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Differential success of somatic embryogenesis in random gene pool of Norway spruce

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ABSTRACT: Somatic embryogenic cultures were established from proembryonal suspensor masses (PEMs) derived from mature seeds of Norway spruce. In this study we used more than 4,300 seeds of *Picea abies* from randomly collected commercial seed lot (originated from open-pollination). Most of the studies are focused on selected genotypes known for higher response to propagation protocols. As indicated in this study, there is a significant variation in success rate of somatic embryogenesis in randomly selected seed lot of Norway spruce. Nutrient GD (1 to 4), LP (1 to 5) media and different level of plant grow regulators (BA, NAA, kinetin and 2,4D) were used for initiation and proliferation of embryogenic cultures. Transfer of embryogenic callus onto medium containing abscisic acid stimulated development of early-established individual embryos. Media GD (5 and 6) and LP (9 to 11) supplemented with ABA (7.5; 20; 38 μM) and PEG 4000 (2%), were used for stadium of maturation. Conversion of somatic embryos to plantlets was stimulated by partial desiccation treatment (HRH-treatment) and by medium changes. On these media plantlets started to regenerate within three weeks.

Keywords: Norway spruce; somatic embryogenesis; *Picea abies*; plant grow regulators

Norway spruce (*Picea abies* [L.] Karst.], ($2n = 2\times = 24$) belonging to the family *Pinaceae*, is one of the most important conifers in Europe (Überall et al. 2004). The natural reproduction of Norway spruce is generative. Norway spruce, in Central Europe is the most abundant species; populations are exposed to industrial pollution and insect outbreaks (Vágner et al. 1998). Unfortunately, a long reproductive cycle, infrequent flowering (response to biotic and abiotic stresses), problematic and slow propagation of genetically improved material are very serious obstacles for clonal propagation and effective tree improvement. The biotechnology of somatic embryogenesis holds considerable promise for clonal propagation and breeding programmes in forestry (Bozhkov et al. 2002). Mastering somatic embryogenesis in Norway spruce could enable this method to be used for large scale multiplication of desired genotypes (Vágner et al. 1998).

Somatic embryogenesis of coniferous species was first reported more than 20 years ago (Stasolla, Yeung 2003). Since then, there has been an expansion of research aimed at developing and optimizing protocols for efficient regeneration of plantlets. Somatic embryogenesis in conifer tissue cultures with the capacity to generate plantlets was first reported from immature embryos of Norway spruce (Hakman et al. 1985; Hakman, Von Arnold 1985; Chalupa 1985). Embryogenic cultures were later also obtained from mature zygotic embryos of Norway spruce (Von Arnold, Hakman 1986; Gupta, Durzan 1986; Krogstrup 1986; Boulay et al. 1988). Growth of somatic embryo plants is under a cumulative influence of a number of treatments during the *in vitro* phase and consequently *ex vitro* establishment phase (Högberg et al. 2001).

A propagation method must be able to produce a large number of high-quality embryos from a range

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of genotypes to be suitable for clonal forestry. To be successful for commercial use, somatic embryogenesis technology must work with a variety of genetically diverse seeds (Pullman, Johnson 2002). The goal of this study was to determine if somatic embryogenesis could be used to provide planting stock for forestry from a large range of randomly-selected genotypes.

MATERIAL AND METHODS

Origin of the cultures

Embryonal suspensor masses were recovered from mature Norway spruce commercial seed mixture. Seeds developed after natural pollination represented a population originated from four different areas. For areas description and more details about SM1 to SM4 (Table 6).

The seeds were surface-disinfested for 20 min in 7.5% (w/v) calcium hypochlorite, in 70% ethanol for 2 min, and finally rinsed twice in sterile distilled water. They were then soaked according to previously published methods (Von Arnold, Hakman 1986).

Induction and maintenance of embryogenic cultures

Mature zygotic embryos were aseptically dissected from the gametophytes and placed onto different types of medium. The dissected embryo was referred to as the primary explant. Embryogenic cultures were initiated on GD medium (Gupta, Durzan 1986) and LP medium (Von Arnold, Ericsson 1981).

Effects of NAA (α-naphthaleneacetic acid), 2,4D (2,4-dichlorophenoxyacetic acid), BA (6-benzyladenine) and kinetin were tested by adding various concentrations (Table 5). At least one hundred twenty embryos were used for each treatment. Experiments were repeated three times. For further development of somatic embryo was used classification of PEMs described by Dyachok et al. (2002).

The tissues were isolated from the primary explants and transferred to fresh medium. Each genotype is a culture line induced from a single zygotic embryo. Active growth was determined by marking the colony edge at the time of transfer and visually determining at the end of the subculture cycle if the colony size increased.

During proliferation were observed ninety cell lines. Experiments were repeated three times for each line. Each cell line – clone has special ID number compounds of the symbols (symbol of medium-code of area/specific numbers). The effect of different conditions on proliferation was investigated by increase of embryogenic callus. The increase was observed on graph paper during 0 to 8 weeks treatment.

For our experiments were used media containing both plant growth regulators – cytokinin and auxin (LP1, LP3, LP4, GD3), also media containing only low concentrations of cytokinin (LP5) (Table 5). The basal initiation and proliferation media employed for the induction and maintenance of embryogenic cultures were supplemented with 30 g/l sucrose, myo-inositol (LP medium – 100 mg/l, GD medium – 1,000 mg/l), 500 mg/l casein hydrolysate, L-glutamin (450 mg/l). pH value was adjusted prior to autoclaving (pH 5.7 ± 0.1).

Somatic embryo development and germination

In order to promote maturation, proembryogenic mass was transferred onto media supplemented with abscisic acid (ABA) and PEG (polyethylene glycol 4000) to test osmotic stress for 4–8 weeks (Table 5). As shown in recent review by Dunstan et al. (1998) ABA plays an important role in the process (and also subsequent germination). The evaluation of mature embryos was used from protocol Webster et al. (1990). Induced embryos were counted as embryos per one square centimetre of embryogenic callus.

For maturation experiment were used forty-two cell lines from area SM1, SM2, SM3 and SM4. Experiments were repeated three times. Described by Roberts et al. (1993) to complete the process and effect a transition to germination upon exposure to suitable conditions, somatic embryos were harvested and subject to a high relative humidity (HRH-treatment) experiments described by Bozkov and Von Arnold (1998). The partial desiccation procedure was tested on half of embryogenic callus from each cell line. Matured embryos from HRH-treatment and without were placed on fresh media (Table 5).

The embryos were placed on germination media containing IBA (indole-3-butyric acid), supplemented with carbohydrate or media lacked plant growth regulators (Table 5).

Germination frequency was estimated as a percentage of embryos that developed radicle and elongated hypocotyls with or without epicotyls. We germinated somatic embryos from forty-two genotypes. *In vitro* germination occurs within 5 to 7 days and proceeds to the development of true needles at 4 to 6 weeks. The basal maturation and germination media employed for the tracking of individual somatic embryo development were supplemented with

Table 1. Initiation raten on GD (1 to 4) and LP (1 to 5) media and two months cultures survival from 4,320 embryos initiated during eyars 2004–2005

Medium	Area	Initiations/total	Initiation ratio (%)	# Survived/total	Survival of initiations (%)
GD1	SM1	19/120	15.83	6/19	31.58
GD1	SM2	22/120	18.33	3/22	13.64
GD1	SM3	10/120	8.33	3/10	30.00
GD1	SM4	20/120	16.67	1/20	5.00
GD2	SM1	13/120	10.83	2/13	15.38
GD2	SM2	23/120	19.17	1/23	4.35
GD2	SM3	19/120	15.83	2/19	10.53
GD2	SM4	14/120	11.67	1/14	7.14
GD3	SM1	2/120	1.67	0/2	0.00
GD3	SM2	10/120	8.33	1/10	10.00
GD3	SM3	5/120	4.17	1/5	20.00
GD3	SM4	6/120	5.00	0/6	0.00
GD4	SM1	11/120	9.17	4/11	36.36
GD4	SM2	14/120	11.67	3/14	21.43
GD4	SM3	8/120	6.67	1/8	12.50
GD4	SM4	20/120	16.67	1/20	5.00
LP1	SM1	3/120	2.50	1/3	33.33
LP1	SM2	10/120	8.33	3/10	30.00
LP1	SM3	5/120	4.17	3/5	60.00
LP1	SM4	14/120	11.67	3/14	21.43
LP2	SM1	1/120	0.83	1/1	100.00
LP2	SM2	8/120	6.67	1/8	12.50
LP2	SM3	3/120	2.50	0/3	0.00
LP2	SM4	9/120	7.50	2/9	22.22
LP3	SM1	4/120	3.33	3/4	75.00
LP3	SM2	9/120	7.50	4/9	44.44
LP3	SM3	8/120	6.67	5/8	62.50
LP3	SM4	16/120	13.33	3/16	18.75
LP4	SM1	3/120	2.50	1/3	33.33
LP4	SM2	14/120	11.67	5/14	35.71
LP4	SM3	4/120	3.33	1/4	25.00
LP4	SM4	12/120	10.00	5/12	41.67
LP5	SM1	6/120	5.00	5/6	83.33
LP5	SM2	16/120	13.33	3/16	18.75
LP5	SM3	3/120	2.50	2/3	66.67
LP5	SM4	19/120	15.83	4/19	21.05
Overall totals		383/4,320	8.87	85/383	28.57

the same level of supplements, see at induction and proliferation treatment.

RESULTS AND DISCUSSION

Induction and proliferation

The induction of embryogenic tissue in coniferous species is strictly dependent upon the choice of explants (Stasolla, Yeung 2003). The proembryonal suspensor mass was initiated from 4–15% of mature zygotic embryos (see Table 1). The GD (GD1 and GD2) medium supplemented with kinetin (2 and 4μM) and BA (2 and 4μM) was more effective in inducting embryogenic tissue than other media (GD4 and LP1-LP5). The number of two month survival embryogenic cultures after initiation treatment was absolutely opposite to compare the initiations rate. Comparisons different areas to cultivation somatic embryogenesis resulted that the highest rate of PEMs were yield on the SM4 area (12.04% the average from GD 1 to 4 and LP1 to 5 media). LP1 medium were used according to previously published methods (Von Arnold, Hakman 1986; Von Arnold 1987; Jain et al. 1988; Kvaalen, Von Arnold 1991). The initiation ratio from this media reached 3–12%.

However, in conifers, the efficiency of somatic embryogenesis from mature embryos has not been as high as that derived from immature embryos. Recently, the highest report yield of embryogenic callus from mature zygotic embryos of *Picea abies* has been reported by Jain et al. (1988) – embryogenic callus (white-mucilaginous callus) developed in 18–32% of the mature embryo explants growing on LP medium.

1 to 20% of the explants (PEMs), cultured during induction, were isolated away from the original non-embryogenic material. 90 stable embryogenic cultures (genotypes) of Norway spruce were established, representing the same number of open-pollinated families. Previous studies have shown that that less than 50% of the explants formed embryogenic cultures. Hence, although these techniques work for a range of genotypes there is a significant reduction in the genetic diversity of the culture collection (Webster et al. 1990).

Data for proliferation stadium were collected after eight weeks from the number of cultures actively growing. The increasing of PEMs were scale red as a difference between stared-up stadium (early stage) and eight weeks old embryogenic culture. In our experiment we observed 90 cell lines. The growth rate among cell lines was very different (from 61% to 2,295%). Results from varying the plant grow regulators level in media is shown in Table 2. The grow rate was watched also after proliferation process as number of survival embryogenic cultures after six month more treatment. Some cell lines continue to grow, although slowly, while other cell lines stop growing (Table 2). The more resistant cell lines are from area SM1, six month after proliferation treatment survival 98% of cell lines from this area. Pullmann and Johnson (2002) observed loblolly pine and discovered, that maintenance of embryogenic tissue from 98 initiated cultures over six months showed a loss of 4/5 cultures.

Nutrient medium GD3 was tested on the basis of publication Vágner et al. (1998, 2000). In this case, this medium was used as maintenance medium for investigating of endogenous levels of plant growth hormones during early stages of somatic embryogenesis of Norway spruce (Vágner et al. 1998) and investigation of endogenous levels of IAA, ABA and cytokines during somatic and zygotic embryogenesis of Norway spruce (Vágner et al. 2000).

Maturation and germination

The maturation of somatic embryos was early identified as a key step in somatic embryogenesis, and one in which losses can be very high (Högberg unpublished data 2003). As the different genotypes (areas) even within a species differ in their culture requirements for growth of maturation shows Table 3. The higher level of ABA (23 and 38μM) induced better result of embryos per cm^2. On LP7 and LP8 media the average moves between 10 to 15 embryos/cm^2.

A common problem is, however, that all embryogenic cell lines contain embryos that can mature (Egertsdotter 1996). The less response for maturation was obtained for SM1 area (6.2 embryos/cm^2). The embryos to mature vary significantly among cell lines. Proembryogenic callus develop embryos on LP media (6 to 8) from area SM4 more often (the average – 11 embryos/cm^2) than from others areas. The best cell lines give hundreds of mature somatic embryos per gram tissue (Nörgaard et al. 1993). The optimal ABA treatment varies from 7 to 60 μM ABA for one to three months depending on cell line (Jalonen, Von Arnold 1991). Albrechtová et al. (unpublished data 2000) achieved final cotyledonary stage after 5 weeks of cultivation with ABA end 3.75% PEG (4000). However, a stimulatory effect of 7.5% PEG on somatic embryo maturation was found for 13 out of 17 genotypes of *Picea abies* (Bozhkov, Von Arnold 1998).

Table 2. Proliferation rates on media GD3 and LP (1 to 5) and three months survial after proliferation treatment

Medium	ID of clone	Early stage (mm^2)	Proliferation after eight weeks (mm^2)	Absolute increment (%)	# Survived/total	Survived of proliferation (%)
GD3	SM1/1-A	12.8	61.3	379.5	30/30	100.0
GD3	SM1/2-A	14.7	45.6	209.2	30/30	100.0
GD3	SM1/3-A	13.9	46.7	236.2	30/30	100.0
GD3	SM1/4-A	14.5	66.2	356.8	30/30	100.0
GD3	SM1/5-A	13.9	34.0	144.1	30/30	100.0
GD3	SM1/6-A	13.9	54.1	287.5	30/30	100.0
GD3	SM1/7-A	14.3	75.7	429.4	30/30	100.0
GD3	SM1/8-A	13.9	41.5	198.4	30/30	100.0
GD3	SM1/9-A	14.0	96.9	588.9	30/30	100.0
GD3	SM1/10-A	14.4	63.2	338.2	30/30	100.0
GD3	SM1/11-A	14.7	88.1	496.7	30/30	100.0
GD3	SM1/12-A	15.1	45.2	199.8	25/30	83.3
GD3	SM2/1-A	15.2	55.0	260.2	26/30	86.7
GD3	SM2/2-A	14.6	40.5	177.6	23/30	76.7
GD3	SM2/3-A	11.2	18.8	66.9	9/18	50.0
GD3	SM2/4-A	15.3	52.6	243.3	25/30	83.3
GD3	SM2/5-A	11.2	18.1	61.3	7/18	38.9
GD3	SM2/6-A	11.3	19.0	68.9	8/20	40.0
GD3	SM2/7-A	14.7	62.8	326.8	28/30	93.3
GD3	SM2/8-A	10.6	17.4	63.1	6/15	40.0
GD3	SM2/9-A	15.0	55.5	268.7	26/30	86.7
GD3	SM2/10-A	14.7	60.5	310.9	25/30	83.3
GD3	SM3/1-A	10.3	16.7	61.3	6/18	33.3
GD3	SM3/2-A	14.7	72.7	393.8	29/30	96.7
GD3	SM3/3-A	14.8	43.7	194.9	22/30	73.3
GD3	SM3/4-A	9.8	15.8	61.0	3/18	16.7
GD3	SM3/5-A	10.0	16.0	59.4	7/21	33.3
GD3	SM3/6-A	14.7	56.5	282.7	30/30	100.0
GD3	SM3/7-A	14.4	66.4	359.1	30/30	100.0
GD3	SM3/8-A	15.4	64.2	316.9	29/30	96.7
GD3	SM3/9-A	14.4	46.2	220.7	25/30	83.3
GD3	SM3/10-A	14.8	67.1	352.9	29/30	96.7
GD3	SM4/1-A	14.8	70.2	374.3	30/30	100.0
GD3	SM4/2-A	14.3	60.8	324.8	28/30	93.3
GD3	SM4/3-A	14.9	35.6	137.8	19/30	63.3
GD3	SM4/4-A	14.8	48.5	227.5	21/30	70.0
GD3	SM4/5-A	10.4	17.2	64.1	5/19	26.3
GD3	SM4/6-A	10.4	17.4	67.3	8/26	30.8
GD3	SM4/7-A	15.0	75.3	402.5	30/30	100.0
GD3	SM4/8-A	14.7	76.4	417.3	30/30	100.0
GD3	SM4/9-A	14.6	36.3	147.9	21/30	70.0
GD3	SM4/10-A	10.4	17.1	64.7	9/30	30.0
LP1	SM1/1-B	7.7	60.5	685.7	10/10	100.0
LP1	SM1/2-B	8.2	95.5	1,064.6	10/10	100.0
LP1	SM1/3-B	8.3	54.7	559.0	10/10	100.0
LP1	SM2/1-B	8.3	84.8	921.7	10/10	100.0

Table 2 to be continued

Medium	ID of clone	Early stage (mm^2)	Proliferation after eight weeks (mm^2)	Absolute increment (%)	# Survived/total	Survived of proliferation (%)
LP1	SM2/2-B	8.2	78.1	852.4	10/10	100.0
LP1	SM2/3-B	8.8	98.2	1,015.9	10/10	100.0
LP1	SM3/1-B	8.1	67.6	734.6	10/10	100.0
LP1	SM3/2-B	8.8	171.1	1,844.3	10/10	100.0
LP1	SM3/3-B	8.4	81.0	864.3	10/10	100.0
LP1	SM4/1-B	9.2	148.9	1,518.5	10/10	100.0
LP1	SM4/2-B	8.1	152.9	1,787.7	10/10	100.0
LP1	SM4/3-B	9.4	186.4	1,883.0	10/10	100.0
LP3	SM1/1-B	8.1	52.2	544.4	9/10	90.0
LP3	SM1/2-B	8.0	66.4	730.0	9/10	90.0
LP3	SM1/3-B	7.9	72.7	820.3	10/10	100.0
LP3	SM2/1-B	8.3	89.6	979.5	10/10	100.0
LP3	SM2/2-B	8.4	88.9	958.3	9/10	90.0
LP3	SM2/3-B	8.5	107.2	1,161.2	10/10	100.0
LP3	SM3/1-B	8.3	65.3	686.7	8/10	80.0
LP3	SM3/2-B	9.1	116.4	1,179.1	10/10	100.0
LP3	SM3/3-B	11.1	122.3	1,001.8	9/10	90.0
LP3	SM4/1-B	9.4	160.3	1,605.3	10/10	100.0
LP3	SM4/2-B	9.5	161.1	1,595.8	10/10	100.0
LP3	SM4/3-B	9.6	182.7	1,803.1	10/10	100.0
LP4	SM1/1-B	8.3	149.8	1,704.8	10/10	100.0
LP4	SM1/2-B	7.8	119.5	1,432.1	10/10	100.0
LP4	SM1/3-B	7.6	109.8	1,344.7	9/10	90.0
LP4	SM2/1-B	8.6	98.2	1,041.9	10/10	100.0
LP4	SM2/2-B	8.5	98.9	1,063.5	9/10	90.0
LP4	SM2/3-B	8.3	111.6	1,244.6	10/10	100.0
LP4	SM3/1-B	9.3	181.7	1,853.8	10/10	100.0
LP4	SM3/2-B	9.4	204.7	2,077.7	10/10	100.0
LP4	SM3/3-B	8.7	188.4	2,065.5	10/10	100.0
LP4	SM4/1-B	10.4	191.1	1,737.5	10/10	100.0
LP4	SM4/2-B	10.3	203.8	1,878.6	10/10	100.0
LP4	SM4/3-B	9.5	215	2,163.2	10/10	100.0
LP5	SM1/1-B	8.1	117.2	1,346.9	10/10	100.0
LP5	SM1/2-B	8.0	76.2	852.5	8/10	80.0
LP5	SM1/2-B	8.2	71.9	776.8	9/10	90.0
LP5	SM2/1-B	8.4	97.1	1,056.0	10/10	100.0
LP5	SM2/2-B	8.4	83.6	895.2	8/10	80.0
LP5	SM2/3-B	8.3	88.9	971.1	9/10	90.0
LP5	SM3/1-B	8.4	166.6	1,883.3	10/10	100.0
LP5	SM3/2-B	9.3	137.8	1,381.7	9/10	90.0
LP5	SM3/3-B	7.7	125.7	1,532.5	9/10	90.0
LP5	SM4/1-B	7.8	186.8	2,294.9	10/10	100.0
LP5	SM4/2-B	8.2	150.7	1,737.8	9/10	90.0
LP5	SM4/3-B	9.1	153.1	1,582.4	10/10	100.0
Overall totals		10.97	88.15	810.66	1,412/1,643	85.94

Table 3. Maturation and desiccation rates on medium GD5 and LP (6 to 8)

Medium	ID of clone	Embryo induction/total size	Embryo inducted (cm^2)	Standard deviation
GD5	SM1/1-A	314/30	11.6	4.0
GD5	SM1/2-A	236/30	8.7	3.3
GD5	SM1/3-A	276/30	10.2	3.7
GD5	SM1/4-A	181/30	6.7	2.9
GD5	SM1/5-A	158/30	5.9	2.3
GD5	SM1/6-A	110/30	4.1	2.5
GD5	SM1/7-A	383/30	14.2	4.4
GD5	SM1/8-A	50/30	1.9	2.7
GD5	SM1/9-A	39/30	1.4	2.7
GD5	SM1/10-A	103/30	3.8	2.4
GD5	SM2/1-A	310/30	11.5	4.1
GD5	SM2/2-A	79/30	2.9	2.4
GD5	SM2/4-A	118/30	4.4	2.5
GD5	SM2/7-A	322/30	11.9	4.2
GD5	SM2/9-A	71/30	2.6	2.6
GD5	SM2/10-A	137/30	5.1	2.5
GD5	SM3/2-A	134/30	15.5	5.7
GD5	SM3/3-A	267/30	9.9	3.8
GD5	SM3/6-A	92/30	3.4	2.3
GD5	SM3/7-A	116/30	4.3	2.1
GD5	SM3/8-A	29/30	1.1	2.7
GD5	SM3/9-A	316/30	11.7	4.3
GD5	SM3/10-A	328/30	12.2	4.1
GD5	SM4/2-A	286/30	10.6	4.2
GD5	SM4/4-A	64/30	2.4	2.8
GD5	SM4/7-A	39/30	1.4	2.6
GD5	SM4/8-A	69/30	2.6	2.3
GD5	SM4/9-A	141/30	5.2	2.3
LP6	SM4/1-B	23/5	4.6	1.4
LP6	SM4/2-B	48/5	9.6	2.4
LP6	SM4/3-B	47/5	9.4	2.2
LP6	SM4/4-B	43/5	8.6	2.0
LP6	SM4/5-B	26/5	5.2	2.0
LP7	SM4/1-B	92/5	18.4	3.3
LP7	SM4/2-B	63/5	12.6	2.2
LP7	SM4/3-B	126/5	25.2	4.1
LP7	SM4/4-B	57/5	11.4	2.4
LP7	SM4/5-B	41/5	8.2	1.7
LP8	SM4/1-B	52/5	10.4	1.9
LP8	SM4/2-B	56/5	11.2	2.3
LP8	SM4/3-B	69/5	13.8	1.6
LP8	SM4/4-B	42/5	8.4	1.0
LP8	SM4/5-B	34/5	6.8	1.6
Overall totals		5,587/915	8.2	5.0

Table 4. Germination rates on medium GD6 and LP (9 to 11)

Clon		Without desiccation treatment		Desiccation treatment			
Medium-area/ID of clone		#c germination/total	germination (%)	#c germination/total	germination (%)	# survived/total	survived desiccation (%)
GD6	SM1/1-A	14/120	11.67	21/120	17.50	105/120	87.50
GD6	SM1/2-A	9/120	7.50	28/120	23.30	86/120	71.67
GD6	SM1/3-A	26/120	21.67	38/120	31.70	98/120	81.67
GD6	SM1/4-A	20/120	16.67	37/120	30.80	112/120	93.33
GD6	SM1/5-A	16/120	13.33	21/120	17.50	97/120	80.83
GD6	SM1/6-A	10/120	8.33	11/120	9.20	87/120	72.50
GD6	SM1/7-A	30/120	25.00	55/120	45.80	88/120	73.33
GD6	SM1/8-A	1/32	3.13	3/28	10.70	21/28	75.00
GD6	SM1/9-A	0/24	0.00	2/27	7.40	18/27	66.67
GD6	SM1/10-A	19/120	15.83	40/120	33.30	113/120	94.17
GD6	SM2/1-A	4/120	3.33	16/120	13.30	106/120	88.33
GD6	SM2/2-A	16/120	13.33	23/120	19.20	110/120	91.67
GD6	SM2/4-A	18/120	15.00	17/120	14.20	73/120	60.83
GD6	SM2/7-A	10/120	8.33	23/120	19.20	85/120	70.83
GD6	SM2/9-A	4/43	9.30	3/38	7.90	33/38	86.84
GD6	SM2/10-A	10/120	8.33	19/120	15.80	91/120	75.83
GD6	SM3/2-A	16/120	13.33	31/120	25.80	89/120	74.17
GD6	SM3/3-A	17/120	14.17	23/120	19.20	84/120	70.00
GD6	SM3/6-A	8/120	6.67	24/120	20.00	97/120	80.83
GD6	SM3/7-A	10/120	8.33	23/120	19.20	102/120	85.00
GD6	SM3/8-A	0/27	0.00	2/31	6.50	24/31	77.42
GD6	SM3/9-A	16/120	13.33	25/120	20.80	83/120	69.17
GD6	SM3/10-A	26/120	21.67	49/120	40.80	108/120	90.00
GD6	SM4/2-A	7/120	5.83	16/120	13.30	75/120	62.50
GD6	SM4/4-A	10/120	8.33	19/120	15.80	80/120	66.67
GD6	SM4/7-A	1/25	4.00	2/26	7.70	22/26	84.62
GD6	SM4/8-A	0/34	0.00	0/31	0.00	21/31	67.74
GD6	SM4/9-A	14/120	11.67	28/120	23.30	93/120	77.50

Table 4 to be continued

Clon		Without desiccation treatment		Desiccation treatment			
Medium-area/ID of clone		#c germination/total	germination (%)	#c germination/total	germination (%)	# survived/total	survived desiccation (%)
LP9	SM4/1-B	2/42	4.76	5/38	13.20	30/38	78.95
LP9	SM4/2-B	1/26	3.85	6/25	24.00	21/25	84.00
LP9	SM4/3-B	3/53	5.66	11/45	24.40	38/45	84.44
LP9	SM4/4-B	2/24	8.33	7/27	25.90	20/27	74.07
LP9	SM4/5-B	0/18	0.00	3/20	15.00	16/20	80.00
LP10	SM4/1-B	3/37	8.11	7/33	21.20	24/33	72.73
LP10	SM4/2-B	3/24	12.50	8/23	34.80	20/23	86.96
LP10	SM4/3-B	5/42	11.90	15/47	31.90	41/47	87.23
LP10	SM4/4-B	3/21	14.29	9/26	34.60	22/26	84.62
LP10	SM4/5-B	1/17	5.88	5/18	27.80	14/18	77.78
LP11	SM4/1-B	1/35	2.86	4/35	11.40	28/35	80.00
LP11	SM4/2-B	0/20	0.00	4/21	19.00	18/21	85.71
LP11	SM4/3-B	2/46	4.35	7/44	15.90	37/44	84.09
LP11	SM4/4-B	1/21	4.76	5/28	17.90	22/28	78.57
LP11	SM4/5-B	0/19	0.00	3/22	13.60	15/22	68.18
Overall totals		359/3,270	10.98	698/3,273	21.33	2,567/3,273	78.40

It has been shown that embryo production from spruce cultures, and subsequent embryo maturation, is enhanced by incorporating ABA into the medium (Becwar et al. 1987; Von Arnold, Hakman 1988; Dunstan et al. 1988).

Konrádová et al. (2002) used for investigation of maturation process GD media.

Roberts et al. (1990) first observe that partial drying of interior spruce somatic embryos at high (> 95%) relative humidity (RH) increased germination frequencies, decreased germination times and improved the synchrony of root and shoot elongation compared to untreated somatic embryos.

Distinct differences in embryo yield and appearance among the variants were found after transfer to germinated media from HRH-treatment and without (Table 4). The lowest difference between HRH treatment records and without is ten percent in the case of evaluation of different media. Previous studies have shown very good results for germination of Interior spruce (Webster et al. 1990). The germination frequency (after 1 week) of the 71 genotypes ranged from 16 to 100%, 48 of the genotypes had frequencies greater than 40% and 12 genotypes exhibited 80 to 100% germination (Webster et al. 1990).

Test with single somatic embryo grown on germination medium showed a strong effect of activated carbon (AC) (Table 4). AC improved initiation in radiata pine and embryo development in Douglas-fir (Pullman, Gupta 1991). However, utilization of somatic embryogenesis for clonal propagation of conifers has been limited by the low frequency of embryo germination and the low yield of acclimatized propagules (Webster et al. 1990).

Plantlets developed from somatic embryos were transplanted into potting mixture and grown under continuous light and high relative

Table 5. Used media and conditions

Growth phase	Medium plant grow regulators (supplements)	Condition	Time (weeks)
Initiation	LP, GD	darkness	4–8
	LP1: 2,4D 10μM, BA 5μM		
	LP2: 2,4D 5μM, BA 5μM		
	LP3: 2,4D 10μM, BA 5μM, NAA 10μM		
	LP4: 2,4D 10μM, BA 5μM, kinetin 5μM		
	LP5: BA 5μM, kinetin 5μM		
	GD1: BA 2μM, kinetin 2μM		
	GD2: BA 4μM, kinetin 4μM		
	GD3: 2,4D 5μM, BA 2μM, kinetin 2μM		
	GD4: 2,4D 10μM, BA 4μM, kinetin 4μM		
	30μM sucrose, 0.54% Difco agar	22–25°C	
	pH 5.7–5.8		
Proliferation	LP, GD	darkness	2, 4, 6, 8
	LP1: 2,4D 10μM, BA 5μM		
	LP3: 2,4D 10μM, BA 5μM, NAA 10μM		
	LP4: 2,4D 10μM, BA 5μM, kinetin 5μM		
	LP5: BA 5μM, kinetin 5μM		
	GD3: 2,4D 5μM, BA 2μM, kinetin 2μM		
	30μM sucrose, 0.54% Difco agar	22–25°C	
	pH 5.7–5.8		
Maturation	LP, GD	darkness	8
	LP6: ABA 7.5μM, PEG 4000 (2%)		
	LP7: ABA 20μM, PEG 4000 (2%)		
	LP8: ABA 38μM, PEG 4000 (2%)		
	GD5: ABA 20μM, PEG 4000 (2%)		
	30μM sucrose, 0.54% Difco agar	22–25°C	
	pH 5.7–5.8		
Germination	LP, GD	desiccation	3
	LP9: without plant grow regulators		
	LP10: active carbon 3 g/l	(darkness 17 ± 1°C)	
	LP11: IBA 2μM		
	GD6: active carbon 4 g/l	x	
	30μM sucrose, 0.54% Difco agar	16 hr photoperiod	3
	pH 5.7–5.8	22–25°C	

humidity for 10 weeks. The results from this experiment were not evaluated yet.

CONCLUSIONS

Somatic embryogenesis was obtained many years ago for a few hardwood species and for many more only in recent years. For conifers, in particular, it is a relatively recent development. Somatic embryogenesis has many advantages over organogenesis. Since somatic embryos have both a shoot and root meristem, a separate rooting step is not required. Somatic embryogenesis generally forms propagules faster and in much larger numbers per explant than organogenesis. Furthermore, it requires less handling and is easier to automate, this saving labour costs. For combined breeding-cloning strategies, somatic embryogenesis offers the advantage that embryogenic cultures are easy to cryopreserve, thereby allowing preservation of clones in a juvenile state during long-term field testing. In addition, somatic embryos can, theoretically, be used for ar-

Table 6. Collected commercial seed lot origin

Code of area	Area	m a.s.l.	Year of collection	Altitudinal vegetation zone	Seed quality (%)
SM1	Brdská vrchovina	700	2002	5	83
SM2	Předhoří Šumavy + Novohradské hory	600	1999	5	75
SM3	Východosudetská oblast	810	2000	6	63
SM4	Českomoravská vrchovina	450	1999	5	80

tificial seed or fluid drilling planting. The performance of germinated somatic embryos (emblings) has generally not been field tested for more than a few years, particularly in the case of conifers. However, in most of these short-term field tests, no genetic or epigenetic abnormalities have yet been observed, i.e., the rate of somaclonal variation appears to be low. A RAPD analysis of populations derived from somatic embryos of *Picea mariana* showed no variation within clones. In comparison to seedlings, somatic embryos may show an initial lag in growth due to an acclimatization requirement but subsequently they behave like seedlings.

There is demonstrated that the initiation of somatic embryogenesis is under strong additive genetic control, with variance due to general combining ability accounting for 42% of the total phenotypic (Kobliha 2002).

In past years, the successful plant regeneration via somatic embryogenesis demonstrated promising prospects for the method in mass clonal propagation or reforestation programmes (see Von Arnold 1987; Chalupa 1989, 1997; Attree, Fowke 1993; Westcott 1994; Tzfira et al. 1998; etc.). For somatic embryogenesis technology to become commercially successful it has to be integrated with breeding programs and has to be successful with a variety of genotypes (Pullman, Johnson 2002).

Somatic embryogenesis from immature zygotic embryos is more efficient, but not so effective for commercial practise. In this study we used mature zygotic embryos and others conditions like in operational laboratory. The goal of this study was to discover if somatic embryogenesis could be used to provide planting stock for forestry in operational laboratory.

Some protocols, which we used in our experiments, were published previously, some media and protocols we modified and tried for the first time. Our approach was to study natural embryo development and changes in medium over time. We expected that well developed protocols would give a great deal of development embryogenic cultures and following plant regeneration. The ratio of survival embryogenic explants after initiation and proliferation stadium (six months treatment) was 25.1% from starting 4,300 zygotic embryos. After two more stages (maturation and desiccation treatment) survived 19.68% of embryos. About 5.35% from the original 4,320 seeds proceeded to the last stage (germination) of somatic embryogenesis. Experiments with the cultivation of Norway spruce indicated, that this techniques is not such effective for randomly selected seeds as we expected.

Somatic embryo plants can be produced from a limited number of genotypes only, when using a standard protocol (Von Arnold et al. 1995). To work most effectively with the breeding program Timmis (1998) concluded that somatic embryogenic technology needs to increase the efficiency of embryogenic tissue establishment. However, before the methods are applied it is important that the plants regenerated via somatic embryogenesis grow as expected, i.e. as seedlings or cuttings (Högberg et al. 2001).

There is large-scale commercial propagation via somatic embryogenesis for some conifers, e.g. *Pinus taeda* (Tang et al. 2001; Chowdhury et al. 2004; Attree 2004) and some well developed protocols, e.g. *Pinus radiata* (Schestibratov et al. 2003; Prehn et al. 2003).

Somatic embryogenesis of conifers, especially some spruces, has been extensively utilized as a model system for investigating many aspects of the embryogenic process as a whole. In recent years, in fact, there have been several studies dealing with developmental, physiological, and biochemical events occurring during the *in vitro* embryo development of coniferous embryos (see reviews by Tautorus et al. 1991; Park et al. 1993; Durzan 1996; Häggman et al. 1997; Godbold, Jentschke 1998; Vágner et al. 1998; Dyachok et al. 2000, 2002; Filonova et al.

2000; Konrádová et al. 2002; Stasolla et al. 2002, 2003; Überall et al. 2004). These studies used for investigation special clones, which are resistant or more sensitive to laboratory conditions. As reported Roberts et al. (1989), in fact, different tissue types within the same plant or the same tissue at various stages of development produce different responses to *in vitro* culture conditions.

References

ATTREE S.M., FOWKE L.C., 1993. Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. Plant Cell, Tissue and Organ Culture, *35*: 1–35.

ATTREE S.M., 2004. Developing a Commercial Somatic Embryogenesis Production Platform for Conifers. 2004 IUFRO Joint Conference of Division 2, Forest Genetic and Tree Breeding in the Age of Genomics: Progress and Future. November 1–5, 2004, Double Tree Guest Suites, Charleston, South Carolina.

BECWAR M.R., NOLAND T.L., WANN S.R., 1987. A method for quantification of the level of somatic embryogenesis among Norway spruce callus lines. Plant Cell Reports, *6*: 35–38.

BOULAY M.P., GUPTA P.K., KROGSTRUP P., DURZAN J.D., 1988. Development of somatic embryos from cell suspension cultures of Norway spruce (*Picea abies* Karst.). Plant Cell Reports, *7*: 134–137.

BOZHKOV P.V., FILONOVA L.H., VON ARNOLD S., 2002. A key developmental switch during Norway spruce somatic embryogenesis is induced by withdrawal of growth regulators and is associated with cell death and extracellular acidification. Biotechnology and Bioengineering, *77*: 658–667.

BOZHKOV P. V., VON ARNOLD S., 1998. Polyethylene glycol promotes maturation but inhibits further development of *Picea abies* somatic embryos. Physiologia Plantarum, *104*: 221–224.

DYACHOK J.V., TOBIN A.E., PRICE N.P.J., VON ARNOLD S., 2000. Rhizobial Nod factors stimulate somatic embryo development in *Picea abies*. Plant Cell Reports, *19*: 290–297.

DYACHOK J., WIWEGER M., KENNE L., VON ARNOLD S., 2002. Endogenous Nod-factor-like signal molecules suppress cell death and promote early somatic embryo development in Norway spruce. Plant Physiology, *128*: 523–533.

DUNSTAN D.I., BEKKAOUI F., PILON M., FOWKE L.C., ABRAMS S.R., 1988. Effects of ABA andanalogues on the maturation of white spruce (*Picea glauca*) somatic embryos. Plant Science, *58*: 77–84.

DUNSTAN D.I., DONG J.Z., CARRIER D.J., ABRAMS S.R., 1998. Events following ABA treatment of spruce somatic embryo. *In vitro* Cellular and Developmental Biology – Plant, *34*: 159–168.

DURZAN D.J., 1996. Asexual reproductive adaptation to simulated cretaceous climatic variables by Norway spruce cells *in vitro*. Chemosphere, *33*: 1655–1673.

EGERTSDOTTER U., 1996. Regulation of somatic embryo development in Norway spruce (*Picea abies*). Agronomie-Paris, *16*: 603–608.

FILONOVA L., BOZKOV P., VON ARNOLD S., 2000. Developmental pathway of somatic embryogenesis in *Picea abies* as revealed by time-lapse tracking. Journal of Experimental Botany, *51*: 343–249.

GODBOLD D.L., JENTSCHKE G., 1998. Aluminium accumulation in root cell walls coincides with inhibition of root growth but not with inhibition of magnesium uptake in Norway spruce. Physiologia Plantarum, *102*: 553–560.

GUPTA P.K., DURZAN D.J., 1986. Plant et regeneration via somatic embryogenesis from subcultured callus of mature embryos of *Picea abies* (Norway spruce). *In vitro* Cellular and Developmental Biology, *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.

HAKMAN I., VON ARNOLD S., 1985. Plantlet regeneration through somatic embryogenesis in *Picea abies* (Norway spruce). Journal of Plant Physiology, *121*: 149–158.

HÄGGMAN H.M., TUIJA S.A., NIKKANEN T.O., 1997. Gene transfer by particle bombardment to Norway spruce and Scots pine pollen. Canadian Journal of Forest Research, *27*: 928–935.

HÖGBERG K.A., BOZHKOV P.V., GNONNOOS R., ANXOLO S.V., 2001. Critical factors affecting *ex vitro* performance of somatic embryo plants of *Picea abies*. Scandinavian Journal of Forest Research, *16*: 295–304.

CHALUPA V., 1985. Somatic embryogenesis and plantlet regeneration from cultured immature and mature embryos of *Picea abies* /L./ Karst. Communicationes Instituti Forestalis Czechosloveniae, *14*: 57–63.

CHALUPA V., 1989. Plant regeneration by somatic embryogenesis in Norway spruce (*Picea abies* /L./ Karst.) and sessile oak (*Quercus petraea* /Matt./ Liebl.). Communicationes Instituti Forestalis Czechosloveniae, *16*: 135–143.

CHALUPA V., 1997. Somatic embryogenesis and plant regeneration in Norway spruce (*Picea abies* /L./ Karst.). Acta Universitatis Carolinae Biologica, *41*: 13–22.

CHOWDHURY M.K., BECWAR M.R., NEHRA N.S., COOK M.J., CLARK J.J., VICTOR J.M., STOUT T.J., EADIE L.M., SAGE J.S., 2004. Improvements in Pine Somatic Embryogenesis Tissue Multiplication, Embryo Production and Embryo Harvest for Large-Scale Production and Deployment. In: 2004 IUFRO Joint Conference of Division 2, Forest Genetic and Tree Breeding in the Age of Genomics: Progress and Future. November 1–5, 2004, Double Tree Guest Suites, Charleston, South Carolina.

JAIN S.M., NEWTON R.J., SOLTES E.J., 1988. Enhancement of somatic embryogenesis in Norway spruce (*Picea abies* L.). Theoretical and Applied Genetics, *76*: 501–506.

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.

KOBLIHA J., 2002. Modern forest tree breeding in the light of foreign experiences. [Study text.] Prague, Czech University of Agriculture in Prague: 195.

KONRÁDOVÁ H., LIPAVSKÁ H., ALBRECHTOVÁ J., VREUGDENHIL D., 2002. Sucrose metabolism during somatic and zygotic embryogeneses in Norway spruce: content of soluble saccharides and localisation of key enzyme activities. Journal of Plant Physiology, *159*: 387–396.

KROGSTRUP P., 1986. Embryo like structures from cotyledons and ripe embryos of Norway spruce (*Picea abies*). Canadian Journal of Forest Research, *16*: 664–668.

KVAALEN H., VON ARNOLD S., 1991. Effects of various partial pressures of oxygen and carbon dioxide on different stages of somatic embryogenesis in *Picea abies*. Plant Cell, Tissue and Organ Culture, *27*: 49–57.

NÖRGAARD J.V., DURAN V., JOHNSON O., KROGSTRUP P., BALDURSSON A., VON ARNOLD S., 1993. Variations in cryotolerance of embryogenic *Picea abies* cell lines and the association to genetic, morphological and physiological factors. Canadian Journal of Forest Research, *23*: 2560–2567.

PARK Y.S., POND S.E., BONGA J.M., 1993. Initiaton of somatic embryogenesis in white spruce (*Picea glauca*): genetic control, culture treatment effects, and implications for tree breeding. Theoretical and Applied Genetics, *86*: 427–436.

PREHN D., SERRANO C., MERCADO A., STAGE C., BARRALES L., ARCE-JOHNSON P., 2003. Regeneration of whole plants from apical meristems of *Pinus radiata*. Plant Cell, Tissue and Organ Culture, *73*: 91–94.

PULLMAN G.S., JOHNSON S., 2002. Somatic embryogenesis in loblolly pine (*Pinus taeda* L.): improving culture initiation rates. Annals of Forest Science, *59*: 663–668.

PULLMAN G.S., GUPTA P.K., 1991. Method for reproducing coniferous plants by somatic embryogenesis using absorbent materials in the development stage media. U.S. Patent No. 5,034,326.

ROBERTS D.R., FLINN B.S., WEBB D.T., WEBSTER F.B., SUTTON B.C.S., 1989. Characterization of immature embryos of interior spruce by SDS-PAGE and microscopy in relation to their competence for somatic embryogenesis. Plant Cell Reports, *8*: 285–288.

ROBERTS D.R., SUTTON B.C.S., FLINN B.S., 1990. Synchronous and high frequency germination of interior spruce somatic embryos following partial drying at high relative humidity. Canadian Journal of Botany, *68*: 1086–1090.

ROBERTS D.R., WEBSTER F.B., FLINN B.S., LAZAROFF W.R., CYR D.R., 1993. Somatic embryogenesis of spruce. In: REDENBAUGH K. (ed.), SynSeeds: Application of Synthetic Seeds to Crop Improvement. Boca Raton, CRC Press: 427–452.

SCHESTIBRATOV K.A., MIKHAILOV R.V., DOLGOV S.V., 2003. Plantlet regeneration from subculturable nodular callus of *Pinus radiata*. Plant Cell, Tissue and Organ Culture, *72*: 139–146.

STASOLLA C., KONG L., YEUNG E.C., THORPE T.A., 2002. Maturation of somatic embryos in conifers: morphogenesis, physiology, biochemistry, and molecular biology. *In Vitro* Cellular and Developmental Biology, *38*: 93–105.

STASOLLA C., YEUNG E.C., 2003. Recent advances in conifer somatic embryogenesis: improving somatic embryo quality. Plant Cell, Tissue and Organ Culture, *74*: 15–35.

TANG W., WHETTEN R., SEDEROFF R., 2001. Genotypic control of high-frequency adventitious shoot regeneration via somatic organogenesis in loblolly pine. Plant Science, *161*: 267–272.

TAUTORUS T.E., FOWKE L.C., DUNSTAN D.I., 1991. Somatic embryogenesis in conifers. Canadian Journal of Botany, *69*: 1873–1899.

TIMMIS R., 1998. Bioprocessing for tree production in the forest industry: conifer somatic embryogenesis. Biotechnology, *14*: 156–166.

TZFIRA T., ZUKER A., ALTMAN A., 1998. Forest-tree biotechnology: Genetic transformation and its application to future forests. Trends in Biotechnology, *16*: 439–446.

ÜBERALL I., VRÁNA J., BARTOŠ J., ŠMEJRA J., DOLEŽEL J., HAVEL L., 2004. Isolation of chromosomes from *Picea abies*. Biologia Plantarum, *48*: 199–203.

VÁGNER M., KUMSTÝŘOVÁ L., VONDRÁKOVÁ Z., EDER J., GÖSSLOVÁ M., SVOBODOVÁ H., ALBRECHTOVÁ J., MACHÁČKOVÁ I., CHALUPA V., 2000. Endogenous IAA and cytokinins in Norway spruce somatic and zygotic embryogenesis. In: Proceedings FESPP Congress, August 2000, Budapest, Hungary. Plant Physiology and Biochemistry, *38* (Supplementum), Abstract: S03–12.

VÁGNER M., VONDRÁKOVÁ Z., STRNADOVÁ Z., EDER J., MACHÁČKOVÁ I., 1998. Endogenous levels of plant growth hormones during early stages of somatic embryogenesis of *Picea abies*. Advances in Horticultural Science, *12*: 11–18.

VON ARNOLD S., EGERTSDOTTER U., EKBERG I., GUPTA P., MO L.H., NÖRGAARD J., 1995. Somatic embryogenesis in Norway spruce (*Picea abies*). In: JAIN S., GUPTA P., NEWTON R. (eds.), Somatic Embryogenesis in Woody Plants. Kluwer Academic Publishers, *3*: 17–36.

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

VON ARNOLD S., HAKMAN I., 1986. Effect of sucrose on initiation of embryogenic callus cultures from mature

zygotic embryos of *Picea abies* (L.) Karst. (Norway spruce). Journal of Plant Physiology, *122*: 261–265.

VON ARNOLD S., HAKMAN I., 1988. Regulation of somatic embryo development in *Picea abies* by abscisic acid (ABA). Journal of Plant Physiology, *132*: 164–169.

VON ARNOLD S., ERICSSON T., 1981. *In vitro* studies of adventitious shoot formation in *Pinus contorta*. Canadian Journal of Botany, *59*: 870–874.

WEBSTER F.D., ROBERTS D.R., MCLNNIS S.M., SUTTON B.C.S., 1990. Propagation of interior spruce by somatic embryogenesis. Canadian Journal of Forest Research, *20*: 1759–1765.

WESTCOTT R. J., 1994. Production of embryogenic callus from nonembryonic explants of Norway spruce *Picea abies* (L.) Karst. Plant Cell Reports, *14*: 47–49.

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Vliv diferencované úspěšnosti somatické embryogeneze smrku ztepilého

ABSTRAKT: Somatické embryogenní kultury byly založeny z proembryogenního suspenzorového pletiva (PEMs), získaného ze zralých semen smrku ztepilého. V práci bylo použito více než 4 300 semen *Picea abies* z různých lokalit (pocházejí z volného opylení). Většina studií je zaměřena na vybrané genotypy, u kterých je známa vyšší úspěšnost propagace. Mezi náhodně vybranými vzorky osiva smrku ztepilého existují významné rozdíly v úspěšnosti somatické embryogeneze. Média GD (1 až 4), LP (1 až 5) o různých koncentracích rostlinných regulátorů růstu (BA, NAA, kinetin a 2,4D) byla použita pro iniciaci a proliferaci embryogenních kultur. Přenesením embryogenního kalusu na médium s přídavkem kyseliny abscisové byl stimulován raný vývoj embryí. Média GD (5 a 6) a LP (9 až 11), obohacená o ABA (7,5; 20; 38 μM) a PEG 4000 (2%), byla použita pro stadium maturace. Konverze somatických embryí v rostliny byla stimulována částečně desikací a změnou média. Na těchto médiích došlo v průběhu tří týdnů k vytvoření rostlin.

Klíčová slova: smrk ztepilý; somatická embryogeneze; *Picea abies*; růstové regulátory

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