transfer to rooting medium. Treatment means were compared with the standard error (SE) of the mean. Shoots with roots were rinsed in water to remove remains of the medium and then transferred to Jiffy 7 peat pellets (AS Jiffy Products, Norway) soaked with water. The shoots were moistened with water to prevent wilting during transplanting. The Jiffy 7 pellets with rooted plants were placed on a greenhouse bench equipped with transparent plastic cover foils (100% air humidity) under the standard greenhouse condition. The plants were gradually acclimated by opening the cover foils over fourteen days.

RESULTS

The results of sterilization procedures and development of shoots from initial explants are recorded in Table 1. The sterilization procedures were successful and bacterial and fungal contaminations were infrequent. Of the 40 explants of both genotypes taken, only one explant of cultivar Altaj was contaminated with fungal infection after the first four weeks of culture. This explant was later discarded. Of the other 39 uncontaminated explants, 18 explants of 20/1 and 9 explants of Altaj produced shoots.

Shoot number varied with the genotype and concentration of BAP (Table 2). The highest multiplication rate was obtained for genotype 20/1 on the medium with BAP concentration 2 mg/l, which produced 10.5 new shoots per explant. A further increase in BAP concentration had an unfavorable effect on shoot number. The lowest multiplication rate was obtained for cultivar Altaj on the medium with the BAP concentration of 4 mg/l; it produced only 1.6 new shoots per explant.

Table 2. Multiplication rates for blue honeysuckle genotypes

BAP (mg/l)	20/1	Altaj
1	4.2 ± 0.2	2.7 ± 0.2
2	10.5 ± 0.7	4.5 ± 0.3
4	7.3 ± 0.2	1.6 ± 0.1

Exposure to a high concentration of IBA (2.5 mg/l) was effective for root induction (Table 3). Root initiation started within 2 weeks. The percentage of rooting was 100% for both genotypes. IBA promoted abundant root development (2–13 good quality roots per shoot) without callus formation. After the rooting procedure, we recorded on average 5.9 roots per rooted shoot for genotype 20/1 and 5.3 for genotype Altaj. Higher survival rates (more than 80%) were obtained after acclimatization of rooted plants under greenhouse conditions. The plants established after period of *in vitro* culture showed no visible morphological differences from conventionally propagated plants.

DISCUSSION

In the present study 39 of 40 explants obtained after disinfection were free of bacterial and fungal contamination. Contaminations were rare probably because the mother plants were not exposed to field microflora in the isolated greenhouse. One half of initial explants of cultivar Altaj did not survive the sterilization procedure probably due to the toxicity to tissues caused by mercuric chloride. It was reported previously that plant explants could be successfully surface-sterilized using solutions of mercuric chloride. Al-Sabbagh et al. (1999) obtained approximately 90% primary explants free of fungal and bacterial contamination for semi-dwarfing cherry rootstock Maxma 14 (*Prunus avium* L.).

Table 3. Rooting on MS medium supplemented with 2.5 mg/l IBA

Genotype	Rooting of *in vitro* shoots		Number of roots per *in vitro* shoot ± SE
	number	(%)	
20/1	100	100	5.9 ± 0.3
Altaj	100	100	5.3 ± 0.1

However, the toxicity to tissues caused by mercuric chloride was high in their experiments and about 50% of initial uncontaminated explants died after sterilization procedure.

In our studies both genotypes of *L. kamtschatica* were micropropagated successfully using shoot culture medium with BAP and rooting medium with IBA. Adding the growth regulator (BAP) was essential for successful shoot initiation and development. Although the multiplication potential of two blue honeysuckle cultivars used in our study was different, BAP level 2 mg/l could be recommended for multiplication of both genotypes used in this study (20/1 and Altaj). Shoots taken from these proliferating cultures were easy to handle in the *in vitro* rooting procedure. It was previously observed that BAP could be used successfully to induce *in vitro* shoot production (Karhu 1997a), and that auxin IBA initiates rooting in *Lonicera* spp. (Karhu 1997b).

In summary, two genotypes of *L. kamtschatica* (Altaj, 20/1) responded favorably to the micropropagation procedure outlined in our study. Micropropagation techniques described in this paper may also be applied to other *Lonicera* species.

References

AL-SABBAGH M., ABDUL-KADER A., KHODER M., KALHOUT A.R., 1999. *In vitro* propagation of semi-dwarfing cherry rootstock. Plant Cell, Tissue and Organ Culture, *59*: 203–208.

BOONNOUR K., WAINWRIGHT H., HICKS R.G.T., 1988. The micropropagation of *Lonicera periclymenum* L. (Honeysuckle). Acta Horticulturae, *226*: 183–189.

KARHU S.K., 1997a. Axillary shoot proliferation of blue honeysuckle. Plant Cell, Tissue and Organ Culture, *48*: 195–201.

KARHU S.K., 1997b. Rooting of blue honeysuckle microshoots. Plant Cell, Tissue and Organ Culture, *48*: 153–159.

KARHU S.K., 2003. Performance of *Lonicera* microcuttings as affected by mineral nutrients and genotype. Acta Horticulturae, *616*: 181–183.

MANSFELD R., 1986. Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen. Berlin, Akademie-Verlag: 1252.

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

Received for publication April 3, 2007
Accepted after corrections June 8, 2007

Množení zimolezu v kulturách *in vitro*

ABSTRAKT: Byla vyvinuta metoda pro rychlé množení zimolezu (*Lonicera kamtschatica* [Sevast.] Pojark) v kulturách *in vitro*. Růstové vrcholy dvou genotypů 20/1 a Altaj byly úspěšně přeneseny do *in vitro* kultur a množeny na médiu typu MS (Murashige a Skoog) s přídavkem různých koncentrací 6-benzylaminopurinu. Bylo dosaženo různých koeficientů multiplikace v závislosti na použitém genotypu a koncentraci BAP. Nejvyššího multiplikačního koeficientu bylo dosaženo u genotypu 20/1, který produkoval 10,5 ± 0,7 nových výhonků (delších než 10 mm) na MS médiu obsahujícím 2 mg/l BAP. Nejnižšího multiplikačního koeficientu bylo dosaženo u genotypu Altaj, který produkoval 1,6 ± 0,1 nových výhonků (delších než 10 mm) na MS médiu obsahujícím 4 mg/l BAP. Dále bylo zaznamenáno kořenění v *in vitro* kulturách na modifikovaném MS médiu obsahujícím 2,5 mg/l kyseliny beta indolylmáselné (IBA). Kořenící výhony byly přeneseny do skleníku pro další hodnocení.

Klíčová slova: *Lonicera*; mikropropagace; ustavení kultury; multiplikace; 6-benzylaminopurin

Corresponding author:

Ing. Jiří Sedlák, Výzkumný a šlechtitelský ústav ovocnářský Holovousy, s. r. o., Holovousy 1,
508 01 Hořice v Podkrkonoší, Česká republika
tel.: + 420 493 692 821, fax: + 420 493 692 833, e-mail: sedlak@vsuo.cz