

MICROPROPAGATION OF *PLANTAGO CAMTSCHATICA* LINK

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ABSTRACT

The Far East medicinal plant – *Plantago camtschatica* was propagated in vitro from tips of shoots (obtained in vitro) and from different explants of 4-week-old seedlings: seedling tips, hypocotyls, cotyledons, roots, first leaves. To our knowledge there is no information in literature about in vitro culture of this plantain. MS basal medium, supplemented with 0.6 µM IAA in combination with various cytokinins (BA, KIN, ZEA), was used. After 6 weeks of culture, micropropagation rate (MR) – mean number of buds and shoots per explant – was calculated. Our study proved that *P. camtschatica* species was amenable to propagation in vitro from different kinds of explants. However, multiplication by adventitious shoot regeneration from hypocotyl explants was found to be the most suitable method for the propagation of this plant. Adventitious shoots could root without stimulation what allows to omit the stage of rooting. The plants obtained as a result of micropropagation were not phenotypically changed.

KEY WORDS: culture in vitro, Kamchatic plantain, organogenesis, Plantaginaceae, root regeneration.

INTRODUCTION

Kamchatic plantain – *Plantago camtschatica* Link, syn. *P. depressa* Wild. subsp. *camtschatica* (Cham. ex Link) Pilg. is one of the East Asian species belonging to the Plantaginaceae family. It is a perennial herb growing on slopes and sand hills near the sea in Japan, Korea, Sakhalin, Kuriles, Kamchatka, Commander Islands and in the regions of Ussuri and Amur (Pilger 1937; Yamazaki 1993; Lee 1996). In the Far East it is a medicinal plant. The phytochemical studies have shown that this plant contains several interesting secondary metabolites, mostly iridoid glucosides: aucubin, geniposidic acid, majoroside and others, phenylethanoid glucoside: verbascoside, isoverbascoside, orobanchoside and others, also sorbitol (Ying et al. 1993; Nishibe et al. 1995; Rønsted et al. 2003).

To our knowledge there have been so far no reports on in vitro culture of *Plantago camtschatica*. However, some other species of the genus *Plantago* were studied in culture in vitro: *P. afra* L. (Sarihan et al. 2005), *P. asiatica* L. (Tu

1996; Makowczyńska and Andrzejewska-Golec 2003; Makowczyńska 2006; Makowczyńska et al. 2008); *P. lanceolata* L. (Fons et al. 1999; Budzianowska et al. 2004; Khawar et al. 2005), *P. major* L. (Brimer 1988; Mederos et al. 1997/1998); *P. maritima* L. (Chang and Locy 1996; Makowczyńska and Andrzejewska-Golec 2007); *P. media* L. (Kunvári et al. 1999); *P. ovata* Forsk. (Wakhlou and Barna 1989; Barot et al. 1994; Pramanik et al. 1995; 1996; Chowdhury et al. 1996).

MATERIAL AND METHODS

Explant sources

– 4-week-old *Plantago camtschatica* seedlings from seeds provided by the Botanical Garden of Martin Luther Universität Halle. Seeds were surface-sterilized by immersion in 2.0% sodium hypochlorite for 10 min., then rinsed 3 times in sterile water and germinated (MS enriched with 0.9 µM KIN and 2.9 µM GA₃), as it was previously described for seeds of *P. asiatica* (Makowczyńska and Andrzejewska-Golec 2003)

– shoots derived from the in vitro culture

Kind of explants

– from seedlings: tips (approximately 0.5 cm long), hypocotyls (approximately 0.5-1.0 cm long), roots (approx-

Abbreviations

BA – 6-bezyladenine; 2,4-D – 2,4-dichlorophenoxyacetic acid; GA₃ – gibberellic acid; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; KIN – kinetin; MS – Murashige and Skoog basal medium; MR – micropropagation rate; NAA – α-naphthaleneacetic acid; TDZ – thidiazuron; ZEA – zeatin.

mately 1 cm long), cotyledons (cultured whole), first leaves (cultured whole)

– from shoots: tips (approximately 0.5 cm long)

Conditions of in vitro culture

Conditions of the in vitro culture were the same as they have been used by us successfully for the micropropagation of the other Far East taxon – Asiatic plantain: MS medium solidified with 0.7% agar, pH adjusted to 5.6-5.9; 26±2°C; light 40 µM/m² s, humidity 80-90% (Makowczyńska and Andrzejewska-Golec 2003).

The cultures were continued in a growth cabinet for 6 weeks.

Growth regulators used in experiments for micropropagation

– auxin: 0.6 µM IAA,
– cytokinins: 2.2, 4.4 or 8.9 µM BA – for shoots formed from tips,
– 8.9 µM BA, 9.3 µM KIN or 9.1 µM ZEA – for shoot regeneration through organogenesis on hypocotyls, cotyledons, roots and first leaves.

Rooting of shoots

MS alone or MS in combination with auxins: 0.6 or 2.8 µM IAA; 0.5 or 2.5 µM IBA; 0.5 or 2.7 µM NAA were used.

Multiplication rate (MR) was evaluated as the mean number of buds and shoots on one explant at the end of the multiplication cycle (6 weeks).

Acclimatisation was conducted in flower pots for two months.

RESULTS AND DISCUSSION

Shoot formation from seedling tips (Table 1)

Micropropagation of the plants through culture shoot-tips is the utilisation of meristem which are already present in explants. This method is commonly employed in in vitro cultures of decorative and therapeutic plants. This method has been described also for few medicinal plants of the ge-

nus *Plantago*. Our study is the first publication of *P. camtschatica* propagation by shoot-tips method. It was appeared to be amenable to propagation in vitro of Kamchatic plantain, but this method is less productive than other methods used in our work. The greatest MR obtained by us for seedling tips of *P. camtschatica* was only 3.7±0.7 for MS containing 0.6 µM IAA and 4.4 µM BA. In our earlier study also in the case of seedling tips for the other Far East taxon of genus *Plantago* – *P. asiatica* – MR was 5.18±0.58 for the same conditions of in vitro culture as in the present study for *P. camtschatica* (Makowczyńska and Andrzejewska-Golec 2003). Pramanik et al. (1995) obtained the best multiplication effect using seedling tips of *P. ovata* with MS medium enriched with the same growth regulators but in higher concentrations (1.1 µM IAA and 22.2 µM BA).

In the base of 90-100% seedling tips the roots were grown.

Shoot formation on tips of shoots derived from in vitro culture (Table 2)

A better effect of *P. camtschatica* shoot formation was obtained by us for the culture of tips of shoots derived from in vitro culture than for seedling tips. MR was then 7.2 for MS with 0.6 µM IAA and 8.9 µM BAP.

Shoot regeneration from hypocotyls, cotyledons, roots and first leaves (Table 3, Figs 1, 2 and 5)

Different cytokinins (BA, KIN, ZEA) with 0.6 µM IAA were tested for their effect on shoot regeneration from various explants of Kamchatic plantain. In the case of each cytokinin the most shoots were regenerated on hypocotyl explants. MR (shoots and buds /explant) about 13 for BA and ZEA, and about 9 for KIN was obtained. Regeneration from root explants was high only when ZEA was applied. A very high multiplication rate in the case of hypocotyl explants from some species of the genus *Plantago* was obtained by some authors, and so: for species *P. lanceolata* – 68.5 shoots per explant (MS + 0.45 µM TDZ + 0.09 µM IBA) by Khawar et al. (2005), and for *P. afra* – 23.17 shoot per explant (MS medium + 0.91 µM TDZ and 0.98 µM IBA) by Sarihan et al. (2005). In our experiments with

TABLE 1. Effect of various concentration of cytokinin BA and 0.6 µM IAA added to MS on shoot micropropagation from seedling tips of *Plantago camtschatica*.

BA (µM)	MR of buds and shoots ±SE	MR of shoots ±SE	MR of normal shoots ±SE	Mean shoot length (cm) ±SE
2.2	2.6±0.6	1.9±0.4	1.7±0.4	2.5±0.3
4.4	3.7±0.7	3.6±0.8	3.2±0.7	2.1±0.2
8.9	2.4±0.4	1.9±0.3	1.9±0.3	2.5±0.4

SE – standard error of the mean

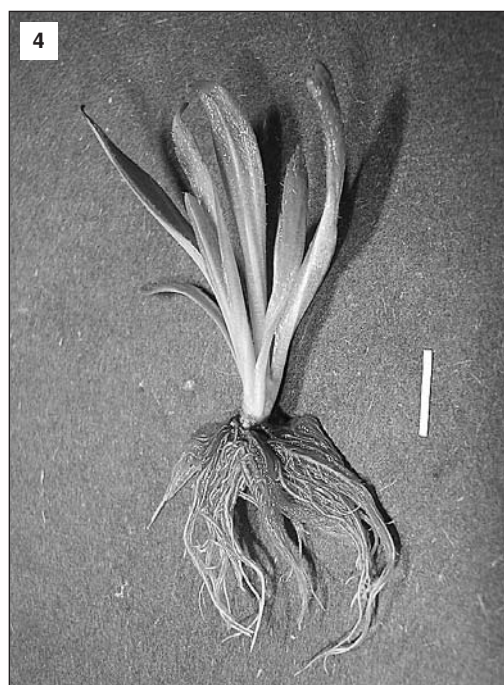
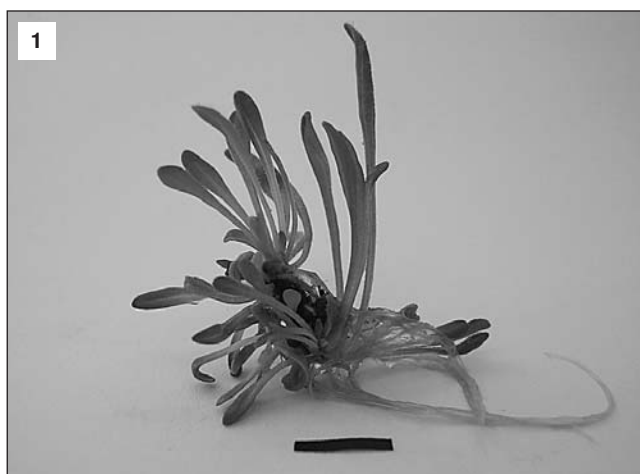
For each treatment about 10-11 explants were used.

TABLE 2. Effect of various concentration of the cytokinin BA and 0.6 µM IAA added to MS on shoot micropropagation from shoot tips of *Plantago camtschatica*.

BA (µM)	MR of buds and shoots ±SE	MR of shoots ±SE	MR of normal shoots ±SE	Mean shoot length (cm) ±SE
2.2	4.2±0.7	2.4±0.4	2.4±0.4	1.8±0.1
4.4	4.9±1.0	3.6±0.8	3.5±0.6	3.3±0.3
8.9	7.2±0.9	4.8±0.8	4.8±0.8	2.4±0.1

SE – standard error of the mean

For each treatment about 17-22 explants were used.



Figs 1-5. *Plantago camtschatica* Link in culture in vitro.

Fig. 1. Direct organogenesis on the dissected fragment of hypocotyl. MS + 0.6 μ M IAA + 9.1 μ M ZEA. Fig. 2. Direct organogenesis on the cotyledo. MS + 0.6 μ M IAA + 9.1 μ M ZEA. Fig. 3. Rooted shoots on the MS after 4-week-old culture. Fig. 4. Rooted shoots on the MS supplemented with 0.5 μ M NAA after 4-week-old culture. Fig. 5. Rooting shoots after 6-week micropropagation on leaf. MS + 0.6 μ M IAA + 9.3 μ M KIN. Bar 1 cm.

Fig. 6. *Plantago camtschatica* Link after two-month pot growth. Bar 1 cm.

P. camtschatica, the greatest effect of regeneration out of other explants was also achieved for hypocotyls, but condi-

tions of the culture were different (also MS, but other combination of growth regulators).

TABLE 3. Effect of medium with various cytokinins and 0.6 μM IAA on shoot micropropagation on various explants of *Plantago camtschatica*.

Kind of explants	Cytokinin	% of explants with buds and shoots	MR of buds and shoots $\pm\text{SE}$	% of abnormal buds and shoots	Mean shoot length (cm) $\pm\text{SE}$	% of shoots with roots
Root	BA	95.0	7.2 \pm 1.1	16.8	2.3 \pm 0.2	10.0
	KIN	60.0	6.9 \pm 1.0	10.8	2.2 \pm 0.1	29.9
	ZEA	100	12.7 \pm 1.1	30.6	2.6 \pm 0.1	16.7
Hypocotyl	BA	90.0	13.2 \pm 1.8	27.4	1.9 \pm 0.1	21.5
	KIN	90.0	8.7 \pm 1.2	11.5	2.0 \pm 0.1	37.3
	ZEA	100	12.6 \pm 1.3	16.7	1.9 \pm 0.1	29.8
Cotyledo	BA	70.0	5.3 \pm 0.7	5.4	2.3 \pm 0.4	59.1
	KIN	80.0	8.1 \pm 1.5	12.3	1.6 \pm 0.1	61.7
	ZEA	90.0	8.2 \pm 1.6	14.3	1.7 \pm 0.1	36.9
Leaf	BA	81.8	5.2 \pm 1.3	25.5	2.4 \pm 0.3	43.8
	KIN	70.0	6.7 \pm 1.4	40.4	2.1 \pm 0.1	66.7
	ZEA	80.0	6.5 \pm 1.4	15.4	2.1 \pm 0.2	17.2

SE – standard error of the mean

Concentration of the cytokinin: 8.9 μM BA, 9.3 μM KIN, 9.1 μM ZEA

For each treatment about 10-20 explants were used.

In our experiments only direct organogenesis occurred, analogous with the case of *P. lanceolata* regenerated by Budzianowska et al. (2004) from segments of leaves and roots on MS with 11.42 μM IAA and 9.29 μM KIN. By indirect organogenesis, *P. afra* from leaves (Sarihan et al. 2005) on MS containing 0.91 μM TDZ and 0.98 μM IBA and *P. ovata* from hypocotyls on MS enriched with 4.5 μM 2,4-D and 4.6 μM KIN (Wakhlu and Barna 1989) were regenerated. Whereas *P. asiatica* from hypocotyls was regenerated by direct and also indirect organogenesis (Makowczyńska 2006).

According to our research results it is possible to micropropagate *P. camtschatica* from different explants, but hypocotyls have been found to be the most effective for induction of shoot formation.

On the part of shoots obtained as a result of organogenesis during 6-week culture adventitious roots developed spontaneously (Fig. 5). The cytokinin KIN was found to be the most effective for induction of the spontaneous root organogenesis during shoot regeneration. The greatest percentage of shoots with adventitious roots was observed for leaves culture with KIN. Rooting of the shoots during the

shoot micropropagation allows to omit the stage of rooting after the shoot micropropagation.

The part of buds and shoots were abnormal (less or more hyperhydrous or deformed). The abnormal behaviour can be attributed to the excess of growth regulators in the explants. These abnormal shoots were rejected and only well-developed shoots were chosen for rooting and acclimatisation.

The rooting of shoots (without roots after 6-week culture) (Table 4, Figs 3 and 4)

The rooting was most effective on shoots cultured on MS without auxin or on MS with 0.5 μM NAA. No callusing or browning was observed during root formation.

Acclimatisation (Fig. 6)

For two months of the acclimatisation in flower pots only 14% plants regenerated from seedling tips and 27% plants regenerated from other seedling explants survived.

The plants obtained as a result of micropropagation were not phenotypically changed (visual observations).

TABLE 4. Effect of medium with various kinds of auxins on rooting of *Plantago camtschatica* shoots.

Concentration of auxin (μM)	Auxin	% shoots with roots	Mean number of roots per explant $\pm\text{SE}$	Mean root length (cm) $\pm\text{SE}$
0.6	IAA	75.0	3.6 \pm 0.6	2.5 \pm 0.2
0.5	IBA	75.0	3.9 \pm 0.2	3.9 \pm 0.2
0.5	NAA	89.7	5.5 \pm 0.6	1.1 \pm 0.1
2.8	IAA	69.0	2.5 \pm 0.3	1.9 \pm 0.1
2.5	IBA	54.8	3.6 \pm 0.5	0.8 \pm 0.1
2.7	NAA	67.7	8.1 \pm 0.9	0.7 \pm 0.0
0	Control	90.0	2.6 \pm 0.2	3.9 \pm 0.2

SE – standard error of the mean

For each treatment about 29-31 explants were used.

The in vitro cultures may be applied for the propagation of the *P. camtschatica* species which do not grow in Poland.

Obtained by us in vitro cultures of *P. camtschatica* can provide plant material for phytochemical analysis. Since regenerated shoots of this plant may contain such important secondary metabolites as iridoid and phenylpropanoid glucosides, the in vitro cultures could be important as the source of these substances.

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