Arch. Biol. Sci., Belgrade, 60 (4), 673-679, 2008 DOI:10.2298/ABS0804673S

FACTORS INFLUENCING GERMINATION AND GROWTH OF ISOLATED EMBRYOS OF *PINUS HELDREICHII*

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Abstract — The effects of nutritional, hormonal, and physical factors on the germination and growth of isolated mature zygotic embryos of *Pinus heldreichii* were studied under in vitro conditions. The optimum medium for embryo germination and adequate conversion into seedlings was basal Gresshoff and Doy (1GD) medium. All tested carbohydrates at the applied concentrations stimulated embryo growth, but only 3% sucrose increased embryo germination compared to the control, while maltose had an inhibitory effect. Among the applied plant growth regulators, only gibberelic acid (GA3) had a few benefits on the development of isolated embryos into plantlets, while the others had a negative effect. Embryos cultured on an agar-solidified medium grew better and the germination percentage was higher than in a liquid medium. Light treatment did not affect embryo germination, but generally stimulated seedling growth.

Key words: *Pinus heldreichii, in vitro* culture, embryo, seed germination

UDC 582.475:581.3:581.14

INTRODUCTION

Pinus heldreichii (Bosnian pine) is a Tertiary relic species endemic on high mountains in the Balkans and Southern Italy. It occurs on steep and dry limestone slopes, most often in pure stands (Vidaković, 1982). The tree is ornamental, up to 30 m high, with pyramidal habit. Although the tree grows slowly, it could be important for afforestation, as it is well adapted to environmental stresses such as low temperature and extreme drought (Jovanović, 1971). In natural stands, trees begin to produce cones at the age of about 40. Seeds are dormant and need chilling treatment of about 6 weeks to germinate (Stilinović, 1985). Abundance of seed production varies greatly, not only from year to year, but also within different trees and cones (Đorđeva et al., 1972; Mičev, 1972). *Pinus heldreichii* is usually propagated by seeds, since the rooting of cuttings has been only partially successful. However, variable seed production may complicate operational reforestation and tree improvement efforts. *In vitro* culture can therefore be used as a significant supplement to conventional methods of vegetative propagation of this pine.

We previously reported plant regeneration of *P. heldreichii* through adventitious buds (Stojičić et al., 1999) and axillary buds (Stojičić and Budimir, 2004). We also demonstrated induction of somatic embryogenesis in culture of this pine (Stojičić et al., 2007). According to the obtained results, micropropagation and somatic embryogenesis have a potential use in the propagation of *P. heldreichii*, but further improvement of the methods is required.

The aim of this study was to investigate the possibility of achieving synchronous and rapid plant production from zygotic embryos using tissue culture methods. To that end, we investigated the effects of nutrients, hormones, and physical factors on germination and growth of isolated mature zygotic embryos.

MATERIAL AND METHODS

Seed sterilization and culture of isolated embryos

Cones of *P. heldreichii* were collected from open pollinated trees in a natural stand located on Lovćen Mountain (Montenegro). Before the experiments, seeds were removed from cones, washed for 24 h under running tap water, surface-disinfected in 20% sodium hypochlorite for 30 min, and rinsed three times with sterile distilled water. Isolated mature zygotic embryos were then placed horizontally on different variations of GD (Gresshoff and Doy, 1972) culture medium as modified by Sommer et al. (1975). If not mentioned otherwise, the medium used was supplemented with 3% sucrose and solidified with 0.7% agar (Torlak, Belgrade). The pH of the media was adjusted to 5.7 prior to autoclaving for 25 min at 115°C. Unless stated differently, cultures were maintained at 25 ± 2°C under conditions of a 16 h/8 h photoperiod.

Variations of media

Medium strength – the effects of five different medium strengths were tested: 0; 0.25; 0.5; 1.0; or 2.0 times those of GD. The other components in all five media were the same as in GD medium.

Effects of carbohydrates - to determine the influence of different carbon sources, GD medium was supplemented with one of four carbohydrates (sucrose, glucose, maltose, or fructose), each at six different concentrations (0; 1; 2; 3; 4; or 5%).

Effect of enzymatic casein hydrolyzate (CH) - as a source of organic nitrogen, CH was tested at the following concentrations 0; 125; 250; 375; or 500 mg l^{-1}.

Effects of plant growth regulators - to examine the effects of different plant growth regulators, basal 1GD medium was supplemented with the following growth regulators at five concentrations: benzyladenine (BA, 0; 0.06; 0.11; 0.22; 0.44 μM), kinetin (KIN, 0; 0.12; 0.23; 0.46; 0.93 μM), gibberellic acid (GA_3, 0; 0.14; 0.29; 0.58; 1.15 μM), α-naphthaleneacetic acid (NAA, 0; 0.07; 0.13; 0.27; 0.54 μM), or indole-3-butyric acid (IBA, 0; 0.06; 0.012; 0.025; 0.49 μM). These media were also supplemented with CH at 500 mg l^{-1}.

Comparison of agar-gelled and liquid media - two different treatments were compared: 0.7% agar-gelled medium and liquid medium. The basal 1GD medium supplemented with CH at 500 mg l^{-1} was used.

Effect of light

Three different treatments were compared in this study: continuous light; 16-h light photoperiods; and continuous darkness.

Data collection and analysis

For each treatment, a total of 36 embryos (six per Petri dish) were used, divided into two replicates. Embryos were scored as germinated if they exhibited root elongation. The dry weight of plantlets was recorded after drying at 70°C for 24 h. Germination percentages were arcsine transformed before analysis. Data collected from experiments were calculated and differences were tested for significance using ANOVA and Duncan's multiple range test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The germination percentage of isolated embryos after four weeks was only slightly affected by medium strength, in spite of significant differences observed during the first two weeks of embryo cultivation (Fig. 1). On the medium without salts and vitamins, radicle elongation was poor, cotyledons did not open, and embryo germination was delayed (Fig. 2). A similar delay was also observed on double-strength (2GD) medium, probably due to high osmotic stress. Supplementation of the medium with salts and vitamins promoted elongation of the radicle, followed by elongation of the hypocotyl and expansion of the cotyledons. The highest frequency of embryo germination was obtained on basal 1GD medium, where an average of 80% of embryos germinated after one week in culture (Fig. 1). Basal 1GD medium stimulated embryo growth, so that at day 28 seedlings were two times longer (42.8 mm) than on control 0GD medium (22.7 mm). Root growth

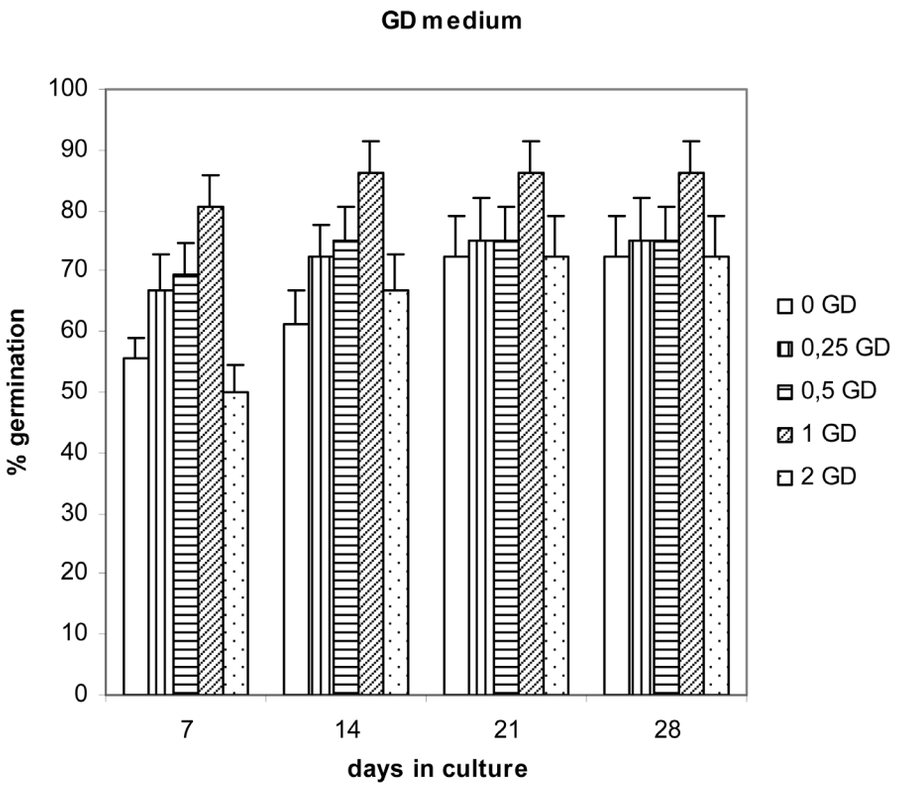

Fig. 1. Effect of medium strength on mean germination percentage of isolated embryos of *Pinus heldreichii*. Error bars indicate the standard error of the mean.

was best on 1GD medium (Table 1) and these roots were branched, with prominent root hairs. The 1GD variant also promoted embryo transformation into seedlings and shoot development (Fig. 3). Lin and Leung (2002) obtained similar results in *Pinus radiata* zygotic embryo culture, where only a few embryos survived on a medium without salts and vitamins, while the highest germination rate and maximum growth were achieved on half-strength medium consisting of Quoirin and Le Poivre salts (von Arnold and Eriksson, 1981) and Schenk and Hildebrandt (1972) vitamins.

Among carbohydrates tested as a carbon source, sucrose was superior in promoting *Pinus heldreichii* embryo germination compared to glucose, fructose, and maltose (Table 2). All sucrose concentrations (1-5%) slightly increased germination frequency compared to the control. In the presence of 5% sucrose, germination was delayed, so that after one week in culture about 60% of embryos germinated, while at the end of the fourth week germination increased to nearly 80%. Glucose and fructose had no effect on germination, while maltose had an inhibitory effect (Table 2). Similar results were obtained in *Pinus radiata*, where sucrose, glucose, and fructose could serve as carbon sources for isolated embryos cultured *in vitro*, while maltose was found to be inferior to these sugars in promoting germination and embryo growth (Lin and Leung, 2002). However, unlike isolated mature embryos of *P. radiata*, which could not germinate on a medium without any carbohydrates, *P. heldreichii* embryo germination in the absence of a carbon source was relatively high (72%). Growth of embryo organs and whole seedlings was stimulated by the presence of the carbohydrates in the medium irrespective of sugar type (Table 2).

Fig. 2. Germinated embryo of *Pinus heldreichii* grown on medium without salts and vitamins (0GD).

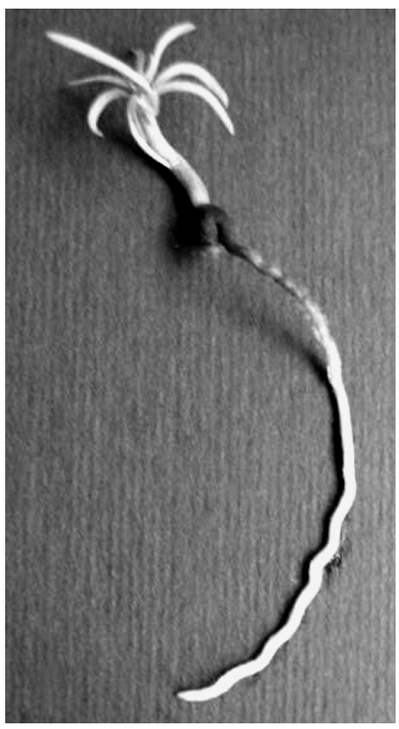

Fig. 3. Seedling of *Pinus heldreichii* grown on medium supplemented with salts and vitamins (1GD).

The addition of organic nitrogen in the form of enzymatic casein hydrolyzate in the medium did not increase germination percentage, but promoted the growth of hypocotyl and cotyledons at concentrations from 250 to 500 mg l^{-1} (data not shown).

None of the plant growth regulators tested affected isolated embryo germination at the applied concentrations (Table 3). However, in the presence of GA_3, germination was synchronous and most embryos germinated after one week of treatment. In addition, GA_3 at lower concentrations slightly promoted hypocotyl elongation, while the highest seedling dry weight was obtained at 1.15 μM GA_3 (Table 3). Gibberellic acid was also shown to increase germination percentages and promote embryo transformation into plants in *P. radiata* and some other woody species, like coconut (Lin and Leung, 2002; Peach et al., 2007). Cytokinins and auxins were generally inhibitory for early seedling growth, especially cotyledon and root growth. The growth of isolated zygotic embryos was significantly reduced

Table 1. Effect of medium strength on the growth of isolated embryos of *Pinus heldreichii.* All data were obtained at day 28. Means in the column followed by different letters are different according to Duncan's multiple range test ($p \leq 0.05$).

Medium strength	Root length (mm)	Hypocotyl length (mm)	Cotyledon length (mm)	Dry weight (mg)
0GD	6.23 ± 0.74^{a}	7.50 ± 0.49^{a}	9.00 ± 0.63^{a}	4.54 ± 0.32^{a}
0.25GD	13.96 ± 3.26^{abc}	8.37 ± 0.73^{ab}	10.89 ± 0.94^{ab}	9.26 ± 1.07^{ab}
0.5GD	16.15 ± 3.60^{bc}	9.85 ± 0.71^{b}	12.22 ± 0.98^{b}	14.52 ± 2.21^{bc}
1GD	20.16 ± 3.47^{c}	9.94 ± 0.89^{b}	12.68 ± 1.21^{b}	31.90 ± 3.74^{d}
2GD	8.58 ± 1.19^{ab}	9.77 ± 0.68^{b}	13.23 ± 1.09^{b}	20.73 ± 2.73^{c}

Table 2. Effect of carbon source on germination and growth of isolated embryos of *Pinus heldreichi*i. All data were obtained at day 28. Means in the column followed by different letters are different according to Duncan's multiple range test ($p \leq 0.05$).

Carbon source	Germination (%)	Root length (mm)	Hypocotyl length (mm)	Cotyledon length (mm)	Dry weight (mg)
Sucrose (%)					
0	72.22 ± 5.56	3.65 ± 0.15^{a}	7.12 ± 0.64^{a}	8.23 ± 0.85^{abc}	4.69 ± 0.37^{a}
1	83.33 ± 6.09	7.33 ± 1.17^{abc}	9.43 ± 0.52^{cdefgh}	13.03 ± 0.84^{j}	9.20 ± 1.01^{b}
2	83.33 ± 8.61	14.07 ± 2.83^{def}	8.20 ± 0.44^{abcde}	11.07 ± 0.72^{ghi}	11.27 ± 0.91^{b}
3	86.11 ± 6.69	20.16 ± 3.47^{fg}	9.94 ± 0.89^{fgh}	12.68 ± 1.21^{ij}	31.90 ± 3.74^{c}
4	83.33 ± 6.09	20.43 ± 2.86^{g}	8.17 ± 0.45^{abcd}	11.33 ± 0.89^{hij}	31.03 ± 1.21^{c}
5	77.78 ± 7.03	13.93 ± 3.32^{de}	7.43 ± 0.47^{ab}	8.71± 0.57^{bcd}	30.71 ± 1.19^{c}
Glucose (%)					
0	72.22 ± 5.56	3.65 ± 0.15^{a}	7.12 ± 0.64^{a}	8.23 ± 0.85^{abc}	4.67 ± 0.37^{a}
1	74.10 ± 8.33	7.48 ± 1.44^{abc}	10.56 ± 0.67^{gh}	10.96 ± 0.72^{fghi}	10.30 ± 0.36^{b}
2	75.00 ± 7.14	16.07 ± 3.60^{efg}	9.52 ± 0.78^{cdefgh}	9.63 ± 0.84^{cdefgh}	10.93 ± 0.35^{b}
3	72.22 ± 3.51	11.15 ± 2.73^{bcde}	8.96 ± 0.75^{bcdefgh}	7.27 ± 0.56^{ab}	9.54 ± 0.29^{b}
4	66.67 ± 6.09	14.52 ± 3.78^{defg}	9.08 ± 0.62^{bcdefgh}	7.36 ± 0.53^{ab}	10.80 ± 0.39^{b}
5	61.11 ± 5.56	14.23 ± 3.38^{defg}	8.62 ± 0.69^{abcdef}	6.58 ± 0.54^{a}	9.96 ± 0.33^{b}
Maltose (%)					
0	72.22 ± 5.55	3.65 ± 0.15^{a}	7.12 ± 0.64^{a}	8.23 ± 0.85^{abc}	4.67 ± 0.37^{a}
1	55.57 ± 5.56	7.60 ± 0.58^{abcd}	9.30 ± 0.56^{bcdefgh}	10.25 ± 0.56^{cdefgh}	9.45 ± 0.74^{b}
2	55.57 ± 5.56	6.75 ± 0.41^{abc}	8.80 ± 0.47^{abcdefg}	10.10 ± 0.53^{cdefgh}	10.05 ± 0.65^{b}
3	50.00 ± 4.30	7.50 ± 0.39^{abcd}	8.72 ± 0.43^{abcdefg}	9.06 ± 0.54^{bcdefg}	9.61 ± 0.44^{b}
4	49.10 ± 8.61	5.72 ± 0.32^{ab}	8.50 ± 0.47^{abcdef}	8.94 ± 0.53^{bcdef}	9.77 ± 0.35^{b}
5	44.43 ± 3.51	5.00 ± 0.32^{ab}	7.69 ± 0.50^{abc}	8.06 ± 0.40^{abc}	10.44 ± 0.39^{b}
Fructose %)					
0	72.22 ± 5.55	3.65 ± 0.15^{a}	7.12 ± 0.64^{a}	8.23 ± 0.85^{abc}	4.67 ± 0.37^{a}
1	74.10 ± 5.70	7.93 ± 0.91^{abcd}	10.74 ± 0.76^{h}	10.89 ± 0.62^{efghi}	9.28 ± 0.35^{b}
2	74.10 ± 5. 70	13.37 ± 2.03^{cde}	10.30 ± 0.90^{fgh}	10.59 ± 0.82^{defgh}	9.91 ± 0.35^{b}
3	72.22 ± 8. 24	13.27 ± 2.09^{cde}	10.58 ± 0.76^{gh}	11.12 ± 0.85^{ghij}	10.21 ± 0.29^{b}
4	75.00 ± 7.14	11.96 ± 1.42^{bcde}	9.93 ± 0.55^{efgh}	8.85 ± 0.61^{bcde}	11.00 ± 0.39^{b}
5	72.22 ± 7.03	11.00 ± 1.09^{bcde}	9.88 ± 0.75^{defgh}	8.96 ± 0.65^{bcdef}	10.90 ± 0.33^{b}

in the presence of either BA or KIN, independently of the concentrations applied (Table 3). However, these cytokinins did not affect shoot development, although they influenced the formation of callus tissue along the hypocotyl. In the presence of BA at 0.44 μM and KIN at 0.23 and 0.46 μM, adventitious buds were formed along the cotyledons. The effects of culture media and BA concentrations on adventitious bud induction and elongation were previously reported by Stojičić et al. (1999). Isolated embryos grown in the presence of auxins (NAA and IBA) showed abnormalities, viz., long and crinkled cotyledons, and short and swollen hypocotyls on which callus tissue was formed. Naphthaleneacetic acid suppressed the growth of isolated embryos, especially root growth. Although inhibition of root growth by low concentrations of auxin is a well-known phenomenon, in *Pinus pinea* zygotic embryos NAA was found to be necessary for root growth (Diamantoglou et al., 1990).

In contrast to germination, which was independent of light (Table 4), the growth of germinated embryos was influenced significantly by different light treatments, among which a 16-h photoperiod appeared to be the best for root and cotyledon growth (Table 4). Seedling dry weight increased significantly when grown in light, compared to continuous darkness. The frequency of embryo germination was higher on agar-solidified medium than in liquid medium (Table 4). In addition, embryos cultured on agar-solidified medium had significantly better growth (Table 4) and developed into healthy-looking plantlets.

The results obtained in this study showed that isolated zygotic embryos germinated readily under *in vitro* conditions after only one week in culture. Although embryos could germinate and grow on a medium without salts, vitamins, and carbohydrates, the highest germination percentage and the best morphological characteristics of the seedlings were obtained on basal 1GD medium supplemented with 3% sucrose. Exogenous growth regulators are not

Table 3. Effect of growth regulators on germination and growth of isolated embryos of *Pinus heldreichii*. All data were obtained at day 28. Means in the column followed by different letters of each growth regulator are different according to Duncan's multiple range test ($p \leq 0.05$).

Growth regulator	Germination (%)	Root length (mm)	Hypocotyl length (mm)	Cotiledon length (mm)	Dry weight (mg)
BA (μM)					
0	86.11 ± 5.12	23.65 ± 2.91^{b}	12.39 ± 0.44^{c}	16.23 ± 1.09^{b}	30.84 ± 1.68^{c}
0.06	74.10 ± 5.69	8.07 ± 1.81^{a}	8.89 ± 0.69^{b}	7.26 ± 0.68^{a}	16.19 ± 0.84^{b}
0.11	80.56 ± 6.69	12.66 ± 2.98^{a}	9.14 ± 0.57^{b}	7.86 ± 0.67^{a}	16.07 ± 0.87^{b}
0.22	83.33 ± 6.09	8.37 ± 1.15^{a}	8.37 ± 0.51^{ab}	7.23 ± 0.63^{a}	14.63 ± 0.82^{ab}
0.44	83.33 ± 4.30	7.37 ± 1.60^{a}	7.03 ± 0.39^{a}	7.43 ± 0.86^{a}	12.47 ± 0.52^{a}
KIN (μM)					
0	86.11 ± 5.12	23.65 ± 2.91^{b}	12.39 ± 0.44^{b}	16.23 ± 1.09^{b}	30.84 ± 1.68^{c}
0.12	80.56 ± 5.12	7.07 ± 1.08^{a}	7.21 ± 0.60^{a}	7.48 ± 0.95^{a}	11.97 ± 0.57^{a}
0.23	86.11 ± 5.12	13.90 ± 3.49^{ab}	7.13 ± 0.43^{a}	7.65 ± 0.65^{a}	15.26 ± 0.87^{b}
0.46	83.34 ± 7.45	13.10 ± 3.18^{a}	7.67 ± 0.37^{a}	8.40 ± 0.77^{a}	17.07 ± 0.91^{b}
0.93	77.78 ± 5.56	7.18 ± 0.75^{a}	7.00 ± 0.48^{a}	7.43 ± 0.89^{a}	12.29 ± 0.52^{a}
GA_3 (μM)					
0	86.11 ± 5.12	23.65 ± 2.91^{a}	12.39 ± 0.44^{a}	16.23 ± 1.09^{b}	30.84 ± 1.68^{a}
0.14	91.67 ± 5.69	20.30 ± 3.13^{a}	14.61 ± 0.60^{b}	11.21 ± 0.68^{a}	30.94 ± 1.32^{a}
0.29	77.78 ± 7.03	24.50 ± 4.68^{a}	14.25 ± 0.77^{ab}	13.71 ± 0.99^{ab}	31.61 ± 1.91^{a}
0.58	72.22 ± 3.51	22.92 ± 2.10^{a}	12.81 ± 0.62^{ab}	12.62 ± 0,91^{a}	35.27 ± 1.21^{ab}
1.15	91.67 ± 5.69	22.42 ± 4.98^{a}	12.94 ± 0.68^{ab}	11.91 ± 0.81^{a}	36.94 ± 1.06^{b}
NAA (μM)					
0	86.11 ± 5.12	23.65 ± 2.91^{b}	12.39 ± 0.44^{b}	16.23 ± 1.09^{b}	30.84 ± 1.68^{c}
0.07	86.11 ± 5.12	6.36 ± 0.90^{a}	6.18 ± 0.44^{a}	11.54 ± 0.92^{a}	15.55 ± 0.74^{a}
0.13	83.33 ± 7.45	5.19 ± 0.73^{a}	5.92 ± 0.48^{a}	12.35 ± 0.92^{a}	22.70 ± 1.07^{b}
0.27	77.78 ± 5.55	7.17 ± 1.55^{a}	6.43 ± 0.64^{a}	12.70 ± 0.89^{a}	31.00 ± 1.21^{c}
0.54	86.11 ± 5.12	4.17 ± 0.21^{a}	5.46 ± 0.58^{a}	12.38 ± 0.92^{a}	33.16 ± 1.61^{c}
IBA (μM)					
0	86.11 ± 5.12	23.65 ± 2.91^{a}	12.39 ± 0.44^{a}	16.23 ± 1.09^{b}	30.84 ± 1.68^{c}
0.06	88.89 ± 5.56	19.19 ± 3.96^{a}	12.00 ± 0.72^{a}	12.25 ± 1.07^{a}	16.75 ± 0.74^{a}
0.12	80.56 ± 5.12	14.48 ± 2.36^{a}	11.59 ± 0.65^{a}	9.48 ± 0.89^{a}	16.55 ± 1.07^{a}
0.25	83.33 ± 6.09	22.10 ± 6.49^{a}	12.03 ± 0.71^{a}	9.60 ± 0.81^{a}	21.63 ± 1.21^{b}
0.49	83.33 ± 6.09	22.83 ± 4.29^{a}	12.73 ± 1.07^{a}	12.40 ± 1.13^{a}	21.33 ± 1.61^{b}

Table 4. Effect of physical factors on the germination and growth of isolated embryos of *Pinus heldreichii*. All data were obtained at day 28. Means in the column followed by different letters are different according to Duncan's multiple range test ($p \leq 0.05$).

Physical factors	Germination (%)	Root length (mm)	Hypocotyl length (mm)	Cotyledon length (mm)	Dry weight (mg)
Light					
24-h light	86.11 ± 6.69	15.59 ± 1.77^{a}	22.63 ± 2.02^{b}	12.03 ± 0.88^{a}	28.53 ± 1.98^{b}
16-h light	86.11 ± 5.12	23.65 ± 2.91^{b}	12.39 ± 0.44^{a}	16.23 ± 1.09^{b}	30.84 ± 1.68^{b}
24-h darkness	88.89 ±5.56	16.47 ± 1.84^{a}	17.72 ± 0.76^{a}	15.34 ± 1.11^{b}	18.53 ± 1.12^{a}
Medium composition					
0.7% agar	86.11 ± 5.12	23.65 ± 2.91^{b}	12.39 ± 0.44^{a}	16.23 ± 1.09^{b}	30.84 ± 1.68^{b}
Liquid	58.33 ± 5.69	12.43 ± 1.10^{a}	13.19 ± 0.92^{a}	10.33 ± 0.79^{a}	20.53 ± 0.76^{a}

necessary for the normal development of isolated mature zygotic embryos. Solid compared to liquid medium was more beneficial for embryo growth. It follows that an *in vitro* culture system could be applied for rapid and synchronous production of healthy seedlings of elite and/or selected genotypes with low seed production. Since the culture of isolated zygotic embryos can provide insight into the requirements for successful transformation of somatic embryos into plants, our further work will be aimed at defining the conditions for *P. heldreichii* somatic embryo maturation and transformation into plantlets, given the knowledge here obtained about factors influencing zygotic embryo development.

Acknowledgment — This work was supported by the Ministry of Science of the Republic of Serbia (Grant No. 143026).

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КЛИЈАЊЕ И РАСТЕЊЕ ИЗОЛОВАНИХ ЕМБРИОНА *PINUS HELDREICHII* У КУЛТУРИ *IN VITRO*

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У култури *in vitro* испитиван је ефекат нутритивних, хормоналних и физичких фактора на клијање и растење изолованих зиготских ембриона мунике (*Pinus heldrechii*). Већина изолованих ембриона мунике клијала је већ током прве недеље након преношења на хранљиву подлогу. Мада ембриони могу да клијају и на подлози без соли, витамина и угљених хидрата, највећи проценат клијања и најбоље морфолошке карактеристике клијанаца постигнуте су на базалној Gresshoff & Doy подлози (1GD) са 3% сахарозом. Од испитиваних регулатора растења само је гиберелна киселина (GA_3) позитивно деловала на развиће ембриона, док су ауксини и цитокинини инхибирали њихово даље развиће. У односу на ембрионе који су расли у течној подлози, ембриони са чврсте агарне подлоге, клијали су у већем проценту и даље се правилно развијали. Мада генерално стимулишу растење клијанаца, светлосни третмани нису имали ефекат на клијање ембриона. Добијени резултати показују да се *in vitro* систем може користити за брзу и синхрону продукцију здравих клијанаца елитних генотипова који се одликују ниском продукцијом семена.