VPLYV KULTIVAČNÉHO MÉDIA NA INICIÁCIU EMBRYOGÉNNYCH PLETÍV A DOZRIEVANIE SOMATICKÝCH EMBRYÍ BOROVICE ČIERNEJ (*Pinus nigra* Arn.)
THE EFFECT OF CULTURE MEDIUM ON THE INITIATION OF EMBRYOGENIC TISSUES AND MATURATION OF *Pinus nigra* Arn. SOMATIC EMBRYOS

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ABSTRACT
The aim of our study was to choose appropriate culture media for initiation of somatic embryogenesis of *Pinus nigra* Arn. and characterization of somatic embryos maturation of different embryogenic cell lines. Embryogenic tissues were initiated from immature zygotic embryos of *Pinus nigra* Arn. in precotyledonary stage of development. Four tested basic culture media were: DCR, LV, MLV a QP, along with their modified variants with lowered contents of plant growth regulators. Experiments showed that the most suitable culture media for induction was DCR, which resulted in the highest percentage of induction. Microscopic observations revealed differences in somatic embryo structure among embryogenic cell lines. Bipolar structures were observed with well organized embryonal part (L14), clusters of meristematic cells without organised suspensor (E235) and less organised bipolar structures (L34). The culture media with 25 mg/L abscisic acid and 6 % of maltose was suitable for maturation of somatic embryos.
Keywords: initiation of somatic embryogenesis, maturation of somatic embryos, *Pinus nigra*, embryogenic cell line, culture medium

INTRODUCTION
Somatic embryogenesis is an asexual developmental process of somatic embryos from vegetative plant tissues. Regeneration of plants through of somatic embryogenesis was described in a huge number of conifers **(Jain et al., 1995, 1999)**. For the first time somatic embryogenesis was observed in *Picea abies* **(Hakman et al., 1985; Chalupa, 1985)**. This process can be divided into four steps: a) intitiation of embryogenic tissues, b) proliferation of embryogenic cultures, c) maturation of somatic embryos and d) plant regeneration from mature somatic embryos.
For initiation of embryogenic tissues different types of primar explants are used. In conifers the embryogenic tissues are produced mostly from juvenile explants. For species belonging to the genus *Pinus* initiation is occures mostly from immature zygotic embryos in precotyledonary developmental stage cultivated within female gametophyte **(Jones, van Staden, 1995; Klimaszewska, Smith, 1997; Salajová et al., 1999)**.
Embryogenic tissue has similar characteristics in all species of conifers – white colour, mucilaginous consistence and contains somatic embryos **(Hakman et al., 1985)** – bipolar structures characterised with embryonal part and suspensor. The embryonal part consists of meristematic cells joined with highly elongated vacuolated suspensor cells. Embryogenic cell lines can belong to three different groups based on morphology of somatic embryos, growth potential and secretion of proteins **(Laine and David, 1990; Bercethe and Paques, 1995; Keinonen-Mettälä et al., 1996; Mo et al., 1996)**. Embryogenic tissues which are part of the first group comprise well developed somatic embryos with high maturation potential. Cell lines of the second group contain relative undeveloped somatic embryos. The suspensor cells are usually not organised into bundles and the embryonal part is less compact **(von Arnold et al., 1996)**. To the third group belong embryogenic cell lines with almost zero capacity of maturation. These lines are composed of clusters of meristematic cells, elongated vacuolated cells and bipolar structures are rarely present **(Salajová and Salaj, 2005)**.
Abscisic acid (ABA) is used as a stimulator of maturation in conifers. Other components of media, for example maltose, which contributes to maturation of somatic embryos, are important, too.

The main goal of our study was to initiate embryogenic tissue on different culture media and to select a suitable culture medium, characterization of chosen embryogenic cell lines and maturation of somatic embryos of selected embryogenic cell lines.

MATERIAL AND METHODS

Unripe zygotic embryos of *Pinus nigra* Arn. enclosed in megagametophytes were used as primary explants for initiation of somatic embryogenesis. Unmatured seeds were collected in locality of Drážovce. We used four basal media for initiation of somatic embryogenesis: DCR **(Gupta nad Durzan, 1985)**, LV **(Litvay et al., 1981)**, MLV – modified LV a QP **(Quoirin and Lepoivre, 1977)** supplemented with 2,4-D (2 $mg.dm^{-3}$) and BAP (0.5 $mg.dm^{-3}$). Variants with reduced content of growth regulators (DCR-R, LV-R, MLV-R and QP-R) contained 2,4-D (0.5 $mg.dm^{-3}$) and BAP (0.5 $mg.dm^{-3}$). For maturation of somatic embryos we used two types of basal DCR medium supplemented with abscisic acid (25mg/L). Sucrose was substituted by maltose (6 % and 9 %) and the content of gerlite was also different (4 $g.dm^{-3}$ and 10 $g.dm^{-3}$).

For cytological characterization of chosen embryogenic cell lines we used staining method with 2 % acetocarmine.

RESULTS AND DISCUSSION

Zygotic embryos cultivated within megagametophytes started to produce embryogenic tissue around the 21st day after isolation. When the tissue reached approximately 5 mm, we isolated it from the primary explants and cultivated separately – we established embryogenic cell lines. Production of embryogenic tissue was observed from the micropylar end of megagametophyte.

We compared influence of four basal media and their variants with reduced content of growth regulators for induction of embryogenic tissues of *Pinus nigra* Arn. The results showed us, that unsuitable media were LV, LV-R, QP and QP-R, on which we noticed almost zero percent of initiation. DCR, MLV, MLV-R belonged to the suitable media for initiation of embryogenic tissue. In case of these media the percentage of initiation reached relatively higher values (9.56 %, 7.35 % and 8.82 %). According to our results the most suitable initiation medium was DCR-R, on which the percentage of initiation reached the highest value – 11.03 %.

Microscopic observations revealed differences in micromorphology of somatic embryos among particular cell lines. We monitored the structure of somatic embryos in 30 cell lines. The micromorphology of somatic embryos was very variable. We observed bipolar structures with well organised embryonal part (Fig. 1) represented by line L14. This cell line belongs into the first group of embryogenic cell lines with the high maturation capacity. Clusters of meristematic cells without organised suspensor (Fig. 2) were observed in cell line E235, which belongs to the third group of embryogenic lines with almost zero maturation capacity. Cell line L34 contains less organised bipolar structures, which represent cell lines of the second group with lowered maturation capacity (Fig. 3). We often observed somatic embryos linked with embryonal part, which are probably result of cleavage (Fig. 4).

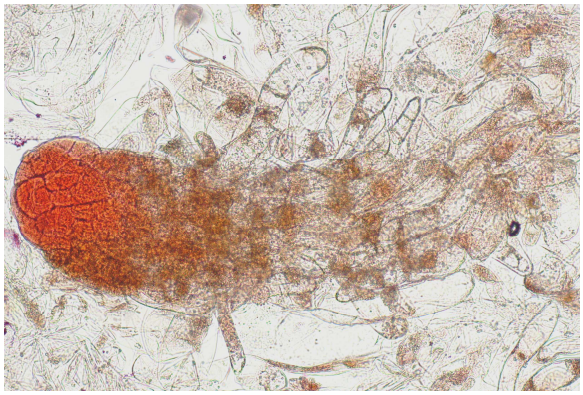

Figure 1 Bipolar structure with well developed embryonal part (line L14)

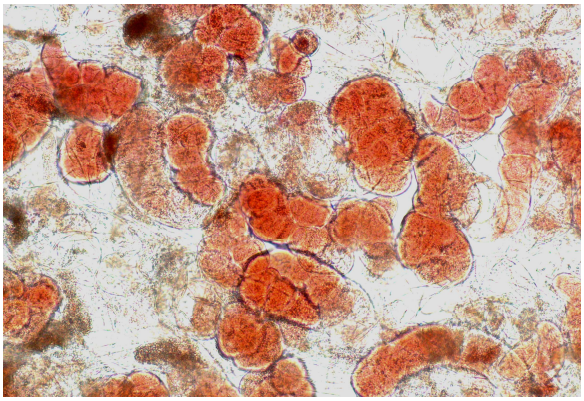

Figure 2 Clusters of meristematic cells without organised suspensor (line E235)

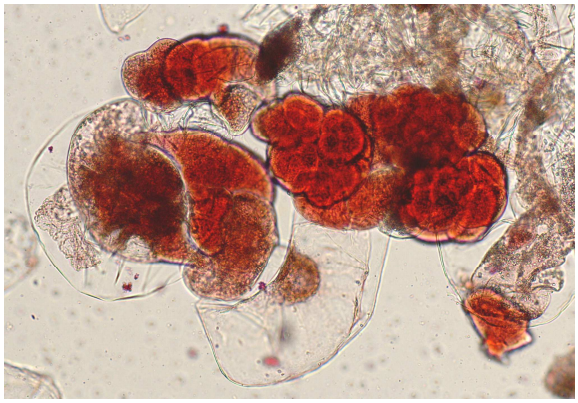

Figure 3 Less developed bipolar structures (line L34)

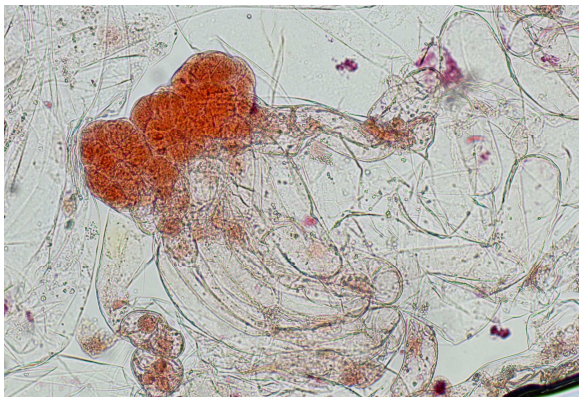

Figure 4 Somatic embryos connected with embryonal parts (line E224)

We selected following cell lines: L14, L29, L34, E235 and E292 for testing of maturation capacity of embryogenic cell lines. We used based DCR media assigned for maturation with different concentration of maltose and gerlite. Embryogenic cell line L14 with high embryogenic potential produced high number of somatic embryos on the both types of media (Fig. 5a, b). Although higher number of somatic embryos was obtained on medium with 1 % gerlite and 6 % maltose. Embryogenic cell line E235 with almost zero capacity of maturation did not produce somatic embryos (Fig. 6). The line L34 produced only low number of somatic embryos on the both types of media but on medium with 1 % gerlite we obtained higher number of somatic embryos (Fig. 7). Embryogenic cell lines L29 and E292 from the beginning of cultivation have not showed any changes.

Categorization of embryogenic cell lines according to the micromorphology of somatic embryos has been given for several *Pinus* species **(Laine and David, 1990; Bercetche and Paques, 1995)**. The micromorfology of somatic embryos is a stable feature and did not change even after cryopreservation **(von Arnold et al., 1996)**. **Mo et al. 1996** supposed et least partly genetic differences, based on the fact that mature somatic embryos of group A produced new cell lines with somatic embryos of the same group. Similarly as in other *Pinus* species **(Keinonen-Mettälä et al., 1996)** in *Pinus nigra* embryogenic cell lines the maturation capacity showed interaction with cytological and morphological features of early stage somatic embryos. The well-structured somatic embryos are capable of maturation on a wide range of maturation treatments **(Ramarosandratana et al. 1999)**.

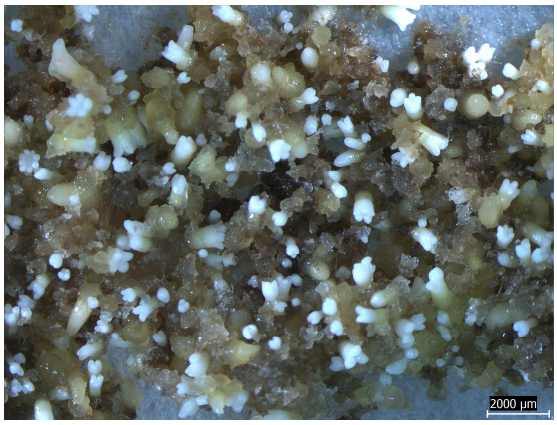

Figure 5a Embryogenic cell line with high embryogenic potential (line L14)

Figure 5b Cotyledonary somatic embryo (line L14)

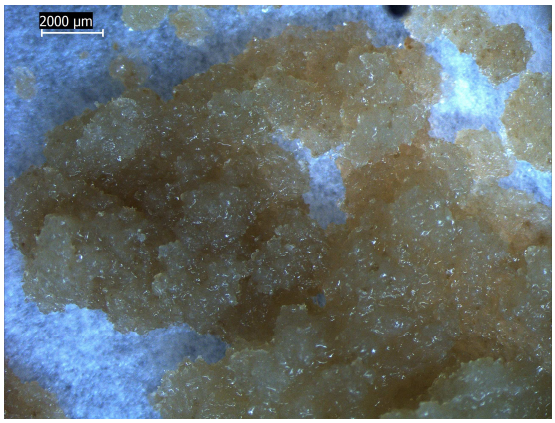

Figure 6 Embryogenic cell line with almost zero capacity of maturation (line E235)

Figure 7 Embryogenic cell line with lowered capacity of maturation (line L34)

CONCLUSION

From our results we can conclude, that the most suitable media for induction of embryogenic tissue from explants of *Pinus* nigra Arn. are basic media DCR (DCR-R) and MLV (MLV-R). Medium with 1 % gerlite and with 6 % maltose showed more positive influence for maturation of somatic embryos.

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