IN VITRO PROPAGATION OF *Lonicera kamtschatica*

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Abstract. The goal of this study was to elaborate a micropropagation protocol for *Lonicera kamtschatica*. Large-sized plantlets were obtained, with long axillary shoots and high proliferation rates by the use of cytokinin CPPU. The use of starch as a gelling agent is an element of novelty and it proved to be very effective and five times cheaper as compared to agar. The most suitable iron source for the in vitro culture of this species proved to be FeNaEDDHA (Sequestrene 138), whereas the microcutting types that yielded the highest multiplication rates were the 3-5 node lateral shoots. The axillary shoots were sucessfully rooted directly *ex vitro* in the acclimatization stage in floating perlite, which is a method routinely used at the Fruit Research Station Cluj.

Keywords: Atut**,** CPPU, Sequestrene 138, starch, micropropagation.

Abbreviations:
IBA - indolyl butyric acid
BAP- 6-benzilaminopurine
CPPU - N-(2-Cloro-4-pyridyl)-N-phenylurea
MS - Murashige and Skoog Media (Murashige and Skoog, 1962)
mT - meta-topoline
WPM - Woody Plant Medium (Lloyd and McCown, 1980)
PR-proliferation rate
MR - multiplication rate

INTRODUCTION

Lonicera kamtschatica is a fruit shrub in Family Caprifoliaceae, from the boreal regions of Russia and Japan. It is extremely frost-tolerant and resistant to pests and diseases. *Lonicera kamtschatica* fruits contain high amounts of vitamins (ascorbic acid, niacin and totopherols), minerals (potassium, calcium, phosphorus), phenolic compounds (protocatechuic, gentisic, elagic, ferulic, cafeic, chlorogenic and coumaric acid) as well as anthocyanins, which represent 0.31 % from the fruit mass. From the anthocyanins, 83.3 % is cyanidin-3 glucoside and the rest are tens of other types, like paeonidin-3 glucoside, cyanidin-3 rutinoside, malvidin-3-arabinoside, petunidin-3 glucoside, petunidin-3-rutinoside (Palikova *et al*., 2008). Having in view its nutritional and medicinal qualities, this species is an extremely important crop plant. Micropropagation could be an effective alternative to the classical, traditional method of propagation by cuttings, as it could be used for the production of large amounts of healthy planting material in a short time.

The cultivar investigated in our study is a very important one, highly productive, with good quality fruit, containing high amounts of ascorbic acid and anthocyanins (Malodobry *et a*l., 2010). The goal of our research was to increase the effectiveness of micropropagation in *Lonicera kamtschatica*, cultivar 'Atut'.

E. Dziedzic (2008) carried out experiments with cultivars 'Czelabinska' and 'Duet'. The cultures were initiated from axillary buds disinfected with 70 % ethanol and 10 % calcium hypochlorite. Initially the explants were cultured on MS media with 1 mg/l BAP. After 6 months of subcultures for stabilization, experiments were carried out using several types of basal media: full-strength MS, MS diluted to 75 %, MS diluted to 50 %. To these, 1-2 mg/l BAP was added. FeNaEDTA concentration was doubled as compared to the usual MS. The best results regarding shoot proliferation as well as fresh biomass were obtained on MS diluted to 75 %. Tens of shoots/initial explant were obtained on these experimental treatments, in cultivar 'Duet' there was the highest proliferation rate, 97.2 shoots/explant on MS diluted to 75 % supplemented with 2 mg/l BAP. Many shoots were hyperhydric and many shoots were very short, less than 0.5 cm in length; most shoots were 0.5-1.5 cm in length. The microcuttings were rooted in various basal media (MS, 50 % MS and WPM) supplemented with 2 mg/l IBA and 5 mg/l IAA. The higest rooting percentages were in the treatments based on WPM. The rooting percentages were very high, 96 % for Czelabinska and 92 % for 'Duet', after 15 days on the rooting media. *Ex vitro* acclimatization was carried out using two treatments: peat and a mixture of peat and AgroAquaGel at 4 g/l. The AgroAquaGel treatment stimulated root growth and development and substantially improved the quality of the plants.

Sedlák and F. Paprštein (2007) worked with two *Lonicera* genotypes, Altaj and 20/1. The initiation of in vitro cultures was done using 5-10 mm shoot tips from 3-year old plants cultured in the greenhouse. The explants were disinfected with mercuric chloride. In both genotypes, survival rates were very high. In genotype 20/1 there was 90 % survival and no contamination. In 'Altaj' there was 45 % survival and regeneration and 5 % contamination. Axillary shoot proliferation was the highest at 2 mg/l BAP in both genotypes: 10.5 shoots/explant in genotype 20/1 and 4.5 shoots/explant in cultivar Altaj. In vitro rooting was successfully carried out on MS media with minerals diluted to 1/3, supplemented with 2.5 mg/l IBA. There was 100 % rooting in both genotypes, resulting 2-13 vigorous roots/plantlet. Average root numbers/plantlet were 5.9 in genotype 20/1 and 5.3 in cultivar 'Altaj'.

Wang et al. (2009) experimented with the micropropagation of *Lonicera macranthoides*, cultivar 'Jincuilei', which is a very important genotype due to its flowers' content in chlorogenic acid. They elaborated an effective protocol based on the regeneration of adventitious shoots from leaf explants. For callus induction they used Gamborg's B5 medium supplemented with 2,4-D and BAP. Adventitius shoot regeneration took place on media supplemented with kinetin and NAA; the optimal concentrations were 0.9 μM and 5.4 μM, respectively. The regenerated adventitious shoots were rooted on half-strength MS media supplemented with 14.8 μM IBA. The rooted plantlets were acclimatized in a shaded greenhouse in a mixture containing clayey soil, coarse sand and charred rice husk.

Karhu (1997) carried out in vitro rooting experiments with *Lonicera caerulea* var. *caerulea* and *L. caerulea* var. *edulis* using MS media with various concentrations of minerals (25 % - 100 %) and various concentrations of NAA and IBA. She also carried out direct *ex vitro* rooting experiments using peat. Another approach was the use of auxin treatment of the microcuttings followed by in vitro rooting in hormone-free media or direct *ex vitro* rooting. The reaction of the two genotypes was very different. In *L. caerulea* var. *caerulea* rooting percentages were higher than 90 % in all treatments. Among the auxins, IBA proved to be more effective than NAA. Half-strength MS combined with IBA at 4 μM proved to be very effective, as it provided high rooting percentages and large numbers

of principal and lateral roots. In *Lonicera caerulea* var. *edulis* on half-strength MS there was no rooting. On MS reduced to 25 % there was only 10 % rooting. On the treatment 25 % MS salts + 4 μM IBA there were optimal results regarding rooting percentages, root length and the number of main roots and lateral roots. If applying IBA as pulse followed by in vitro rooting on hormone-free media, the rooting percentage was high in Lonicera caerulea var. edulis (more than 85 %) and no calli were generated at the base of the plantlets.

Karhu (1997, B) studied the in vitro multiplication of *Lonicera caerulea* var. *caerulea* and *L. caerulea* var. *edulis*. She carried out several independent experiments, studying various factors: explant type; growth regulators (BAP compared with kinetin); various BAP concentrations; the effect of temperature above in vitro proliferation, MS basal media (either full or half strength, with increased amounts of FeNaEDTA). In these studies, proved to be more effective than kinetin. Increaseing BAP concentrations from 1.1 to 17.8 μM lead to increased proliferation rates, but the lenth of axillary shoots was decreased, whereas the calli at the plantlet's base increased in diameter. High temperatures, up to 28 °C increased proliferation rates, also. The author considered 8.9 μM BAP and 26 °C as optimal. Decreasing the concentration of minerals in the culture media stimulated axillary shoot proliferation, but also increased the frequency of apex necrosis.

Hui et al. (2012) set up an efficient protocol for *Lonicera japonica* micropropagation. Culture initiation was done using apical buds. For multiplication they used WPM supplemented with various concentrations of auxins and cytokinins, among which four combinations gave good results, with more than 5 shoots regenerated per explant. These consisted of combinations of NAA at 2, 6 and 9 μM or IAA at 9 μM with BAP at the concentrations of 2, 4.5 and 8.5 μM. Rooting was carried out using IAA and IBA lat various concentrations (0-5.5 μM IAA combined with 0-3 μM IBA). All these concentrations gave rooting percentages of more than 80 %. The highest number of roots resulted on media with 2.0 μM IBA + 2.5 μM IAA. The plantlets were successfully acclimatized *ex vitro*, with more than 70 % survival.

Osburn et al. (2009) experimented with *Lonicera japonica* and *L. maackii*. Among the basal media, Driver and Kuniyuki proved to be the most effective. High axillary shoot proliferation rates were obtained, in both species, by using BAP. For optimal proliferation, the cultures had to be incubated for 12 weeks. The axillary shoots were successfully rooted *ex vitro* with or without PGR treatment in *L. japonica*. In *L. maackii* good results were obtained by treatment with 200 mg/l IBA. From the facts presented above it results that *Lonicera* species, especially *L. kamtschatica* represent a challenge for micropropagation due to the relatively low proliferation rates in some cases, short shoots, physiological problems like necrosis of the apices, hyperhydricity on media with high BAP concentration, which necessitate the further optimization of micropropagation technologies for Lonicera.

MATERIALS AND METHODS

Plant material. This study was carried out with *Lonicera kamtschatica*, cultivar 'Atut', using two-year old container-grown plants cultured in the greenhouse.

***In vitro* culture initiation.** For *in vitro* culture initiation, two experimental series were carried out.

Young, semi-lignified shoots were harvested, the leaves were cut off and the shoots were washed for 30 minutes under cold running water, followed by three rinses in sterile deionized water on the magnetic stirrer. The following operations were done under the laminar airflow hood. The shoots were disinfected for 20 minutes with a mixture of 20 % ACE bleach in sterile deionized water, followed by four rinses in sterile deionized water. The apical and axillary buds were excised and inoculated onto modified MS media, with 0.5 mg/l BAP (Table 1, MSm). The culture vessels used for *in vitro* culture initiation were test-tubes conaining 5 ml of culture media. The cultures were incubated for two months in the growth room at 23 $\pm$ 3 °C and 2400 Lux (36 μmol m^{-2} s^{-1}) light intensity.

In the second experimental series, modified MS media were used, with macroelements' concentration reduced to 1/2, as well as full strength WPM (Table 1, MS/2, respectively WPM). Both treatments contained 0.5 mg/l BAP. The explants consisted of apices excised from the apical buds. In most of the apical buds 2-3 apices were found.

Table 1.

The composition of culture media for the initiation and stabilization of *Lonicera kamtschatica in vitro* cultures and for the preliminary tests of *in vitro* multiplication

Components	Concentration		
	MSm	MS/2	WPM
MS macroelements	Full strength	Half strength	-
MS microelements	Full strength	Full strength	-
WPM macroelements	-	-	Full strength
WPM microelements	-	-	Full strength
FeNaEDTA	36.7 mg/l	36.7 mg/l	36.7 mg/l
Myo-inositol	100 mg/l	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l	2 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l	1 mg/l
Nicotinic acid	0.5 mg/l	0.5 mg/l	1 mg/l
BAP	0.5 mg/l	0.5 mg/l	0.5 mg/l
Sugar	30 g/l	30 g/l	30 g/l
Plant Agar	6 g/l	6 g/l	6 g/l
pH adjusted to 5.8			

The *in vitro* multiplication stage

The *in vitro* culture stabilization stage and preliminary tests for the induction of *in vitro* proliferation.

The viable plantlets obtained in the multiplication stage were subcultured on MS media with macroelements reduced to 1/2 as well as on modified MS media with full concentration of minerals and on WPM. The culture media were supplemented with 0.5 and 0.7 mg/l BAP (Table 1). The culture vessels were 320 and 720 ml glass jars with vented caps, containing 50, respectively 100 ml of media.

Due to the slow growth and insignificant proliferation on the media presented previously, for the maintenance of *in vitro* cultures modified MS media were tested, gelled with 50 g/l wheat starch instead of agar and supplemented with 0.5 mg/l BAP (Table 2, A).

Due to obvious leaf chlorosis, a similar MS medium was tested, but containing 100 mg/l Sequestrene 138 (FeNaEDDHA) instead of FeNaEDTA (Table 2, B). Due to negligible axillary shoot proliferation in the presence of 0.5 mg/l BAP we tested 1 mg/l BAP, based on the information from scientific literature.

Table 2.

The composition of modified MS culture media used in the multiplication experiments

Component MS* salts	A Full strength	B Full strength	C Full strength
FeNaEDTA	36.7 mg/l	-	-
FeNaEDDHA (Sequestrene 138)	-	100 mg/l	100mg/l
Myo-inositol	100 mg/l	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l	1 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l	0.5 mg/l
Nicotinic acid	0.5 mg/l	0.5 mg/l	0.5 mg/l
BAP	0.5 mg/l	0.5 mg/l	1 mg/l
Sugar	30 g/l	30 g/l	30 g/l
Wheat starch	50 g/l	50 g/l	50 g/l

* Macroelements and microelements, without iron source

For the *in vitro* multiplication experiements we used 720 ml jars containing 100 ml of culture media/vessel. The jars were fitted with vented caps. The explants were inoculated into the culture media in such a way that 2/3-3/4 of their basal portion was immersed into the culture media.

The influence of explant type in the *in vitro* multiplication stage

The types of explant we used were:

V1- shoot fragments with three nodes;

V2- short lateral shoots (2-3 cm in length), with 3-5 nodes;

V3- whole shoots, about 5 cm in length.

The culture medium was modified MS, supplemented with FeNaEDDHA and 1 mg/l BAP, gelled with 50 g/l wheat starch (Table 2, C). There were 5 explants/vessel, six vessels per experimental treatment.

Testing various types of cytokinins

The experimental treatments for testing cytokinins were:

C1- BAP 1 mg/l (control) C3- zeatin 1 mg/l C5- CPPU 1 mg/l

C2- mT 1 mg/l C4- CPPU 0.5 mg/l

The basal medium was the one in Table 2, B or C; BAP was substituted with the cytokinins specified for treatments C1-C5. The explant type consisted of shoot fragments with three nodes. Five microcuttings/vessel were used. The experimental treatment C1, with 1 mg/l BAP was considered control. Seven vessels per experimental treatment were examined (35 plantlets/treatment).

Establishing the optimal CPPU concentration for the *in vitro* multiplication of *Lonicera kamtschatica*. Two experimental series were set up for testing the influence of CPPU. In the first series this cytokinin was tested in several concentrations (0.5, 0.7, 1

and 1.5 mg/l) in order to optimize the *in vitro* multiplication stage. The basal medium was modified MS (Table 2B). BAP was substituted with CPPU. The explant type was 3-node shoot fragments. Five microcuttings/vessel were used. Seven vessels per experimental treatment were examined (35 plantlets/treatment). The source of microcuttings was represented by plantlets propagated on media with 1 mg/l BAP. In the second experimental series the treatments containing 0.5 and 0.7 mg/l CPPU were re-tested in order to establish the optimal concentration of this growth regulator in the multiplication stage. The plant material consisted of microcuttings excised from plantlets regenerated on media with 0.7 mg/l CPPU. Two types of explants were used:

- microcuttings with 3 nodes, as in the earlier experiments;
- short lateral shoots, under 2 cm in length, containing 1-2 nodes and the apical bud.

For each type of explant, five microcuttings/vessel were used. Seven vessels per experimental treatment were examined (35 plantlets/treatment).

The direct *ex vitro* rooting and acclimatization of Lonicera cultivar 'Atut' shoots obtained in the multiplication stage. As a method of direct *ex vitro* rooting and acclimatization, the floating perlite method was used (Fira *et al*, 2012; Clapa *et al*., 2013).

Three treatments were tested for *ex vitro* rooting in floating perlite. The plant material consisted of shoots excised from plantlets grown on media with 0.7 mg/l CPPU. The cultures were kept in the growth room for one month.

R1- hormone-free

R2 - 0.5 mg/l IBA;

R3 - 1 mg/l IBA

Statistical data analysis. Monofactorial ANOVA was used ($p<0.05$). The culture vessel was considered as experimental unit for the comparisons regarding the number of shoots resulted/vessel and the number of inocula (microcuttings) resulted/vessel. For the comparisons regarding plantlet height, multiplication rates and proliferation rates the plantlet was considered experimental unit.

Multiplication rates were considered to be the number of standard-sized microcuttings that could be obtained per plantlet (three-node shoot fragments or whole shoots at least 2 cm in length, containing at least three nodes). Axillary shoot proliferation rates were considered to be the number of standard-sized axillary shoots resulted/plantlet (at least 2 cm in length, containing at least three nodes). In many cases MR was higher than PR because two microcuttings could be obtained from some of the shoots.

RESULTS AND DISCUSSIONS

***In vitro* culture initiation.** In the first experimental series of *in vitro* culture initiation all the explants underwent necrosis. In the second experimental series, six cultures/treatment were contaminated, from the total number of 30 inocula/treatment. All the explants consisting of axillary buds underwent necrosis. Only the explants consisting of apices survived, resulting 5 viable plantlets/treatment.

The *in vitro* multiplication stage.

The *in vitro* culture stabilization stage and preliminary tests for the induction of *in vitro* proliferation. On all the MS and WPM treatments (Table 1) there was little growth and negligible proliferation. Leaf chlorosis anappeared and necrosis of apices.

After transfer onto MSm media with full nutrient concentration, supplemented with 0.5 mg/l BAP the plantlets grew fast, but axillary shoot proliferation was negligible. Long shoots regenerated, which grew up to the caps of the culture vessels in two months. Chlorosis persisted. After transfer to modified MS media containing FeNaEDDHA as iron source, the plantlets looked healthier, with a more intense green colour. Chlorosis was greatly reduced, apparently. In the *in vitro* culture stabilization stage for *Lonicera kamtschatica* it was established that the basal medium should be modified MS with FeNaEDDHA as iron source and 50 g/l wheat starch as gelling agent.

The influence of explant type in the *in vitro* multiplication stage

The best results regarding proliferation and multiplication rates were obtained in treatment V2, where lateral shoots containing 2-3 nodes were used (Table 3). The differences regarding *in vitro* proliferation were significant statistically. The differences regarding the viability percentage of the inocula were not significant statistically.

Testing various types of cytokinins.

BAP at 1 mg/l gave mediocre results regarding proliferation and multiplication rates, like in the preceding experiment. Meta-topolin (Treatment C2) gave poorer results than the control. The worst results were recorded in treatment C3, with 1 mg/l zeatin (Table 4), in which there was also abundant callus growth at the plantlets' base (Figure 1). Like in the previous experiment, there was apex necrosis in some shoots.

Table 3.

The biometrical values per experimental treatment regarding *in vitro* multiplication in *Lonicera kamtschatica* cultivar 'Atut'

Treatment	Shoots resulted/ vessel	No. of inocula resulted/ vessel	Average plant height (cm)	Average PR	Average MR
V1 - control	40	48.14	6.56	8	9.63
V2	**60.43**	**73.57 ****	**8.05 n. s**	**12.09 ***	**14.71****
V3	38.80	50.40 n. s	5.89 *	7.76 n. s	10.08 n. s

* - statistically significant ($p<0.05$), ** - distinctly significant statistically ($p<0.01$); n. s - not significant statistically

Table 4

The average values regarding the *in vitro* multiplication of *Lonicera kamtschatica* under the influence of some cytokinins

Treatment	No. of shoots resulted/ vessel	No. of inocula resulted/vessel	Average PR	Average MR	Viability percentage (%)
C1 (control)	17.5	27.66	3.5	5.53	100
C2	9**	16.66**	1.8***	3.33***	100
C3	6**	8.33*	1.2***	1.66***	90*
C4	42.33n.s	50.83n. s	8.47*	10.16*	90*
C5	58.83*	71.83n. s	11.77**	14.36*	66.66n. s

* - statistically significant ($p<0.05$), ** - distinctly significant statistically ($p<0.01$);
*** - very significant statistically ($p<0.001$), n. s - not significant statistically

The best results regarding proliferation and multiplication rates were recorded in treatments C4 and C5, where the cytokinin was CPPU. In these treatments, also, callus

was present at the explants' base, especially in treatment C5 with 1 mg/l CPPU. In many plantlets there was an exaggerated amount of callus. In the treatments C4 and C5 the multiplication rates were 10.16 and, respectively 14.36, superior to the ones obtained by Sedlák and Paprštein (2007), who obtained 10.5 shoots/explant in genotype 20/1 and 4.5 shoots/explant in cultivar 'Altaj'. The shoots were also well developed and had a healthy appearance.

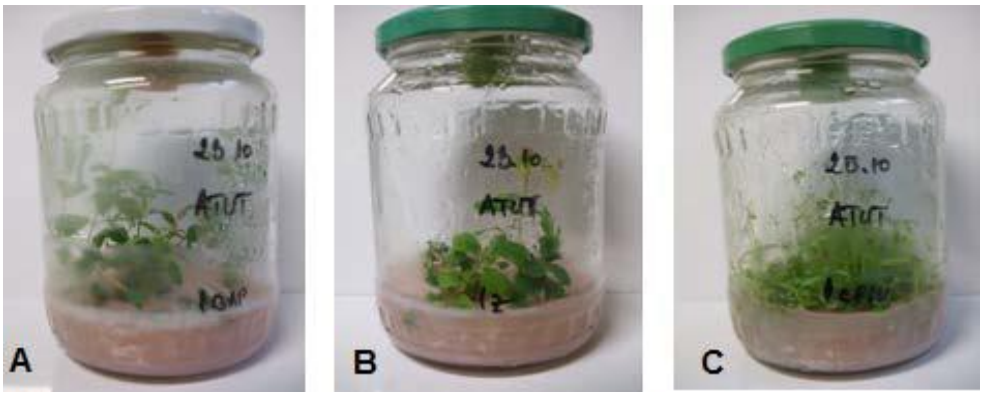

Figure 1. *In vitro* cultures of *Lonicera kamtschatica*, cultivar 'Atut' on various culture media: A- MSm+ 1 mg/l BAP; B- MSm+1mg/l zeatin;
C- MSm+1 mg/l CPPU.

Establishing the optimal CPPU concentration for the *in vitro* multiplication of *Lonicera kamtschatica*. In the first experimental series all the CPPU concentrations (0.5, 0.7, 1 and 1.5 mg/l) provided intense shoot proliferation, especially at 1 and 1.5 mg/l. But the high CPPU concentrations also had bad side effects: short shoots, many of them deformed, abundant calli generated at some plantlets' bases and many of the microcuttings generated non-viable plantlets due to deformation and callusing, as well as uneven growth and proliferation. Low plantlet viability lead to lower average proliferation rates than in the treatment with 0.7 mg/l CPPU, due to the fact that many inocula generated just a few shoots or no shoots at all (Table 5). In the treatments with 0.5 and 0.7 mg/l CPPU the plantlets were well developed, with longer shoots and healthier appearance.

Table 5

The average values regarding the *in vitro* multiplication of *Lonicera kamtschatica*, cultivar 'Atut' under the influence of cytokinin CPPU

Treatment	No. of shoots resulted/vessel	No. of inocula resulted/vessel	Average PR	Average MR
0.5 mg/l CPPU	37.5	43.16	7.5	8.63
0.7 mg/l CPPU	82.16	98.16	16.43	19.63
1 mg/l CPPU	60.83	73.33	12.17	14.67
1.5 mg/l CPPU	17.33	21.66	3.47	4.33

In the second experimental series the treatments with 0.5 and 0.7 mg/l CPPU were re-tested in order to establish optimal concentration. The treatments with 1 and 1.5 mg/l CPPU were abandoned due to the low quality of the plantlets. In the treatment with 0.7 mg/l CPPU superior results were obtained regarding axillary shoot proliferation and

other biometrical characteristics (the number of shoots resulted/vessel, te number of microcuttings resulted/vessel, proliferation rate, multiplication rate and the results were in most cases significant statistically as compared to the treatment with 0.5 mg/l CPPU, which was considered control or reference (Table 6). Both CPPU concentrations provided the regeneration of well-branched and well-developed plantlets, with normal, healthy appearance. Callus was generated at the plantlets' base, especially at 0.7 mg/l CPPU, but not in excessive amounts.

Table 6

The results regarding axillary shoot proliferation *in vitro* in *Lonicera kamtschatica*, cultivar 'Atut' under the influence of two CPPU concentrations

Treatment	Plantlet height (cm)	No. of shoots resulted/ vessel	No. of inocula resulted/ vessel	Average PR	Average MR
0.5 mg/l CPPU	9.16	38	48	7.6	9.6
0.7 mg/l CPPU	6.74**	80.29**	94.85**	16.06***	8.97***
0.5 mg/l CPPU-short shoots	7.78	36.42	45.71	7.28	9.14
0.7 mg/l CPPU-short shoots	8.21 n. s	56.57 n. s	71 n. s	11.31 n. s	14.2*

* - statistically significant ($p<0.05$); ** - distinctly significant statistically ($p<0.01$);

*** - very significant statistically ($p<0.001$); n. s - not significant statistically.

The standard-sized microcuttings (shoot fragments with three nodes) as well as the ones consisting of short lateral shoots proved to be adequate for *in vitro* multiplication and we recommend the use of both types of microcuttings. But the short shoots developed unevenly, with great variability regarding proliferation rates and plantlet height.

The direct *ex vitro* rooting and acclimatization of Lonicera cultivar 'Atut' shoots obtained in the multiplication stage. In the shoots excised from plantlets regenerated on media supplemented with 0.7 mg/l CPPU, IBA stimulated direct *ex vitro* rooting in floating perlite (Table 7). The treatment with 1 mg/l IBA proved to be optimal as this provided superior rooting percentages as compared to the ones obtained in the hormone-free perlite treatment. Root length could not be measured and the roots could not be counted because they were entangled and dispersed on the shoots.

The differences between the control (perlite without IBA) and the treatment with 1 mg/l IBA were significant statistically ($p<0.05$).

Table 7

The influence of IBA upon the *ex vitro* rooting of axillary shoots of *Lonicera kamtschatica*, cultivar 'Atut' in floating perlite

Treatment	Vessel	Total no. of shoots	Rooted shoots	Non-rooted live shoots	Shoots with necrosis	Rooting percentage (%)
R3- (control)	1	175	35	68	72	20
hormone-free	2	171	43	58	70	25.15
R4-	1	202	98	59	45	48.51
0.5 mg/l IBA	2	196	107	28	61	54.59
R5-	1	89	55	14	20	61.79
1 mg/l IBA	2	146	115	4	27	78.76

CONCLUSIONS

The results obtained in the the initiation, multiplication and acclimatization stages in *Lonicera kamtschatica*, cultivar 'Atut' show that micropropagation can be a means for the production of container-grown planting material in this species.

The *in vitro* multiplication stage was optimized, as intense axillary shoot proliferation was achieved. The shoots wewre well developed, in some cases exceeding 6-8 cm, unlike the results obtained by other researchers.

As a basal medium for *Lonicera kamtschatica in vitro* culture, MS media gelled with 50 g/l wheat starch can be used, as the media gelled with Plant Agar proved to be inadequate for the multiplication stage.

Intense proliferation of axillary shoots was provided by the presence of cytokinin CPPU in the culture media gelled with wheat starch. The optimal CPPU concentration proved to be 0.7 mg/l.

For the rooting and acclimatization of *Lonicera kamtschatica* shoots in floating perlite we recommend a solution of 1 mg/l IBA.

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