Acta Universitatis Latviensis, Biology, 2004, Vol. 676, pp. 257–260

Propagation of some *Abies* species by somatic embryogenesis

Božena Vooková, Andrej Kormuťák

Institute of Plant Genetics and Biotechnology, Slovak Academy of Science, Akademická 2, Nitra 950 07, Slovak Republic
*Corresponding author, E-mail: nrgrvook@savba.sk

Abstract

Somatic embryogenesis was used for propagation of *Abies cilicica*, *Abies numidica* and *Abies concolor*. The same experimental conditions were suitable for plantlets regeneration of all tested species. Initiation of embryogenic tissue was obtained from immature embryos on Schenk and Hildebrandt (SH) initiation medium with 1 mg l^{-1} benzylaminopurine and 20 g l^{-1} sucrose. The best maturation of somatic embryos was achieved on modified Murashige and Skoog medium supplemented with 40 g l^{-1} maltose, 100 g l^{-1} polyethylene glycol-4000, 10 mg l^{-1} abscisic acid and 500 mg l^{-1} L-glutamine and casein hydrolysate. After three weeks of partial drying, mature embryo germination occurred on medium containing ½-SH medium salts, 10 g l^{-1} sucrose, 10 g l^{-1} charcoal and 3 g l^{-1} Phytagel. Plantlets with cotyledons, hypocotyl and radicula were obtained and transferred to small pots containing autoclaved peat / vermiculite / perlite mixture. Plantlet regeneration of other *Abies* species can be achieved under the same experimental conditions, but with optimizing for individual species to affect the yield of regenerants.

Key words: *Abies*, embryogenic tissue, firs, induction, germination, maturation.

Introduction

Somatic embryogenesis is a very convenient method for mass multiplication of conifers including of the genus *Abies*. The technology to initiate, maintain, and develop somatic embryos and emblings of conifer species via somatic embryogenesis is now well established. Somatic embryogenesis in *Abies* with limited success or succesfull regeneration has been reported for some species (for review see Vooková, Kormuťák 2001). *A. cilicica* Carr. is a fast growing species whose natural distribution is in Asia Minor (Bozkus 1987). *A. numidica* De Lann. is a North African species, and the *A. concolor* (Gord. et Glend) distribution is North America (Liu 1971).

The objective of the present study was to apply somatic embryogenesis technology and try to generalize micropropagation conditions of three *Abies* species growing in Arboretum Mlyňany, Slovakia.

Materials and methods

Cones containing immature seeds of *A. cilicica* Carr., *A. numidica* De Lann. and *A. concolor* (Gord. et Glend) was colected in June 8th, 1997 from the Arboretum Mlyňany,

Slovakia. Imature seeds were surface-sterilized for 10 min in 10 % H_2O_2. Megametophytes containing embryos were cultured on Schenk, Hildebrandt (1972; SH) medium with 1 mg l^{-1} 6-benzylaminopurine (BAP). Induced embryogenic tissue was maintained at 24 ºC in the dark on proliferation medium supplemented with 1 mg l^{-1} BAP, 1 g l^{-1} casein hydrolysate, 500 mg l^{-1} L-glutamine and 3 g l^{-1} Phytagel.

To assess the most beneficial medium for somatic embryo maturation, embryogenic tissue of one cell line per species was cultured on SH, Gresshoff, Doy (1972; GD) and modified Murashige and Skoog (1962; MS) media. SH and GD media contained the original macro, micro-elements, FeEDTA and vitamins; MS medium contained ½ strength MS macro elements, original micro elements and FeEDTA, and modified vitamins: 1 mg l^{-1} nicotinic acid, 1 mg l^{-1} thiamine HCl, 1 mg l^{-1} pyridoxin HCl, 2 mg l^{-1} glycine, 100 mg l^{-1} *myo*-inositol. All media contained 10 mg l^{-1} abscisic acid (ABA), 40 g l^{-1} maltose, 100 g l^{-1} polyethylene glycol, 500 mg l^{-1} casein hydrolysate and 500 mg l^{-1} L-glutamine. The maturation medium was gelled with 3 g l^{-1} Phytagel.

Pieces of embryogenic tissue with approximate weight of 350 mg were cultured in 60 mm plastic Petri dishes on maturation medium in the dark at 21 to 23 °C. Mature somatic embryos were subjected to partial desiccation during three weeks at 24 ºC in the dark (Vooková et al. 1997/1998). The embryos were allowed to germinate on SH medium containing ½-SH medium salts, 10 g l^{-1} sucrose, 10 g l^{-1} charcoal and 3 g l^{-1} Phytagel. Six replications of ten embryos were cultivated in Erlenmayer flask with 50 ml media per treatment. Germination percentages were evaluated after 40 d of cultivation. Plantlets with a root were transferred to small pots containing autoclaved peat / vermiculite / perlite mixture.

Results and discussion

Embryogenic tissue was induced in all of the studied species of *Abies*. After four to eight weeks of cultivation on SH induction medium with BAP, white, mucilaginous extrusions were observed (Fig. 1A). This embryogenic tissue consisted of single elongated highly vacuolated cells, clumps of small and densely cytoplasmic cells and somatic embryos in an early stage of development (Fig. 1B). The initiation percentage of embryogenic tissue is shown in the Table 1. We obtained 26 cell lines of *A. cilicica*, three lines of *A. numidica* and two cell lines of *A. concolor* with different growth characteristics. The same SH induction medium was suitable also for initiation of embryogenic tissue in some hybrids (Gajdošová et al. 1995). Unlike other genera in the *Pinaceae*, *Abies* requires only cytokinin for induction of embryogenic tissue from zygotic embryos (Salajová et al. 1996).

The duration of maturation treatment was eight to ten weeks. The cell lines differed in their response to the three maturation media. Somatic embryos reached the cotyledonary

Table 1. Percentage of embryogenic tissue from immature zygotic embryos of *Abies* species

Species	Number of explants	Initiation (%)	Number of cell lines
Abies cilicica	63	65.5	26
Abies numidica	44	6.8	3
Abies concolor	64	5.6	2

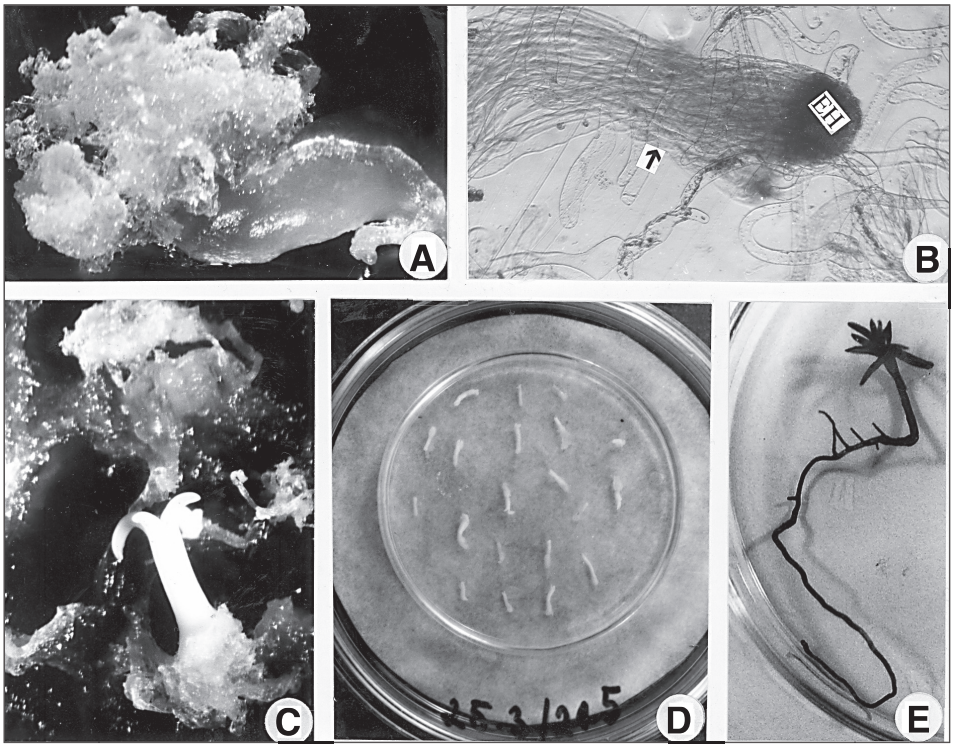

Fig. 1. Embryogenic tissue induction and plantlet regeneration of *Abies species*. A, Initiation of embryogenic tissue from immature seeds of *Abies cilicica*. B, Early somatic embryo of *Abies numidica* with embryonal head (EH) and long suspensor cells (arrow). C, Developing cotyledonary somatic embryo of *Abies concolor* after eight weeks on MS maturation medium with ABA. D, Partial desiccation of mature somatic embryos placed in Petri dishes (ø 60 mm). Petri dish was open and placed on moist filter paper in a Petri dish (ø 90 mm), which was sealed with parafilm. E, *Abies numidica* plantlet with developed root system and primary shoot five months after transfer into the soil.

stage of development on MS and SH medium (Fig. 1C). The number of mature cotyledonary embryos per g of embryogenic tissue was different in individual species (Table 2). The tendency for better maturation on MS medium was typical for all tested species. GD medium was not suitable because maturation was slow and only the precotyledonary stage of development was achieved.

Table 2. The number (± SE) of cotyledonary somatic embryos of *Abies* species (per g of embryogenic tissue) matured on Shenk and Hildebrandt (SH), Murashige and Skoog (MS) and Gresshoff and Doy (GD) media

Species	SH medium	MS medium	GD medium
Abies cilicica	6 ± 2	16 ± 2	0
Abies numidica	16 ± 5	26 ± 3	1 ± 1
Abies concolor	-	61 ± 8	0

Table 3. Germination of somatic embryos of *Abies* species on germination medium. Means ± SE, n = 6

Species	Embryos forming roots (%)
Abies cilicica	75.0 ± 6.8
Abies numidica	85.5 ± 4.1
Abies concolor	77.1 ± 5.2

After partial desiccation (Fig. 1D) mature embryos underwent germination on medium with charcoal. They developed into plantlets with green cotyledons, red hypocotyl and white radicula. A high rooting percentage was achieved for all tested species (Table 3). In previous work (Vooková, Kormuťák 2001) the efficacy of the same germination medium was tested also for some Abies hybrids and its general application was confirmed. Plantlets that had formed a radicle were transferred to a soil / peat / perlite mixture. During the seven-months-period after transfer to the soil some of them had survived (Fig. 1E).

Our results showed that the same experimental conditions were suitable for plantlet regeneration of the all tested species. It seems that plantlet regeneration of more *Abies* species can be achieved using the same experimental conditions, but probably optimizing for individual species can significantly affect the yield of regenerants.

Acknowledgements

The work was supported by the Slovak Grant Agency for Science, No. 2/7250/20.

References

Bozkus H.F. 1987. The natural distribution and silvicultural characteristics of *Abies cilicica* Carr. in Turkey. Yayjn No 660, Ser. No 60. L.U.Orman Fakultesi Silvikultur Anabilim Dalt, Ankara.

Gajdošová A., Vooková B., Kormuťák A., Libiaková G., Doležel J. 1995. Induction, protein composition and DNA ploidy level of embryogenic calli of silver fir and its hybrids. *Biol. Plant*. 37: 169–176.

Gresshoff P.M., Doy CH. 1972. Development and differentiation of haploid *Lycopersicum esculentum* (tomato). *Planta* 107: 161–170.

Liu T. S. 1971. *A Monograph of the Genus* Abies. National Taiwan University, Taipei, Taiwan, China. 608 p.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 15: 473–497.

Salajová T., Jásik J., Kormuťák A., Hakman I. 1996. Embryogenic culture initiation and somatic embryo development in hybrid firs (*Abies alba* × *Abies cephalonica* and *Abies alba* × *Abies numidica*). *Plant Cell Rep*. 15: 527–530.

Schenk R.U., Hildebrandt A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. For. Res*. 50: 199–204.

Vooková B., Gajdošová A., Matúšová R. 1997/1998. Somatic embryogenesis in *Abies alba* and *Abies alba* × *Abies nordmanniana* hybrids. *Biol. Plant*. 40: 523–530.

Vooková B., Kormuťák A. 2001. Effect of sucrose concentration, charcoal, and indole-3-butyric acid on germination of *Abies numidica* somatic embryos. *Biol. Plant*. 44: 181–184.