DENDROBIOLOGY
2003, vol. 50, 17–24

Teresa Hazubska, Krystyna Szczygieł

Induction of somatic embryogenesis in spruce: *Picea omorika, P. pungens* 'Glauca', *P. breweriana* and *P. abies*

Received: 3 July 2003, Accepted: 26 September 2003

Abstract: The studies were carried out on seed explants (mature zygotic embryos) of four spruce species: *Picea omorika* (Pancić) Purk., *P. pungens* 'Glauca' Beisnn., *P. breweriana* S. Watson and *P. abies* (L.) Karst. The explants required darkness during the induction of embryogenic tissue. Temporary exposure of explants to light did not increase their embryogenic capacity. It was observed that the temperature of 25°C was the optimal for embryogenic tissue induction from mature zygotic embryos of *Picea omorika*. Cold treatment at 4°C during one week improved embryogenic tissue initiation in the explants of *P. pungens* 'Glauca'. Seed explants of *Picea omorika* produced embryogenic tissue on medium lacking glutamine. Only explants of *Picea abies* and *P. omorika* showed embryogenic tissue formation. For effective proliferation of embryogenic tissue in *Picea abies* and *P. omorika* it was necessary to supply the medium with 2,4-D (4.5–9 μM) and BA (2.25 μM). Two of three analized lines showed that ABA promote somatic embryo maturation, at the ABA concentration 20 μM in the medium.

Additional key words: maturation, somatic embryos, Norway spruce, Serbian spruce, blue spruce

Addresses: T. Hazubska, Polish Academy of Science, Institute of Dendrology,
Parkowa 5, 62-035 Kórnik, Poland, e-mail: hazubska@o2.pl
K. Szczygieł, Forest Research Institute Department of Genetics and Physiology of Forest Trees,
Bitwy Warszawskiej 1920 roku 3, 00-973 Warsaw, Poland, e-mail: k.szczygiel@ibles.waw.pl

Introduction

Somatic embryogenesis is potentially the most promising method of clonal propagation of conifers, mainly providing for the needs of forestry and horticulture. It makes possible to micropropagate several species of coniferous trees, mainly of the following genera: *Picea, Abies, Larix, Pinus,* and *Pseudotsuga* (Gupta and Grob 1995, Dunstan et al. 1995).

The process of somatic embryogenesis in species belonging to the genus *Picea* was first discovered in mid 1980s in Norway spruce *P. abies* (Hakman et al. 1985; Chalupa 1985). In the 1990s plant regeneration through somatic embryogenesis was attempted in: Serbian spruce *P. omorika* (Budimir and Vujicic 1992, Vujicic and Budimir 1995) and blue spruce *P. pungens* (Afele et al. 1992).

The successful research on somatic embryogenesis was obtained in Norway spruce (*Picea abies*), used in our experiments as "positive control". Other species such as: *Picea omorika, P. pungens* 'Glauca' and *P. breweriana* for example, are still very difficult to propagate by this method. These species of spruce are an excellent material for planting in cities because of their decorative value and resistance to air pollution, drought (*P. omorika*, *P. pungens* 'Glauca') and frost (Seneta and Dolatowski 1997).

P. breweriana is rare in Poland, and mainly grown in Botanical Gardens and Arboreta. Only a few of the specimens growing in Poland produce cones. The seeds of this species do not germinate easily and the seedlings grow poorly.

Numerous studies indicated that the induction of embryogenic tissue is determined by the origin of the explants, i.e. by the genotype of the original material (Park et al., 1998). According to Cheliak and Klimaszewska (1991) the potential to initiate embryogenic tissue in e.g. black spruce (*Picea mariana*) depended on the origin of the seeds from which the explants had been obtained.

Embryogenic tissue cultures of different species of spruce were most frequently obtained from immature and mature zygotic embryos (Bozhkov et al. 1992, Misra et al. 1993) as well as different parts of seedlings such as cotyledons or hypocotyls (Mo and von Arnold 1991, Vujicic and Budimir 1995). For embryogenic callus initiation of *Picea abies* hypocotyls and cotyledons were used from one month plantlets and from needles of 14 months old somatic plants (Ruaud et. al. 1992, Ruaud 1993). Recently Harvengt et al. (2001) have obtained embryogenic tissue from needles 3 year old somatic seedlings of Norway spruce.

To improve the induction of embryogenic tissue from mature zygotic embryos a specific requirements of this four species of spruce we have been studied. It is known, that plant growth regulators (PGRs) play a key role in the process of somatic embryogenesis (Klimaszewska et al. 2001). We have been tested the influence of 2,4-D and BA on the proliferation of embryogenic tissue of *P. abies* and *P. omorika*, and the influence of ABA on the maturation of somatic embryos. It was impossible to study the proliferation and maturation of the somatic embryos of *P. pungens* 'Glauca' and *P. breweriana*, because of death of the embryogenic tissue in the short time after initiation stage.

The objectives of this work were to study:

- the influence of the light, explants pretreatment with low temperature and lack of glutamine in the basal medium on the embryogenic potential of the embryos of spruces,
- the influence of various types and concentrations of PGRs supplied in the basal medium on the growth of embryogenic tissue in *Picea abies* and *P. omorika*,
- the influence of different concentrations of abscisic acid (ABA) on *Picea abies* and *P. omorika* somatic embryos development and maturation.

Materials and methods

Plant material

The explants (mature zygotic embryos) for somatic embryogenesis initiation were derived from seeds of studied species of spruce. The seeds sources were following: *P. abies* from the Forest District of Augustów (Poland) as "positive control", *P. omorika* from Kórnik Arboretum (Poland), *P. pungens* 'Glauca' from the nursery of J. Z. Byczkowski in Kostrzyn and *P. breweriana* in Przelewice Arboretum (Poland) and in Oregon, USA (collected by Florpak company).

The seeds were sterilized in 33% (v/v) H_2O_2 (hydrogen peroxide solution) containing 2 drops of Tween 20 for 10–15 minutes. Next they were rinsed three times in sterile distilled water and stored in containers with a small volume of sterile distilled water for 14–16 hours, in the dark at 4°C. Mature zygotic embryos were excised from the seeds and placed onto initiation medium.

Initiation

Two types of media were used to induce SE: BM 3 (Gupta and Durzan 1986) and NS III (Jain et al. 1988). The effect of several factors on the induction of embryogenic tissue was tested in three separate experiments.

Experiment 1 involved studies of the effect of light and dark on the SE induction. There were four variants of the intervals of exposure of cultures to different light conditions: I/dark (4 weeks), II/light (1 week) and dark (3 weeks), III/light (2 weeks) and dark (2 weeks), IV/light (3 weeks) and dark (1 week). The cultures were maintained at 25° C under a 16-h photoperiod, at a light intensity 7.5 W/m^2, provided by 40 W mercury-discharge lamps.

Experiment 2 involved studies of the effect of high and low temperatures on the SE induction. There were four of intervals of exposure to different temperatures: I/25°C (4 weeks), II/4°C (1 week) and 25°C (3 weeks), III/4°C (2 weeks) and 25°C (2 weeks), IV/4°C (3 weeks) and 25°C (1 week). The embryos were incubated in the dark.

In both experiments the cultures were maintained on BM 3 medium, supplemented with: 2,4-D (9 μM) and BA (4.5 μM) and with sucrose (10 g/l), casein hydrolysate (N-Z Amine A from bovine milk, Sigma 1 g/l) and L-glutamine (450 mg/l).

Experiment 3 involved studies of the effect of the lack of glutamine in medium on the induction of SE in the selected species of spruce. Explants were cultured on NS III medium supplemented with: 2,4-D (10 μM) and BA (5 μM), as well as sucrose (10 g/l), and lacking glutamine. Glutamine was added to the control medium (450 mg/l). The cultures were placed in the dark at 25°C.

Three to 5 Petri dishes 100 × 15 mm with 10 embryos in each in two replicates in this experiments were used. They were subcultured onto fresh medium (the same composition) after six weeks of induction. The tissue was examined microscopically (visual) and transferred onto fresh medium for proliferation.

Proliferation

For proliferation of embryogenic tissue BM 3 medium was used in which the concentration of sucrose was lowered to 5 g/l and casein hydrolysate was eliminated. Three PGRs variants were used: a) 9 μM 2,4-D and 2.25 μM BA, b) 4.5 μM 2,4-D and 2.25 μM BA and c) 9 μM BA. In this experiment two embryogenic lines of *Picea abies* and two lines of *P. omorika* were tested. Nine pieces of embryogenic tissue have been placed in four Petri dishes for each PGRs variant.

To determine the influence of different PGRs components on the increment of embryogenic tissue the pieces were weighed on first and twelfth days of culture. The experiment was repeated four times. Embryogenic tissue was maintained in the dark at 25°C.

The increment of the fresh weigh of embryogenic tissue was statistically analysed for each variant and for each species.

Maturation

The maturation of somatic embryos of *Picea abies* and *P. omorika* was carried out on BM 3 medium lacking PGRs but supplemented with activated charcoal (Sigma, Cell Culture Tested, 10 g/l) for one week. During the following six weeks somatic embryos were cultured on a medium BM 3 supplemented with sucrose (34 g/l), IBA (1 μM) and ABA at: 20, 40, and 60 μM. To evaluate the effect of different concentrations of ABA the total number of somatic embryos (TE) and the number of the mature embryos at the cotyledonary stage (CE) it has been determined. The observation was made in the fifth week of culture. The lenght of culture period on the total number of somatic embryos and the number of cotyledonary embryos on the medium BM 3 supplemented with 20 μM ABA was tested. The observations were made in the third, fourth and fifth week of culture. Five pieces approximately 80–150 mg FW of embryogenic tissue was placed on two Petri dishes for each ABA variant. The cultures were cultivated at temp. ±25°C, at a light intensity 7.5 W/m^2 (40 W mercury-discharge lamps), photoperiod 16-h day.

Initiation, proliferation and maturation media used in the respective experiments were sterilized at 121°C, under the pressure of 1 atmosphere for 20 min. Gellan gum (Phytagel, Sigma) (4 g/l) was used as a solidifying agent, pH of all the media were adjusted to 5.8 before autoclaving. Filter sterilized (±) ABA, IBA, L- glutamine were added into cooled, liquid medium at the maturation stage.

The statistical analysis of the experimental data was conducted with the assistance ANOVA/MANOVA program from the STATISTICA package. The one-way variance analysis based on Tukey test was used to study the differences between respective variants, with the level of significance $\alpha = 0.05$.

Results and discussion

Initiation

All studied species of spruce showed embryogenic tissue formation. It was posssible to proliferate the embryogenic tissue of *P. abies* and *P. omorika* only. The embryogenic tissues of *P. pungens* 'Glauca' and *P. breweriana* decayed after their initiation in a short period of time.

The highest embryogenic tissue frequency for *P. abies* was achieved in the explants cultured only in the dark – 4.4% without any light treatment (Table 1) and in the one week cold period – 3.4% (Table 2). The explants of *Picea abies* regenerated embryogenic tissue in the medium NS III without glutamine (Fig. 1), but the number of explants with embryogenic tisssue was very low – 2%.

In Serbian spruce the highest embryogenic frequency – 6.7% was observed in the variant of 4 weeks exposure to dark (Table 1). The explants of *P. omorika* reached also the highest frequency of embryogenic tissue induction after cultivation at 25°C continuously. Temporary exposure to lower temperature negatively affected the induction of embryogenic tissue of this species (Table 2).

Table 1. Effect of light conditions on the induction of embryogenic tissue in *Picea abies* and *P. omorika*, cultured on BM 3 medium (average ± standard deviation)

Culture conditions	Embryos with embryogenic tissue *Picea abies* (%)	Embryos with embryogenic tissue *Picea omorika* (%)
I/dark 4 weeks	4.4 a* ± 1.8	6.7 a ± 3.3
II/light 1 week + dark 3 weeks	3.3 a ± 1.7	0.0 a ± 0.0
III/light 2 weeks + dark 2 weeks	2.2 a ± 2.2	4.4 a ± 2.4
IV/light 3 weeks + dark 1 week	1.1 a ± 1.1	2.2 a ± 1.5

* Values marked with the same letters are not significantly different (p=0.05)

Table 2. Effect of temperature conditions on the induction of embryogenic tissue in four study species cultured on BM 3 medium

Culture conditions	Embryos with embryogenic tissue (%)			
	Picea abies	*Picea omorika*	*P. pungens* 'Glauca'	*P. breweriana*
I/25°C 4 weeks	3.3 a	6.7 b	4.4 b	1.1 a
II/4°C 1 week + 25°C 3 weeks	3.4 a	1.1 a	6.8 b	0.0 a
III/4°C 2 weeks + 25°C 2 weeks	2.2 a	0.0 a	1.1 a	1.1 a
IV/4°C 3 weeks + 25°C 1 week	0.0 a	0.0 a	0.0 a	0.0 a

During the culture of explants of *Picea omorika* on NS III medium supplemented with glutamine, only 3.3% of them showed the ability to produce embryogenic tissue (Table 3). The lack of glutamine in the medium positively affected the induction of embryogenic tissue in *P. omorika* – embryogenic frequency was 18% (Fig. 1).

The zygotic embryos of *P. pungens* 'Glauca' did not induce embryogenic tissue under light treatment. The explants of this species cultivated at temp. 25°C and subjected to 1 week of lower temperature showed higher frequency of embryogenic tissue formation – 6.8%. In the case of *P. pungens* 'Glauca' one week explants cooling had a positive effect on the frequency of embryogenic tissue induction, which was slightly higher than in the culture conducted only at a constant temp. 25°C. The difference, however, was not statistically significant (Table 2).

The explants of *P. pungens* 'Glauca' cultured on NS III medium lacking glutamine showed low induction, reaching the frequency of only 4.2% (Fig. 1).

The explants of *P. breweriana* did not regenerate embryogenic tissue after light treatment. Zygotic embryos of this spruce produced embryogenic tissue at 1.1% in the culture conducted at a constant 25°C and in the 2 week cold period (Table 2) and at 6.6% in the culture on NS III medium without glutamine (Fig. 1).

In coniferous trees the first two phases of somatic embryogenesis (initiation and proliferation of

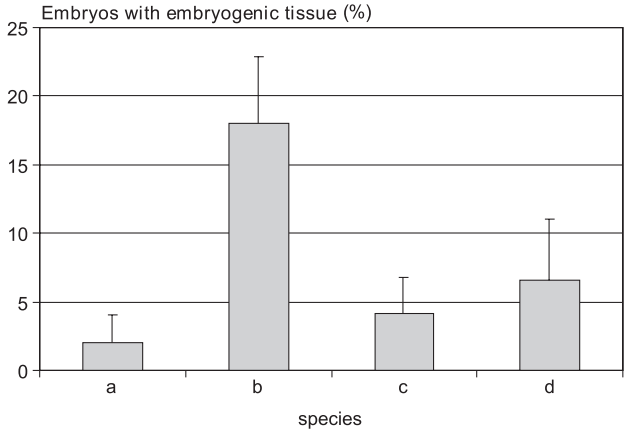

Fig. 1. Induction of embryogenic tissue from mature zygotic embryos cultured on NS III medium lacking glutamine (percentage ± standard deviation)

a – *Picea abies*, b – *P. omorika*, c – *P. pungens* 'Glauca', d – *P. breweriana*

embryogenic tissue) are usually conducted in the dark, at temperature 20–27°C (Norgaard et al. 1991, Roberts 1991, Bellarosa et al. 1992), and the remaining phases (maturation and germination of somatic embryos) in light. In the experiments testing the effect of external factors on the induction of embryogenic tissue, formation of this tissue was obtained in the temporary lighting and cooling of the explants. Temporary light treatment of the explants did not increase their embryogenic potential. Probably the mechanisms steering the process of somatic embryogenesis operate much better in the dark than in light. Short term light exposure did not stimulate the embryogenic tissue initiation.

The effect of temporary exposure of explants to low temperature on the presence of embryogenic tissue was observed in zygotic embryos of all the studied species. The treatment of spruce explants with the temperature of 25°C during culture, proved to be most beneficial. Exposure to low temperature did not increase the embryogenic potential of the cultured explants, with the exception of *P. pungens* 'Glauca'. Prolongation of exposure to low temperature decreased of embryogenic potential.

During the culture of explants on NS III medium supplemented with glutamine, the presence of embryogenic tissue was observed only in the zygotic embryos of *Picea omorika*. The explants of the remaining species of spruce did not induce embryogenic tissue (Table 3). However, the explants of all tested species, cultured on NS III medium lacking glutamine induced embryogenic tissue. Statistically significant differences were observed in the reaction of the respective species of spruce to the lack of glutamine in the NS III medium, which manifested itself in different embryogenic potential of the explants. The lack of glutamine in the medium most positively affected the induction of embryogenic tissue in *P. omorika*. Budimir and Vujicic (1992) were induced similarly embrygenic tissue on the shoot explants of *P. omorika*, cultivating without any amino acids on the medium. The explants of the other species of spruce showed lower induction on the same medium (Fig. 1). These results would indicate that not all species of spruce require media containing enriched sources of nitrogen in the form of glutamine for the initiation and growth of embryogenic tissue. It is widely assumed

Table 3. Induction of embryogenic tissue from mature zygotic embryos cultured on NS III medium supplemented with glutamine (percentage ± standard deviation)

Species	Percentage of embryos with embryogenic tissue
Picea abies	0.0 a ± 0.0
P. omorika	3.3 a ± 3.3
P. pungens 'Glauca'	0.0 a ± 0.0
P. breweriana	0.0 a ± 0.0

that glutamine positively affects the induction of embryogenic tissue and the maturation of somatic embryos of coniferous trees (Hristoforoglu et al. 1995, Khlifi and Tremblay 1995), since it provides nitrogen in the reduced form. Organic nitrogen is assimilated easier than the nitrogen in the form of nitrate or ammonium (Leustek and Kirby 1988). So far only von Arnold (1987) has shown that the level of initiation of embryogenic cultures of *Picea abies* was higher without glutamine, in the medium containing NH_4NO_3.

Proliferation

Most frequently used PGRs combination in SE in conifers has been: 2,4-D (9.5–15 μM) and BA (2.2–5.0 μM). It was noted that the level of growth regulators in the initiation and proliferation medium affected further ability of respective embryogenic lines to mature of somatic embryos (Klimaszewska et al. 2001). In order to improve the quality of somatic embryos different concentrations of growth regulators were used in the initiation and proliferation media.

At the proliferation stage higher increase in the fresh weight of embryogenic tissue was obtained for the two lines of *P. abies* than for the two embryogenic lines of *P. omorika* (Table 4). The increase in the fresh weight of embryogenic tissue of the two studied lines was on average highest for the PGR variant (A), with 2,4-D at 9 μM and BA at 2,25 μM and for the PGR variant (B) with a lower concentration of 2,4-D was used (4.5 μM) (Table 5). The embryogenic tissue cultured on the medium containing these two PGR combinations was whitish to transluscent and was

Table 4. Increment of embryogenic tissue fresh weight (lines of *Picea abies* and *P. omorika*), on BM 3 medium

Species – line	Fresh weight of embryogenic tissue (g)
Picea abies I	0.157 b
P. abies II	0.163 b
P. omorika I	0.102 a
P. omorika II	0.098 a

characterized by vigorous growth. A significantly slower growth of embryogenic tissue was observed for cultures grown on the medium without auxin and supplemented only with BA (9 μM). Depriving the medium of auxin and supplementing it only with cytokinin led to the death of embryogenic tissue.

Maturation

Abscisic acid is necessary for effective maturation process of somatic embryos (Becwar et al. 1987; Jalonen and Arnold von 1991). The exogenous ABA has influence on the accumulation of storage reserves in embryos (proteins, lipids and carbohydrates) and contributes to the inhibition of cleavage polyembryony. Abscisic acid in medium synchronized the process of maturation of somatic embryos and supported proper germination of these embryos (Misra 1994). For *P. abies* ABA has been used in the concentration of 7–60 μM (von Arnold et al. 1995). We have studied the effect of three concentrations of ABA on the maturation of somatic embryos of *Picea abies* and *P. omorika*. It was observed the differences in the reaction to ABA between the studied genotypes and species of spruce.

From the two tested embryogenic lines of *P. abies* only one regenerated somatic embryos in the presence of ABA. The other line of embryogenic tissue necrotized on all media with ABA. Both embryogenic lines of *P. omorika* regenerated somatic embryos.

In the case of *P. abies* there was no statistically significant difference in the total number of regenerated somatic embryos – TE on media with different concentrations of ABA (Table 6). However, an obvious influence of ABA concentration on the number of somatic embryos at the cotyledonary stage – CE was observed. The highest number of somatic embryos at the cotyledonary stage, average 61/g FW of embryogenic tissue,

Table 5. Fresh weights of embryogenic tissues of *Picea abies* and *P. omorika* cultured on the media with various concentrations of 2,4-D and BA

Hormone	Fresh weight of embryogenic tissue (g)			
	Picea abies		*Picea omorika*	
	Line I	Line II	Line I	Line II
A/2,4-D 9 μM	0.174 b	0.217 c	0.103 ab	0.110 b
BA 2,25 μM				
B/2,4-D 4,5 μM	0.183 b	0.184 b	0.117 b	0.108 b
BA 2,25 μM				
C/BA 9 μM	0.115 a	0.088 a	0.087 a	0.077 a

was observed on the medium containing 20 μM of ABA. The lowest number of cotyledonary embryos, average 7/g FW of embryogenic tissue, was observed on the medium containing 60 μM of ABA. A similar tendency was noticed in line I of *P. omorika*. The concentration of ABA had influence on the total number of somatic embryos regenerated by line II of *P. omorika*, but did not significantly affect the number of somatic embryos at the cotyledonary stage. The highest number of somatic embryos was regenerated by line II, at ABA concentration of 60 μM, the average 375/g FW of embryogenic tissue and at an ABA concentration of 20 μM, average 290/g FW of embryogenic tissue.

It was noted that the time significantly influenced on the total number of somatic embryos only in the case of line II *P. omorika*. The highest average number of somatic embryos – 357 was noted in the fifth week of culture. During culture the number of somatic embryos at cotyledonary stage increased significantly as well both in *P. abies* and in the two lines of *P. omorika* (Table 7).

The highest number of somatic embryos was obtained in *P. omorika* (line I), the total number of embryos was 717 with 45 of embryos at the cotyledonary stage (per 1g of the fresh mass). Slightly lower number was achieved for *P. abies*, and the poorest regeneration of somatic embryos took place in line II of *P. omorika* (Table 8).

These results revealed that concentration of ABA and duration of growth on maturation medium have significant influence on the number of cotyledonary stage somatic embryos, and less on the total number of somatic embryos. The time of contact the embryogenic tissue with maturation medium has influence on the yield of mature somatic embryos and future growth of somatic seedlings of *Picea abies*. Somatic embryos maturated for 5-weeks produced about twice as large plants as those recovered from 7-week embryos (Bozhkov and von Arnold 1998). By giving the embryogenic tissue a prematuration medium (medium lacking growth hormones) a short 5-week ABA treatment is sufficient for the development of somatic embryos to mature forms (Hogberg et al. 2001). Many studies demonstrated that only the cotyledonary somatic embryos guaranteed well conversion to plants (Becwar et al. 1987). In *P. abies* and *P. omorika* line I the lowest concentration of ABA (20 μM) had a more positive effect on the maturation of somatic embryos than the higher one. The increase of concentration of ABA in the maturation medium inhibited the ability of somatic embryos to develop from the early stages to the cotyledonary stage.

Table 6. Effect of ABA concentration on the total number of somatic embryos (TE) and the number of somatic embryos at the cotyledonary stage (CE), per 1 gram of fresh weight of embryogenic tissue

Species-line	ABA concentration μM	TE/1g	CE/1g
Picea abies	20	568 a	61 b
	40	427 a	51 b
	60	492 a	7 a
P. omorika I	20	674 a	70 b
	40	785 a	45 ab
	60	693 a	20 a
P. omorika II	20	290 ab	19 a
	40	184 a	19 a
	60	375 b	26 a

Conclusions

1. The highest frequency of induction of somatic embryogenesis was obtained from zygotic embryos of *Picea abies* and *P. omorika* cultured in the darkness.
2. The best conditions for *P. omorika* embryogenic tissue induction were: temp. 25°C for four weeks, on the other hand explants of *P. pungens* 'Glauca' needed temp. of 4°C during one week and 25°C during three weeks.
3. The highest frequency of *P. omorika* embryogenic tissue induction was obtained on medium lacking of glutamine.
4. Continuous proliferation of *Picea abies* and *P. omorika* embryogenic tissues required both: auxin

Table 7. Effect of the length of culture period on the total number of somatic embryos (TE) and the number of somatic embryos at the cotyledonary stage (CE), per 1 gram of fresh weight of embryogenic tissue, cultured on BM 3 medium, supplemented with 20 μM ABA

Species-line	Culture period (week)	TE/1g	CE/1g
Picea abies	3	454 a	18 a
	4	490 a	28 a
	5	544 a	73 b
P. omorika I	3	712 a	18 a
	4	720 a	58 b
	5	720 a	58 b
P. omorika II	3	194 a	2 a
	4	299 ab	20 b
	5	357 b	42 c

Table 8. Number of 5-weeks somatic embryos (per 1 gram of fresh weight of embryogenic tissue): TE –total number of somatic embryos, CE – embryos at cotyledonary stage

Species-line	TE/1g	CE/1g
Picea abies	496 b	40 b
P. omorika I	717 c	45 b
P. omorika II	283 a	21 a

2,4-D (4.5–9.0 μM) and cytokinin BA (2.25 μM) in the medium.

5. For maturation of somatic embryos it is necessary to supply the basal medium with ABA. Abscisic acid (at concentration 20 μM) promoted the process of maturation of somatic embryos two of three analyzed lines of spruce.

References

Afele J.C., Senaratna T., Mc Kersie B.D., Saxena P.K. 1992. Somatic embryogenesis and plant regeneration from zygotic embryo culture in blue spruce (*Picea pungens* Engelman.). Plant Cell Reports 11: 299–303.

Arnold von S. 1987. Improved efficiency of somatic embryogenesis in mature embryos of *Picea abies* (L.) Karst. Journal of Plant Physiology 128: 233–244.

Arnold von S., Egertsdotter U., Ekberg I., Gupta P., Mo H., Norgaard J. 1995. Somatic embryogenesis in Norway spruce (*Picea abies*). Somatic Embryogenesis in Woody Plants Kluwer. Academic Publishers 3: 17–36.

Becwar M.R., Noland T.L., Wann S.R. 1987. Somatic embryo development and plant regeneration from embryogenic Norway spruce callus. Tappi Journal 70: 155–160.

Bellarosa R., Mo L.H., von Arnold S. 1992. The influence of auxin and cytokinin on proliferation and morphology of somatic embryos of *Picea abies* (L.) Karst. Annals of Botany 70: 199–206.

Bozhkov P.V., Arnold von S. 1998. Polyethylene glycol promotes maturation but inhibits further development of *Picea abies* somatic embryos. Physiologia Plantarum 104: 211–224.

Bozhkov P.V., Lebedenko L.A., Shiryaeva G.A. 1992. A pronounced synergistic effect of abscisic acid and 6-benzyladenine on Norway spruce (*Picea abies* L. Karst) somatic embryo maturation. Plant Cell Reports 11: 386–389.

Budimir S., Vujicic R. 1992. Benzyladenine induction of buds and somatic embryogenesis in *Picea omorika* (Pancic) Purk. Plant Cell, Tissue and Organ Culture 31: 89–94.

Chalupa V. 1985. Somatic embryogenesis and plantlet regeneration from cultured immature and mature embryos of *Picea abies* (L.) Karst. Communicationes Instituti Forestralis 14: 57–63.

Cheliak W.M., Klimaszewska K. 1991. Genetic variation in somatic embryogenic response in open-pollinated families of black spruce. Theoretical and Applied Genetics 82: 185–190.

Dunstan D.I., Tautorus T.E., Thorpe T.A. 1995. Somatic Embryogenesis in Woody Plants. In: In Vitro Embryogenesis in Plants. Kluwer Academic Publishers, Dordrecht: 471–538.

Gupta P.K., Grob J.A. 1995. Somatic embryogenesis in conifers. In: Jain S., Gupta P., Newton R. (eds), Somatic Embryogenesis in Woody Plants. Kluwer Academic Publishers, Dordrecht 1: 81–98.

Gupta P.K., Durzan D.J. 1986. Plantlet regeneration via somatic embryogenesis from subcultured callus of mature embryos of *Picea abies* (Norway spruce). In Vitro Cellular and Developmental Biology – Plant 22: 685–688.

Hakman I., Fowke L.C., von Arnold S., Eriksson T. 1985. The development of somatic embryos in tissue cultures initiated from immature embryos of *Picea abies* (Norway spruce). Plant Science 38: 53–59.

Harvengt L., Trontin J.F., Reymont I., Canlet F., Paques M. 2001. Molecular evidence of true- to-type propagation of 3-year-old Norway spruce through somatic embryogenesis. Planta 213: 823–832.

Hogberg K.A., Bozhkov P.V., Gronnroos R.von Arnold S. 2001. Critical factors affecting ex vitro performance of somatic embryo plants of *Picea abies*. Scandinavian Journal of Forest Research 16: 259–304.

Hristoforoglu K., Schmidt J., Bolhar-Nordenkampf H. 1995. Development and germination of *Abies alba* somatic embryos. Plant Cell, Tissue and Organ Culture 40: 277–284.

Jalonen P., von Arnold S. 1991. Characterization of embryogenic cell lines of *Picea abies* in relation to their competence for maturation. Plant Cell Reports 10: 384–387.

Jain S. M., Newton R.J., Soltes E.J. 1988. Enhacement of somatic embryogenesis in Norway spruce (*Picea abies L.*). Theoretical and Applied Genetics 76: 501–506.

Khlifi S., Tremblay F.M. 1995. Maturation of black spruce somatic embryos. I Effect of L-glutamine on the number and germinability of somatic embryos. Plant Cell, Tissue Organ Culture 41: 23–32.

Klimaszewska K., Park Y-S., Overton C., Maceacheron I., Bonga J.M. 2001. Optimized somatic embryogenesis in *Pinus strobus* L. In Vitro Cellular and Developmental Biology- Plant 37: 392–399.

Leustek T., Kirby E.G. 1988. The influence of glutamine on growth and viability of cell suspension cultures of Douglas – fir after exposure to ethylene glycol. Tree Physiology 4: 371–380.

Misra S., Attree S.M., Leal I., Fowke L.C. 1993. Effect of abscisic acid, osmoticum and dessication on synthesis of storage proteins during the development of white spruce somatic embryos. Annals of Botany 71: 11–22.

Misra S. 1994. Conifer zygotic embryogenesis, somatic embryogenesis and seed germination: Bio-

chemical and molecular advances. Seed Science Reseach 4: 357–384.

Mo L.H., von Arnold S. 1991. Origin and development of embryogenic cultures from seedlings of Norway spruce (*Picea abies*). Journal of Plant Physiology 138: 223–230.

Norgaard J.V., Krogstrup P. 1991. Cytokinin induced somatic embryogenesis from immature embryos of *Abies nordmanniana* Lk. Plant Cell Reports 9: 509–513.

Park Y.S., Barret J.D., Bonga J.M. 1998. Application of somatic embryogenesis in high-value clonal forestry: deployment, genetic control, and stability of cryopreserved clones. In Vitro Cellular and Developmental Biology – Plant 34: 231–239.

Roberts D.R. 1991. Abscisic acid and mannitol promote early development, maturation and storage protein accumulation in somatic embryos of interior spruce. Physiologia Plantarum 83: 247–254.

Ruaud J.N., Bercetche J., Paques M. 1992. First evidence of somatic embryogenesis from needles of one-year-old *Picea abies* plants. Plant Cell Reports 11: 563–566.

Ruaud J.N. 1993. Maturation and conversion into plantlets of somatic embryos derived from needles and cotyledons of 7–56-day old *Picea abies*. Plant Science 92: 213–220.

Seneta W., Dolatowski J. 1997. Dendrologia PWN. Warszawa.

Vujicic R., Budimir S. 1995. Somatic embryogenesis and plant regeneration in *Picea omorika*. Somatic Embryogenesis in Woody Plants. Kluwer Academic Publishers Netherlands 3: 81–97.