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In Vitro Organogenesis of *Fagopyrum esculentum* Moench (Polygonaceae) as a Method to Study Seed Set in Buckwheat

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ABSTRACT

In vitro organogenesis may provide a method to reduce the very large number of observations required for *in planta* studies on seed set in *Fagopyrum esculentum*. Plants were regenerated from leaf petiole explant tissue of buckwheat seedlings. Maximum shoot regeneration was induced on Murashige and Skoog (MS) medium containing Gamborg's vitamins, 6-benzylaminopurine (BA), 6-γ,γ-dimethylallyl-amino-purine (2iP) and 2,3,5-triiodobenzoic acid (TIBA) at 1 mg L^{-1} each, and sucrose at 30 g L^{-1}. Whole plants were obtained at high frequencies (up to 98%) when the shooting explants were transferred to half-strength MS media without growth regulators for rooting and then to vermiculate followed by soil mix in greenhouse conditions. Regenerated plants were studied for vegetative, floral and seed characteristics in comparison with buckwheat plants developed from seeds. Plants regenerated by organogenesis were 33% shorter than those developed from seeds, with lower pollination frequency and smaller seeds but the number of seeds per raceme and percentage of seed set did not show any significant differences. In addition, the number of aborted seeds per raceme was significantly lower (by 40%) in plants developed from *in vitro* organogenesis than those grown from seeds. This regeneration system may be valuable for studying the problem of low seed set in common buckwheat.

Keywords: buckwheat, *in vitro* culture, leaf petiole explants, organogenesis, seed set
Abbreviations: ABA, abscisic acid; **BA**, 6-benzylaminopurine; **2iP**, 6-γ,γ-dimethylallylamino-purine; **TIBA**, 2,3,5-triiodobenzoic acid

INTRODUCTION

Fagopyrum species are diploid (2n=16), but tetraploid buckwheat either occurs spontaneously or can be induced. Buckwheat has for centuries remained a crop with low seed set due to certain characteristics which prevent the application of conventional breeding methods (Kreft 1983). More specifically, seed set is highly variable among individual plants, from 2.8 to 52% of the flowers pollinated, a range of 18.7 fold (Obendorf *et al*. 1993). The causes of low seed set are unknown but have been attributed to high temperature, plant water stress (Slawinska and Obendorf 1993, 2001), plant age, incompatibility caused by heterostylism, defective reproductive organs, failure of fertilization, and embryo and/or endosperm abortion (Pomeranz 1983; Adachi 1990; Guan and Adachi 1992; Obendorf *et al*. 1993; Taylor and Obendorf 1993; Guan and Adachi 1994). Buckwheat flowers are incomplete (lacking a corolla), perfect and heterostylous (Marshall and Pomeranz 1982). Half of the plants have pin-type flowers with long styles and short filaments, and the other half have thrum-type flowers with short styles and long filaments. Flowers within each type are self- and cross-incompatible. Only legitimate cross-pollination, pin by thrum or thrum by pin, results in fertilization in most cultivars. About 20% of the flowers have defective megagametophytes and are female sterile (Obendorf *et al*. 1993; Taylor and Obendorf 1993). A fertile flower can produce a single seed – an achene – at maturity (Steadman *et al*. 2001).

Modern biotechnology may provide means to address these problems (Nešković *et al*. 1990). The study of organogenesis provides a method to reduce the very large number of observations required for *in planta* studies on seed set in buckwheat. Regenerated plants from explants of a single plant have identical genotypes and, therefore, could be used to precisely characterize the influence of a single factor, such as temperature, on seed set. By sampling a number of plants ranging in response to temperature, a precise evaluation of treatments is possible with a minimum number of observations using matched sets of regenerated plants across the experimental treatments.

In vitro organogenesis has been reported in buckwheat and other plants (Kong *et al*. 1992; Miljuš-Djukić *et al*. 1992; Rumyantseva *et al*. 1992; Zhang *et al*. 1992). Different types of explants of buckwheat have been tested for organogenesis: pollen (Adikari and Campbell 1998), root parts of young seedlings (Tůmová *et al*. 2007), cotyledon tissue (Woo *et al*. 2000), hypocotyls and nodal segments (Jin *et al*. 2002; Klčová and Gubišová 2008), and immature inflorescence culture (Takahata 1988). In other plant species, exogenous auxins and cytokinins affect organogenesis *in vitro*, and levels of endogenous hormones in explants also have a regulatory role in shoot and root formation (Hempel 1979; von Arnold and Tillberg 1987). Shoot regeneration in explants of other species have been achieved previously using high cytokinin/low auxin media (Paek and Yeung 1991). 2,3,5-Triiodobenzoic acid (TIBA) also may control shoot initiation in other species, possibly by contracting the presumably high level of endogenous auxins (Genga and Allavena 1991).

The purpose of this study was to investigate the response of buckwheat explants to different concentrations and combinations of 6-benzylaminopurine (BA), 6-γ,γ-dimethylallylamino-purine (2iP) and TIBA. In addition, the plant height, the number of seeds per raceme, the number of seeds aborted per raceme, and the length, width and dry weight of mature seeds (achenes) of plants regenerated by organogenesis were compared to plants grown from seeds.

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Original Research Paper

Table 1 Percent of small leaf petiole explants forming shoots, number and length of shoots per explant, as a function of plant growth regulators in media, and plantlet survival after transfer to vermiculite and then soil mix.

Plant growth regulator (mg L^{-1})	Explants plated	Explants with shoots (%)	Shoots per explant (Mean no. ± SD[a])	Length of longest shoot[b] (Length in mm ± SD)	Plantlet survival (%)
2iP(1)	26	4 d[c]	1.8 ± 0.4 d	35.1 ± 9.3 a	68 cd
2iP(2)	26	4 d	2.2 ± 0.3 cd	33.0 ± 8.7 a	87 b
BA(1)	26	26 c	2.8 ± 0.3 bcd	26.2 ± 5.1 a	86 b
BA(2)	26	18 c	3.1 ± 0.4 abcd	24.9 ± 5.2 a	87 b
TIBA(1) + 2iP(1)	26	66 b	4.1 ± 0.8 ab	27.8 ± 6.3 a	98 a
TIBA(1) + BA(1)	26	68 b	4.6 ± 1.4 ab	27.3 ± 6.1 a	63 d
TIBA(1) + 2iP(1) + BA (1)	26	84 a	5.0 ± 0.5 a	26.0 ± 5.9 a	74 c

[a] SD = standard deviation
[b] Length of the longest shoot at 30 days after incubation
[c] a–d column wise comparison
All comparisons are done using the Tukey-Kramer's test at the 5% level of significance

MATERIALS AND METHODS

Young, expanding leaves with about 6 – 8 mm leaf blade were removed with petiole from upper nodes of primary branches or main stems of plants grown in the greenhouse (Horbowicz and Obendorf 1992) and used as explants. In the laboratory, these immature leaves were thoroughly washed for 3 min under running tap water, disinfected for 5 to 10 seconds in 80% ethanol, and surface sterilized for 15 min in a 0.5% (v/v) solution of NaOCl containing a drop of the surfactant Tween 80. Explants tissues were rinsed three times with sterile distilled water to remove the residual chlorine. Young-expanding-leaf-petiole explants were re-cut near the junction of petiole and blade leaving about 3 mm of petiole attached to the blade, and the blade was cut about 2 mm from the petiole to form the explant.

After preparation, equal numbers of explants were placed on medium containing MS (Murashige and Skoog 1962) basal salt mixture (Sigma) supplemented with B5 vitamins (Gamborg *et al.* 1968), sucrose (30 g L^{-1}) as a carbon source, and plant growth regulators (PGRs). Seven combinations, as indicated in **Table 1**, with varied concentrations of BA, 2iP and TIBA at 1 mg L^{-1}, were tested for organogenesis efficiency. The pH was adjusted to 6.5 before autoclaving, and the media were solidified with agar at 7 g L^{-1}. The cultures were incubated at 25°C in a growth chamber with fluorescent light at 20 to 60 µmol m^{-2} s^{-1} with a photoperiod of 16 hrs. Cultured explants were checked weekly for organogenesis, and the final observations were made 30 days after incubation. Morphological changes were recorded on the basis of visual observations. When possible, the effects of different treatments were quantified on the basis of the proportion of cultures showing the response and the degree of response.

Shoot organogenesis medium contained modified MS basal salt mixture, Gamborg B5 vitamins, 30 g of sucrose L^{-1}, 1.0 mg of TIBA L^{-1}, 1.0 mg of 2iP L^{-1}, 1.0 mg BA L^{-1} and agar at 7 g L^{-1}. Groups of adventitious shoots, without separation into individual shoots, were excised and transferred to fresh shoot organogenesis medium, without BA in culture tubes. After 3 weeks, individual shoots were separated and transferred to root organogenesis medium. Root organogenesis medium contained PGR-free half-strength MS basal salt mixture, B5 vitamins, and agar at 7 g L^{-1}. Each shoot was inserted into root organogenesis media and incubated at 25°C under continuous fluorescent light at 20 to 60 µmol m^{-2} s^{-1}. After 2 to 3 weeks when roots were well-developed, plantlets were transferred to vermiculite medium in Styrofoam cups covered with perforated polytene bags to maintain a humid environment for 14 days. The vermiculite medium was wetted with half-strength Hoagland's nutrient solution No. 1 (Hoagland and Arnon 1950) which was continuously supplied through a cotton wick connecting a reservoir of nutrient solution in a beaker to the vermiculite in the Styrofoam cup above. When the roots permeated the vermiculite medium, young plants were transplanted to a greenhouse potting soil-mix for growth to plant maturation in the greenhouse (Horbowicz and Obendorf 1992).

The experiment was conducted as a completely randomized design with seven treatments and 26 replicates per treatment. The results were expressed as percentage of explants that initiated shoots, mean number of shoots per explant and mean length of longest shoot. The data were analyzed by one-way ANOVA followed by the separation of mean values with the Tukey-Kramer HSD test on the SAS JMP 8 statistical software program. In addition, means comparisons for plant height, number of flowers pollinated per raceme, number of seeds per raceme, % of seed set per raceme, number of aborted seeds per raceme, seed length and width, and dry weight per seed among plants that were developed from seeds and from organogenesis were tested for all pairs (from seeds vs. from organogenesis) for all treatments using the Tukey-Kramer HSD test on the SAS JMP 8 statistical software program.

Fig. 1 Formation of adventitious shoot buds on the proximal end of young leaf petiole explants at 4 weeks after culture on MS + B5 medium supplemented with hormones.

RESULTS

Petiole explants from small expanding leaves were placed on solidified MS+B5 vitamins media with BA, 2iP or TIBA (1 or 2 mg L^{-1}) either singly or in combination and shoots formed on the cut end of the petiole of young-expanding-leaf-petiole explants (**Fig. 1**). Of the seven different media tested, media supplemented with BA, 2iP and TIBA (each at 1 mg L^{-1}) were found to be the most suitable for shoot induction (**Table 1**) given that 84 % of the explants formed shoots which grew rapidly and increased in vigor within 2 to 3 weeks of culture. This percentage was significantly different from those observed for the other media combinations tested. It is worth noticing that BA appeared to be a more important PGR for shoot formation than 2iP and that increased or decreased levels of BA had no significant effect on shoot initiation. Also, TIBA was probably the catalytic growth regulator for shoot formation when it was combined with BA and 2iP.

To evaluate the growth rate on shoot forming media, the number of shoots per explant and the length of shoots were measured. The results indicate that a combination of PGRs enhanced shoot development since the number of shoots per explant is higher for media containing TIBA with BA and 2iP than for media that contained either BA or 2iP alone. The length of the longest shoot was not significantly different among any of the media combinations and ranged from 26.0 mm (± 5.9) to 35.1 mm (± 9.3). To promote rooting in buckwheat, half-strength MS without PGRs was used (**Fig. 2**). A vigorous root system was observed by 2-3 weeks, and the frequency of root regeneration was almost

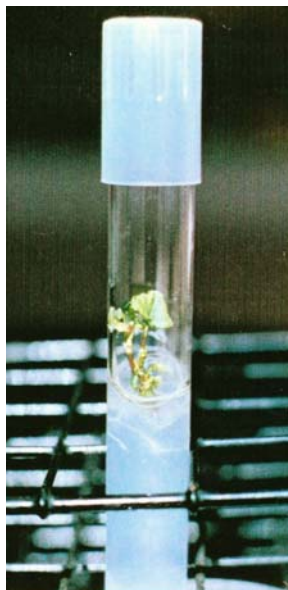

Fig. 2 Elongated shoot regenerated from callus and rooted on half-strength MS + B5 medium. Lower portion of regenerated shoot showing rooting.

Fig. 3 Regenerated plant in the reproductive stage.

100%. After 3 weeks in PGR-free media, well-rooted plantlets were separated, washed and transplanted to vermiculite medium in Styrofoam cups in which the survival rate ranged from 63 to 98% (**Table 1**).

After acclimation, the hardened plants were transferred to soil mix under greenhouse conditions (**Fig. 3**). The regenerated plants were morphologically normal (**Fig. 4**) compared to plants grown from seeds (**Table 2**). Seed-grown plants formed flowers from 4 weeks after seeding until plant senescence at 10 to 12 weeks after seeding. Plants regenerated by organogenesis started flowering at 2 weeks after transfer to soil mix, but these plants senesced at 6 weeks after transfer to soil mix. Plants regenerated by organogenesis were significantly shorter, with lower pollination frequency and smaller seeds that those obtained from seeds (**Table 2**). On the other hand, it is interesting that the number of seeds per raceme and percentage of seed set did not show any significant differences between plants grown from seeds and those developed through organogenesis. The number of aborted seeds per raceme was significantly lower (by 40%) in plants developed from *in vitro* organogenesis than those grown from seeds.

Fig. 4 Morphologically normal flowers and seeds produced on a regenerated plant.

Table 2 Plant heights, number of flowers pollinated per raceme, number of seeds, percent of seed set per raceme, number of seeds aborted per raceme, and length, width and dry weight of mature seeds (achenes) from seed-grown plants and plants regenerated by organogenesis.

Parameter	Plants from	
	Seeds (Mean ± SD[a])	Organogenesis (Mean ± SD)
Plant height (cm)	153.0 ± 20.0 a[b]	102.0 ± 15.0 b
No. of flowers pollinated per raceme	47.4 ± 3.0 a	25.7 ± 1.9 b
No. of seeds per raceme	9.7 ± 1.0 a	7.2 ± 1.3 a
Seed set per raceme (%)	20.5 ± 3.8 a	28.0 ± 3.9 a
No. of aborted seeds per raceme	2.2 ± 0.4 a	1.3 ± 0.3 b
Seed length (mm)	7.5 ± 0.1 a	6.1 ± 0.1 b
Seed width (mm)	5.0 ± 0.1 a	4.3 ± 0.1 b
Dry weight per seed (mg)	34.6 ± 1.1 a	38.1 ± 3.4 a

[a] SD = standard deviation
[b] a-b row wise comparison
All comparisons are done using the Tukey-Kramer's test at the 5% level of significance

DISCUSSION

This study tested the responsiveness of buckwheat explants to the PGRs in the culture medium. Organogenesis of *F. esculentum* was achieved on media containing different PGR combinations. This occurred with relatively high frequency (84%) on media containing TIBA (1 mg L^{-1}), 2iP (1 mg L^{-1}) and BA (1 mg L^{-1}). In the presence of auxin, the meristem of other species responds by maintaining a small cytoplasmic zone and retarding leaf formation (Paek and Yeung 1991). Cytokinins generally reduce rhizome growth but can induce shoot formation *in vitro*. For other dicotyledonous plants (Saka *et al.* 1980; Mangat and Roy 1986), a low amount of auxin promoted callus formation, but shoot differentiation was totally suppressed. This tendency for callus and root formation suggests the presence of a high level of endogenous auxin that might interfere with shoot regeneration (Cambecèdes *et al.* 1991). Addition of TIBA or ABA to the medium increased the effectiveness of embryogenesis in *Phaseolus* (Genga and Allavena 1991). Effectiveness of BA on direct shoot organogenesis from explants has been reported previously in other species (Economou and Maloupa 1995). Variation in the activity of different cytokinins can be explained by their differential uptake rate reported in different genomes (Blakesey 1991), and varied translocation rates to meristematic regions and metabolic processes in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds (Kamínek 1992). In the present research, TIBA and 2iP stimulated shoot organogenesis on buckwheat young-expanding-leaf-petiole explants. This treatment

would be expected to inhibit endogenous auxins and enhance the exogenous cytokinin/auxin balance. TIBA may control shoot initiation, possibly by contracting the presumably high level of endogenous auxins. The correct hormonal balance to achieve such a goal must also involve a control of the endogenous auxin level in explant tissues.

Another interesting result of this study was that the plants taken from *in vitro* organogenesis showed a significantly reduced rate of seed abortion than those developed from seeds. This observation indicates that modern biotechnology may provide a method to address the problem of low seed set in buckwheat.

CONCLUSIONS

The procedure developed in this work allowed regeneration of buckwheat plants from leaf petiole explants by organogenesis in a simple three-step process. First, shoot initiation was induced on basal medium supplemented with TIBA, 2iP and BA. Second, root organogenesis on individual shoots was readily induced on growth-regulator-free basal medium. Finally, regenerated plantlets were transferred to vermiculite and then to soil mix for morphologically normal plant growth, seed development and maturation. It is expected that this biotechnological approach will be an alternative to reproduce *F. esculentum* in order to satisfy the need of reducing the large number of observations required for *in planta* studies on seed set in buckwheat.

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REFERENCES

Adachi T (1990) How to combine the reproductive system with biotechnology in order to overcome the breeding barrier in buckwheat. *Fagopyrum* **10**, 7-11

Adikari KN, Campbell CG (1998) *In vitro* germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. *Euphytica* **102**, 87-92

Blakesey D (1991) Uptake and metabolism of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. *Plant Cell, Tissue and Organ Culture* **25**, 69-74

Cambecèdes J, Duron M, Decourtye L (1991) Adventitious bud regeneration from leaf explants of the shrubby ornamental honeysuckle, *Lonicera nitida* Wils. cv. 'Maigrun': Effects of thidiazuron and 2,3,5,-triiodobenzoic acid. *Plant Cell Reports* **10**, 471-474

Economou AS, Maloupa EM (1995) Regeneration of *Elaeagnus angustifolia* from leaf segments of *in vitro*-derived shoots. *Plant Cell, Tissue and Organ Culture* **40**, 285-288

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**, 151-158

Genga A, Allavena A (1991) Factors affecting morphogenesis from immature cotyledons of *Phaseolus coccineus* L. *Plant Cell, Tissue and Organ Culture* **27**, 189-196

Guan LM, Adachi T (1992) Reproductive deterioration in buckwheat (*Fagopyrum esculentum*) under summer conditions. *Plant Breeding* **109**, 304-312

Guan LM, Adachi T (1994) Ultrastructural changes of the mature embryo sac in buckwheat (*Fagopyrum esculentum*) as a result of high temperature exposure. *Cytologia* **59**, 237-248

Hempel M (1979) Application of growth regulators for *in vitro* propagation of ornamental plans. *Acta Horticulturae* **91**, 4-32

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *California Agricultural Experimental Station Bulletin* **347**, 4-32

Horbowicz M, **Obendorf RL** (1992) Changes on sterols and fatty acids of buckwheat endosperm and embryo during seed development. *Journal of Agricultural and Food Chemistry* **40**, 745-750

Jin H, Jia J-F, Hao J-G (2002) Efficient plant regeneration *in vitro* in buckwheat. *Plant Cell, Tissue and Organ Culture* **69**, 293-295

Kamínek M (1992) Progress in cytokinin research. *Trends in Biotechnology* **10**, 159-162

Klčová L, Gubišová M (2008) Evaluation of different approaches to buckwheat (*Fagopyrum esculentum* Moench.) micropropagation. *Czech Journal of Genetics and Plant Breeding* **44**, 66-72

Kong F, Shong Y, Wang Z, Yang L (1992) Study on plant regeneration from anther culture in common buckwheat (*Fagopyrum esculentum*). In: Lin R, Zhou M, Tao Y, Li H, Zhang Z (Ed) *Proceeding of the 5th International Symposium on Buckwheat*, 20-26 August 1992, Taiyuan, China. Agriculture Publishing House, Beijing, pp 309-314

Kreft I (1983) Buckwheat breeding perspectives. In: Nagatomo T, Adachi T (Eds) *Buckwheat Research. Proceedings of the 2nd International Symposium on Buckwheat*. Miyazaki University, Japan, pp 3-12

Mangat BS, Roy MK (1986) Tissue culture and plant regeneration of okra (*Abelmoschus esculentus*). *Plant Science* **47**, 57-61

Marshall HG, Pomeranz Y (1982) Buckwheat: description, breeding, production, and utilization. *Advances in Cereal Science and Technology* **5**, 157-210

Miljuš-Djukić J, Nešković M, Ninković S, Crkvenjakov R (1992) *Agrobacterium*-mediated transformation and plant regeneration of buckwheat (*Fagopyrum esculentum* Moench). *Plant Cell, Tissue and Organ Culture* **29**, 101-108

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco cultures. *Physiologia Plantarum* **15**, 473-497

Nešković M, Vinterhalter B, Miljuš-Djukić J, Ghalawenji N (1990) Micropropagation of recessive determinate genotypes of buckwheat (*Fagopyrum esculentum* Moench) as an alternative approach to uniform seed production. *Plant Breeding* **105**, 337-340

Obendorf RL, Horbowicz M, Taylor DP, Slawinska J (1993) Buckwheat seed development and regulation of seed set. In: Cóme D, Corbineau F (Eds) *Proceedings of the 4th International Workshop of Seeds: Basic and Applied Aspects of Seed Biology*, Angers, France, 20-24 July 1992, ASFIS, Paris, pp 30-46

Paek KY, Yeung EC (1991) The effects of 1-naphthaleneacetic acid and N^6-benzyladenine on growth of *Cymbidium forrestii* rhizomes *in vitro*. *Plant Cell, Tissue and Organ Culture* **24**, 65-71

Pomeranz Y (1983) Buckwheat: Structure, composition, and utilization. *CRC Critical Reviews in Food Science and Nutrition* **19**, 213-258

Rumyantseva NI, Sal'nikova VV, Fedoseeva NV, Lozovaya VV (1992) Morphogenic peculiarities in buckwheat calli cultivated for a long time. *Fizjologiya Rastenii* **39**, 143-151

Saka H, Voqui-Dinh TH, Cheng TY (1980) Stimulation of multiple shoot formation on soybean stem nodes in culture. *Plant Science Letters* **19**, 193-201

Slawinska J, Obendorf RL (1993) Buckwheat seed set and development by *in vitro* inflorescenece culture. In: Cóme D, Corbineau F (Eds) *Proceedings of the 4th International Workshop of Seeds: Basic and Applied Aspects of Seed Biology*, Angers, France, 20-24 July 1992. ASFIS, Paris, pp 61-66

Slawinska J, Obendorf RL (2001) Buckwheat seed set *in planta* and during *in vitro* inflorescence culture: Evaluation of temperature and water deficit stress. *Seed Science Research* **11**, 223-233

Steadman KJ, Burgoon MS, Lewis BA, Edwardson SE, Obendorf RL (2001) Buckwheat milling fractions: Description, macronutrient composition, and dietary fiber. *Journal of Cereal Science* **33**, 271-278

Takahata Y (1988) Plant regeneration from cultured immature inflorescence of common buckwheat (*Fagopyrum esculentum* Moench) and perennial buckwheat (*F. cymosum* Meisn.) *Japanese Journal of Plant Breeding* **38**, 409-413

Taylor DP, Obendorf RL (1993) Limits to seed set on buckwheat. In: Lin R, Zhou M, Tao Y, Li H, Zhang Z (Eds) *Proceeding of the 5th International Symposium on Buckwheat*, 20-26 August 1992, Taiyuan, China. Agriculture Publishing House, Beijing, pp 236-239

Tůmová L, Pichová M, Dušek J (2007) *Fagopyrum esculentum in vitro*. *Ceska Slovenska Farmacie* **56**, 125-128

von Arnold S, Tillberg E (1987) The influence of cytokinin pulse treatments on adventitious bud formation on vegetative buds of *Picea abies*. *Plant Cell, Tissue and Organ Culture* **9**, 253-261

Woo SH, Nair A, Adachi T, Campbell CG (2000) Plant regeneration from cotyledon tissues of common buckwheat (*Fagopyrum esculentum* Moench). *In Vitro Cellular and Developmental Biology – Plant* **36**, 358-361

Zhang J, Chen R, Qi J (1992) Effect of phenyl urea derivatives (PUD) on organ formation and plantlet regeneration of tartary buckwheat. In: Lin R, Zhou M, Tao Y, Li H, Zhang Z (Eds) *Proceeding of the 5th International Symposium on Buckwheat*, 20-26 August 1992, Taiyuan, China. Agriculture Publishing House, Beijing, pp 301-305