Properties of Rescued Embryonal Suspensor Masses of Norway Spruce Determined by the Genotype and the Environment *In Vitro*

Kari J. Jokinen and Don J. Durzan

Jokinen, K.J. & Durzan, D.J. 1994. Properties of rescued embryonal suspensor masses of Norway spruce determined by the genotype and the environment *in vitro*. Silva Fennica 28(2): 95–106.

Fifty three genotypes of embryonal suspensor masses (ESMs) rescued from mature seeds of Norway spruce (*Picea abies* L. Karst.) were examined for their pattern of growth and development under standardized culture conditions *in vitro*. Patterns were classified according to the color of the colonies grown in darkness, clarity of cell masses and proembryos in the mucilaginous ESM, surface boundary topology of colonies, structure of suspensors, growth rate of the ESM, and recovery of mature embryos. Five distinctive major growth patterns were observed among ESM colonies under standardized culture conditions. The multiplication of proembryos and early embryos by cleavage and budding polyembryony was the main factor contributing to proliferation and colony growth and further determined the morphology of the colonies. Callus and teratological structures were induced from early embryos by changing the standardized culture conditions i.e. inadequate subculture, excessive dose of 2,4-D in the medium and premature exposure of the colonies to light. Results enable the selection of ESM genotypes for the more predictable recovery of mature somatic embryos.

Keywords Norway spruce, *Picea abies*, growth, somatic embryogenesis, callus.
Authors' addresses *Jokinen* (corresponding author), Kemira Agro Oy, Espoo Research Centre, P.O. Box 44, FIN-02271 Espoo, Finland; *Durzan*, Dept. of Environmental Horticulture, University of California, Davis, CA 95616-8587, USA.
Accepted October 5, 1994

1 Introduction

In northern temperate forests, the polyembryony in seeds is related to the local climate and the short growing season (Simak 1973). Polyembryony represents a survival mechanism scheduled around stressful environment (Klekowski 1988). The adaptive plasticity of the new generations shown by polyembryony has not been studied under tissue culture conditions. Studies of the adaptive plasticity are important because the environment in which a genotype gives rise to the new generation under tissue culture conditions is very different from the environment where the genotypes are deployed for timber production.

Systems for large scale clonal propagation of conifers by *in vitro* embryogenesis are also under development (e.g. Hämäläinen and Jokinen 1992, Gupta et al. 1993, Attree and Fowke 1993). This will enable wider genotype × environment testing. Basic studies to recognize, screen and select of various ESM genotypes under tissue culture conditions are needed. Guidelines from results make it easier to stabilize and control the reconstitution processes for cloning purposes. This will improve the recovery of true-to-type embryos and possibly minimize clonal variation. Moreover, any lethal genes from inbreeding and introgression associated with the genotypes may be detected more conveniently. Consequently more information on the performance of different ESMs *in vitro* is required.

In our approach, whole embryonal suspensor masses (ESMs) of Norway spruce are rescued from a population to reconstitute new generation of embryos by polyembryogenesis. Reconstitution is based on the well-known cleavage and development of coniferous zygotic embryos (Sinnott 1960, Singh 1978).

In several laboratories, the recovery of embryos is reported to be based on a callus phase using an "embryogenic callus" or "tissue" (Hakman and von Arnold 1985, Jain et al. 1988, Becwar et al. 1989, Verhagen and Wann 1989, Hakman et al. 1990, Lelu et al. 1990, Mo and von Arnold 1991, Egertsdotter and von Arnold 1993). In the present investigation our aim was to define the cellular attributes that contribute to overall growth and morphology in Norway spruce ESM colonies under standardized culture conditions in the absence of a callus phase. Our purpose was also to show how the specific attributes of colonies and the growth patterns relate to the recovery of somatic embryos.

2 Materials and Methods

2.1 Origin of the Cultures

Embryonal suspensor masses (ESMs) were recovered from mature Norway spruce (*Picea abies* L. Karst.) commercial seed mixture collected in 1985 from Finnish sources (Seed lot no. P 27-85-01 provided by Enso-Gutzeit Company, Imatra, Finland). Seeds developed after natural pollination in a natural population representing a population of trees ranged from latitudes and longitudes of 61° to 62° N and 27° to 29° E, respectively. This range covers a population of Norway spruce at the southern to middle part of the forest region in Finland.

2.2 Selection of ESM, Culture Media and Culture Conditions

One thousand seeds were used. ESMs were dissected from individual seeds by utilizing the development of an ESM directly on isolated zygotic embryos not attached to female gametophyte without a callus phase (Hong et al. 1991). Only one of several possible ESMs found in each seed was rescued under the microscope and thus each ESM represents a genotype.

Rescued ESMs were subcultured on half-strength LP medium (von Arnold and Eriksson 1981, von Arnold 1987) modified with NH_4NO_3 at 15 mM on 2.0 g/l Gelrite Gellan Gum (Merck & Co., Inc.). The LP medium contained sucrose (60 mM), L-glutamine (3.5 mM), 2,4-dichlorophenoxyacetic acid (2,4-D) at 10 μM and N^6-benzyladenine (BA) at 5 μM. This medium was used for the initiation and maintenance of all cultures.

In the study of colony properties of ESMs, two Petri dishes (Falcon 1005, 100 × 20 mm, containing 30 ml/dish of medium) each with 10 colonies were established for each genotype. The diameter of each colony was ca. 1.5 cm at the start of each subculture. The cultures were kept in darkness at 23 ± 2 °C and subcultured three times at 20-day intervals. The colony properties of each ESM genotype were examined on day 10 to 15 from the start of each subculture period to ensure that the observed properties were stable.

Optimal subculture rate and recrudescence of the ESMs were determined by subculturing the colonies twice at 35-day intervals and then twice at 20-day interval (see terminology and observations). Recrudescence was observed at the end of the culture period. All the ESM genotypes were always cultured in the same tests to prevent variations caused by batches of media or experimental conditions such as fluctuations in temperature, harvest date, etc. No less than two Petri dishes, each with 10 colonies were always subcultured for each genotype (i.e. two replicates for each genotype).

For mature embryo formation the colonies were derived from the dishes which were continuously subcultured at 20-day intervals in the maintenance medium. In this study the two Petri dishes each with five colonies were initiated for each genotype. These were not subcultured during the formation of mature embryos. The experiment was employed on a modified LP medium without the plant growth regulators 2,4-D and BA (see above), but with 7.6 μM abscisic acid (ABA) and 5 μM ß-indolebutyric acid (IBA). Abscisic acid was filter sterilized. Colonies were grown for 2 weeks in darkness before transfer to continuous light at a photon flux density of 60 $\mu molm^{-2}s^{-1}$ supplied by 20 W fluorescent lamps (General Electric cool white). After another 4 weeks (6 weeks from the start of the mature embryo formation experiment), the number of embryos with cotyledons and root primordia that could be recovered was counted from an area of 5 × 5 mm of each colony (altogether 10 colonies of each genotype). The experiment has been repeated several times during the last four years to ensure the selection of the most stable ESM genotypes in terms of mature embryo recovery.

Responses of one ESM genotype to the changing culture conditions were determined in three separate experiments. This genotype represented normal developmental pattern since it has produced well germinating somatic embryos for several years under standardized culture conditions.

First, the effect of 2,4-D on cleavage polyembryony of ESM was examined by varying the concentration of the 2,4-D (5μM, 10μM, 20μM, 50μM) in the maintenance media. Two Petri dishes per treatment each with 10 colonies were established. The exposure time was 20 days in darkness.

Second, responses of ESMs to inadequate subculture were investigated. Two Petri dishes each with 10 colonies were subcultured twice at 40-day interval in the maintenance medium and then the colonies were subcultured as in the study of embryo formation.

Third, the effect of light on the performance of ESMs was determined. Two Petri dishes each with 10 colonies were prematurely exposed to continous light (a photon flux density of 60 $\mu molm^{-2}s^{-1}$) in the maintenance medium or at the start of the embryo formation.

The abnormal tissues i.e. callus and teratological structures induced by changing the standardized culture conditions were subcultured further in maintenance medium both in darkness and in continous light at a photon flux density of 60 $\mu molm^{-2}s^{-1}$.

The interaction between different ESMs was examined by culturing several ESM genotypes on the same plate. The combinations of the genotypes were a) 6, 7, 28, 31, and 222, and b) 28, 31, and 222. Of both combinations, two plates with one colony of each genotype were used. Culture media and culture conditions were equal to maintenance. The exposure time was 30 days. The experiment was repeated twice.

2.3 Terminology and Observations

In this study the morphological use of the term proembryo refers to all the tiered developmental stages before elongation of the suspensor (Singh 1978). In a technological sense, proembryo is sometimes referred to embryonal groups of cells from rescued and cultured ESMs, that through cleavage and budding have reconstituted new embryos without a callus phase (Durzan and Durzan 1991). Proembryonal cells are strongly reactive to acetocarmine. They are capable of restoring a new axial tier and may represent steps

in apomictic parthenogenesis (Durzan et al. 1994).

Early embryony in this study and in zygotic development covers all stages after the elongation of suspensors and before the establishment of a root generative meristem (Singh 1978). Early embryos derives from proembryonal cells and involves the establishment of a polar axial tier of embryonal tube and suspensor cells.

Late embryony is the establishment of the polar meristems and the development of the embryo following early embryony.

Microscopic observations on cell types in the ESM relating to proembryonal and embryonal development were aided by the double-staining method, using acetocarmine with or without Evan's blue (Gupta and Durzan 1986), and by inspection with natural and polarized light.

The colony morphology of ESMs was defined by comparison with a chart specifying three-dimensional geometrical patterns for microbial colony formation presented by Clifton (1958). Matching of colony patterns for all three dimensions was done visually. Wherever possible, these attributes were compared to the classical features of early zygotic embryology in *Picea* sp. (Singh 1978). A classification of the types (see Table 1) was based on the following properties of the colonies:

A. Color of the colonies grown in darkness, i.e. white, creamy, light brown (pathological).
B. Translucence of the colonies (early embryos) in the mucilaginous ESM.
C. General growth habit of colonies, based on the dominant developmental pattern of proembryos, i.e. tendency for polyembryony of the cleavage and/or budding types, or tendency to produce callus.
D. Colony characteristics.
   a) the surface type: smooth, opaque, translucent, transparent, arborescent, filamentous, wavy interlaced, coarsely granular or finely granular.
   b) the surface topology: raised, convex, low convex, diffuse, pulvinate, umbonate, raised with beveled edges, convex rugose or convex papillate.
   c) the circumferential boundary: undulate, crenate, erose, smooth (entirely), ramose, lacerate, fimbriate, ciliate or lobate.
E. Growth rates of ESMs were estimated by circumferential growth and mass under identical culture conditions for 20 days per subculture. Comparisons were made among rescued genotypes as superior, average, and poor. This could not be made in terms of equivalent rates of zygotic development.
F. Consistency and coherence within each colony, in terms of synchrony, composition, mucilage production, etc.
G. The subculture time leading to the deterioration and browning of ESMs. This was determined visually and based on the ESM response to 20-day and 35-day subculture.
H. Normal and complete late embryo formation (with cotyledon and root primordia) per unit area of ESM at the time of individual embryo differentiation (see also Hämäläinen and Jokinen 1992). This estimates the efficiency of individual proembryos and early embryos to outgrow the environment of the ESM. Criteria were based on recovery of
   a) more than three normal well-developed mature embryos and abnormal embryos at low frequency per 0.25 cm^2 (high)
   b) less than three normal well-developed mature embryos and several abnormal embryos per 0.25 cm^2 (low).
I. Maturation properties for specific features during colony deterioration in the maintenance medium.
J. Recrudescence was determined visually and is the ability of a genotype to produce a new ESM after prolonged subculture (35-day interval) followed by subculture of 20-day interval in the maintenance medium.

3 Results and Discussion

3.1 Classification and Development of ESM Colonies under Standardized Conditions

Fifty three Norway spruce ESM genotypes out of 1000 started to proliferate on the initiation medium. The survey of these ESM genotypes revealed five distinctive major growth patterns (types I to V). Colony attributes of the types are summarized in Table 1. Over half of the ESM genotypes (66 %) had the properties of type III.

Table 1. Colony properties of Norway spruce embryonal suspensor masses (ESMs) rescued from seeds and cultured *in vitro*. Fifty three ESM genotypes were evaluated. Terminology for criteria is based on Clifton (1958).

Criterion	Types/Colony properties				
	I	II	III	IV	V
Color	creamy	creamy to light brown	creamy	white	white
Clarity	translucent	opaque, wavy interlaced	opaque	translucent	opaque
Mucilage[1]	proembryos escape	proembryos escape	suspensors partly embedded	suspensors embedded	suspensors embedded
Colony traits:					
consistency	smooth elastic tensile compact	rough elastic tensile compact	smooth compact with "hills"	smooth loose	smooth loose
surface (upper)	rugose	rugose	convex papillate	convex papillate	convex papillate
circumference	ramose	fimbriate	lobate	undulate	undulate
suspensors	thick and stacked	thin and individual	thick and short	undeveloped (short)	thick
cleavage	strong, with budding	strong	weak	rare	weak
Growth rate per 10 days	high	moderate	high	low	low
Optimal subculture time in days	15–20	15–20	20–25	15–20	15–20
Embryo recovery[2]	high	high	high	low	low
Maturation properties	dark brown	indefinite color	light brown	no color changes	dark brown color spots
Recrudescence	high	moderate	weak	poor	poor
Distribution (n = 53)	13 %	5 %	66 %	8 %	8 %

[1] Embryos with their suspensors escape the mucilaginous ESM by growing into the air rather than agar. In some genotypes, the suspensors remain embedded in the mucilaginous ESM.
[2] Based on recovery of late embryos from a 5 × 5 mm diameter of ESM after 45 days culture high > 3, low < 3.

Light microscopic examinations showed that the multiplication of proembryos and early embryos was by cleavage (Fig. 1) and budding polyembryony (Fig. 2) although Norway spruce is considered a non-cleavage species (Dogra 1967). Budding polyembryony is distinguished from cleavage by a distinctive free-nuclear stage in embryonal tube cells. The free-nuclear stage re-establishes a "basal plan" for early ontogeny (Dogra 1980, Gifford and Foster 1989). In our cultures, the "basal plan" comprised a two-cell stage, arbitrarily designated pE and pU. The heavier nucleus formed an acetocarmine-reactive neocytoplasm and a new proembryonal cell (Fig. 2B). This is confirmed recently by Durzan et al. (1994).

According to Dogra (1980), the cleavage performance of cells in the pro- and early zygotic embryo falls into eight categories. In our study, all categories were reaffirmed in the cultures maintained at 23 °C ± 2. Light microscopic observations facilitated the recognition of variations in the cleavage process as being:

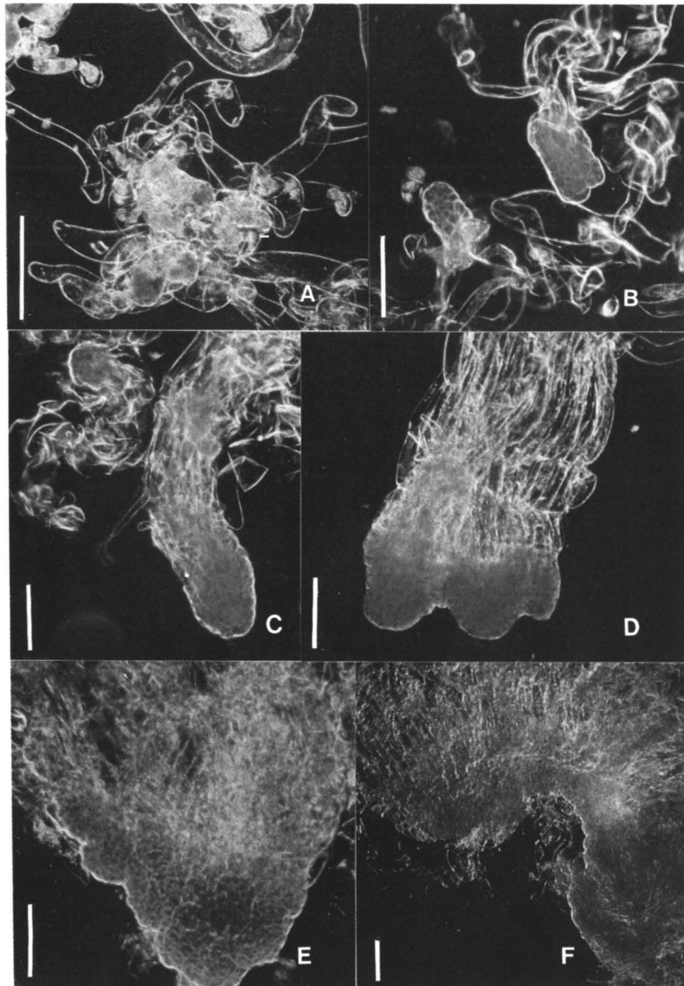

Fig. 1. Cleavage polyembryony in embryonal suspensor masses of Norway spruce under tissue culture conditions. It is important to note that Norway spruce is not normally considered a cleavage species (Dogra 1967) but when the embryonal suspensor mass (ESM) is rescued, cleavage is expressed. Cleavage polyembryony in ESM responds to high 2,4-D by inhibition of the cleavage process and fasciation of early embryos. All preparations stained with acetocarmine. Bar = 0.2 mm.

A. Early embryos at 5 μM 2,4-D in a loose array of cells in the ESM.
B. Early embryonal group development prior to the development of the axial suspensor.
C. Individual early embryos with well-developed suspensors at 5 μM 2,4-D.
D. Three cleaved, but not separated, early embryos.
E. Fasciation increases at 20 μM 2,4-D in the culture medium.
F. Distortion effect of 50 μM 2,4-D in the culture medium.

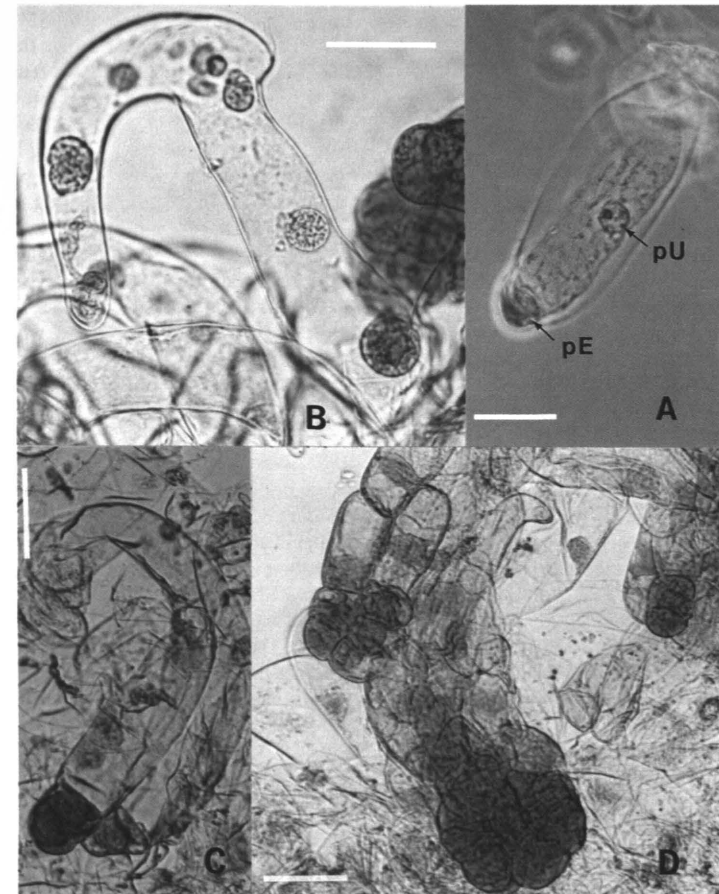

Fig. 2. Early embryos in embryonal suspensor masses can reset their development through a free-nuclear stage. This stage is susceptible to necking i.e. the formation of protoplasts that trap organelles within the cell as revealed by acetocarmine staining (Durzan 1991).

A. Products of necking sometimes appear in budding cylindrical cells on the flanks of early embryos when examined in live preparations by the light microscope. Staining with acetocarmine reveals protoplast fragments inside the cell, some of which may trap a nucleus. Bar = 50 μm.
B. Two-cell basal plan established after migration of free nuclei during budding polyembryogenesis. Cells are arbitrarily designated as pE – proembryonal cell, and pU – a free nucleus with at least two fates: one that may determine the differentiation of the new axial tier of the early embryo or another that could represent the basal plan concept of Dogra (1967). Note weak acetocarmine staining of upper nucleus and lack of acetocarmine-reactive neocytoplasm. Bar = 50 μm.
C. Establishment of two primary early embryos by the budding process. Bar = 0.1 mm.
D. Asynchrony in cleavage polyembryony as revealed by tiered early embryo development (cf. Durzan and Durzan 1991). Bar = 0.1 mm.

1) Determinate, i.e. the early establishment of a successful single early embryo (Fig. 1C).
2) Dominant and/or asynchronous, i.e. one embryonal unit outgrows the others (Fig. 2D).
3) Incipient and/or proliferative, i.e. the formation of multicellular embryonal cell masses from one or all proembryonal cells that are not yet in a cleavage stage (Fig. 1F). This was predominant in type IV colonies. High levels of 2,4-D (> 20 μM) contributed to the expression of this type.
4) Indeterminate, i.e. very little or no early indication of individual early embryo supremacy (Fig. 1D). Observations revealed that this predominates in type III colonies.
5) Inhibitory of two types: physical, based on failure of the embryonal suspensor or elongation of cells to establish another embryonal unit, and chemical, due to the release of diffusible inhibitors acting on other proembryonal units. Inhibition was significant in type V colonies.
6) Partial and/or asynchronous, where two or more embryonal units remain joined while other units separate from one another. This model was dominant in type I colonies.
7) Unitary lobing where the early embryonal mass becomes lobed with the development of embryonal tubes. Embryonal tubes sometimes pass through a free-nuclear stage before early ontogeny (Fig. 2B) (Durzan et al. 1994).
8) Total cleavage. Here all proembryonal and early embryonal units separate completely. Total cleavage was predominant in type II colonies.

The present results indicate that the outcome of cleavage and budding polyembryony was the main factor contributing to clonal proliferation and colony growth and consequently determined the morphological traits of the colonies on gelrite plates in the absence of a callus phase. The occurrence of cleavage and budding polyembryony in ESMs of Norway spruce *in vitro* was reported earlier by Gupta and Durzan (1986), Durzan (1988a,b), Boulay et al. (1988) and Hong et al. (1991) but no detailed classification of the colonies was given.

On the semi-solid medium, an excessive mucilage was observed to cover the developing embryos on type IV and V colonies (Table 1). The mucilage was produced when the embryonal tubes and suspensors developed in response to 2,4-D in the medium. The growth rate and the late embryo formation of these ESM types were low (Fig. 3). This suggests that the mucilage may contain compounds which retard the cell growth and development under tissue culture conditions. According to Durzan (1988c), the mucilage is acetocarmine-reactive being largely pectinaceous and containing several proteins and glycoproteins. In developing seeds, it is thought that the mucilage contributes enzymes for the digestion of the female gametophyte.

When five different ESM genotypes were cultured on the same plate for 30 days, four of these deteriorated (severe browning), while one remained viable. The viable one had, however, lost its ability to reconstitute embryos. Upon repeating the study, the exlusion of two types from the plate increased the viability of the others. This suggests that ESMs may release chemical compounds that suppress the cleavage and/or budding of the others. Consequently more phytochemical research on ESM genotype characterization is needed.

The ESM genotypes classified as types I to III yielded high rates of late embryos (Table 1 and Fig. 3). The recovery of late embryos from these types by inhibition of cleavage and budding polyembryony seems to be a continual and stable process since it is maintained in our laboratories for over 5 years. High recovery is obtained by subculturing ESMs every 10–15 days under the standardized conditions. However, to optimize the performance of recalcitrant genotypes belonging to type IV or V, our culture conditions need to be modified further e.g. by analyzing the composition of the developing seeds (Durzan and Chalupa 1968, Chalupa and Durzan 1972, Durzan and Durzan 1991). For example, the composition of the amino acid pool in the female gametophyte may be substituted for by amides and amino acids in acid casein hydrolysate in the LP culture medium.

Results indicate that genotypic variations among the different ESM genotypes clearly identify the need for early, stabilizing selection for colony establishment and evaluation in the cloning process. According to Falconer (1989), selection acts first through viability and then through fertility. In our case, the ability of ESM to produce a new ESM (viability) and then to

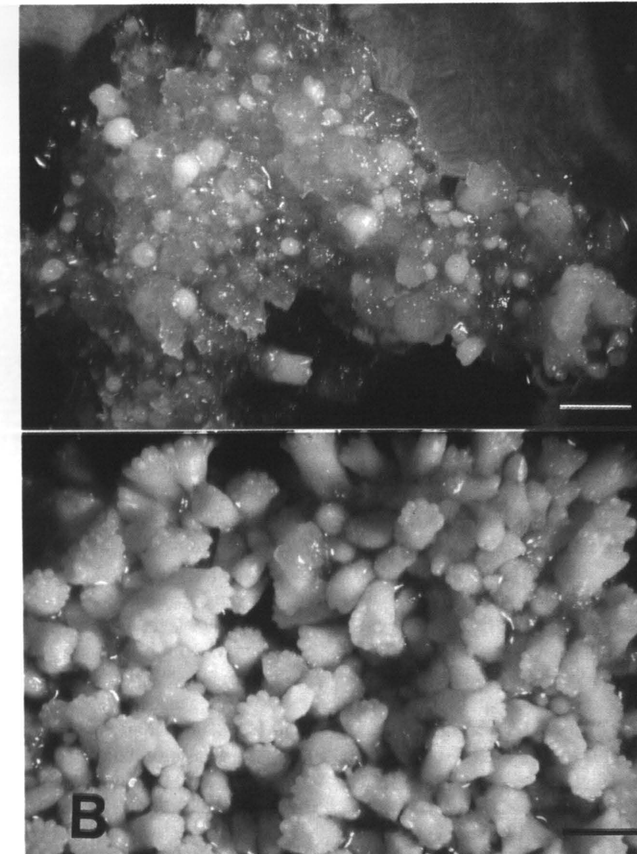

Fig. 3. Late embryos of Norway spruce recovered in embryonal suspensor mass (ESM) under standardized tissue culture conditions. Bar = 2 mm.
A. Low rate of embryos on the type IV colony.
B. High rate of embryos on the type III colony.

produce mature embryos (fertility) can now be estimated soon after fertilization with ESMs arising by polyembryony (Dogra 1980). Our classification also establishes a baseline for correlations between culture performance in the maintenance medium and recovery of mature embryos under different culture conditions. Baseline studies may be extended to progeny and clonal testing procedures under field conditions where correlations are sought as a time-saving measure in tree improvement.

3.2 Non-Polyembryonic Differentiation from ESMs

In the present study callus and teratological structures were induced from early embryos by chang-

ing the standardized culture conditions, i.e. inadequate subculture, excessive dose of 2,4-D in the maintenance medium and premature exposure of the colonies to light. Unstained callus, which differentiated from ESMs, had the following distinguished characteristics:

A. Color and clarity in darkness were initially white and opaque, followed by rapid browning. In light, tissues became green and/or red, followed again by rapid browning, especially from red cells.
B. General growth habit varied from compact to friable or nodular, with irregular arrays of autofluorescing cells.
C. Growth rates in darkness were usually half to two-thirds the rate of ESMs under conditions favoring the growth of ESMs. However, under light callus growth was stabilized with the formation of embryogenic meristemoids at the expense of ESM growth.
D. Callus cells were round, elongated and often highly vacuolated and lacking extracellular mucilage.

Some isodiametric cells, callus and tissues derived from callus are potentially embryogenic (Hong et al. 1991, Jalonen and von Arnold 1991). Cells in embryogenic cultures and tissues derived from callus initially had starch grains that, together with nuclei, showed weak but increased reactivity with acetocarmine. These cells were interspersed with others bearing vacuoles filled with tannins and phenols, and a cytoplasm that did not react with acetocarmine (see Hong et al. 1991).

In cases of somatic embryogenesis in Norway spruce where polyembryony (cleavage and budding) has not yet been reported, a different classification of embryogenic cultures was proposed (Jalonen and von Arnold 1991, Egertsdotter and von Arnold 1993). In that system, cell colony patterns showed a polar type development, with distinct embryo heads and well-developed suspensors. In addition, a solar type, with embryos showing a radial symmetry, and an undeveloped type, where embryogenic cells were intermingled with clusters of vacuolated cells, were also described.

In our polyembryogenic cultures, polar development and axial tier differentiation represented the only type of embryonic development. The solar types were not seen unless culture conditions were changed and 2,4-D levels were high i.e. under conditions which induced non-polyembryonic differentiation from ESMs. The loss of axial polarity normally associated with embryony would decrease viability and eliminate the role of monozygotic polyembryony as a survival process (cf. Klekowski 1988).

Both polyembryonic and nonpolyembryonic patterns of growth require further study in terms of the origins, maturation processes, precocious behaviour and adaptive phenotypic plasticity under 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.

Becwar, M.R., Noland, T.L. & Wyckoff, J.L. 1989. Maturation, germination and conversion of Norway spruce (Picea abies L.) somatic embryos to plants. In Vitro Cellular & Developmental Biology 25: 575–580.

Boulay, M.P., Gupta, P.K., Krogstrup, P. & Durzan, D.J. 1988. Development of somatic embryos from cell suspension cultures of Norway spruce (Picea abies Karst.). Plant Cell Reports 7: 134–137.

Chalupa, V. & Durzan, D.J. 1972. Differences in the seed size and in the biochemical composition of seed of various provenances of jack pine (Pinus banksiana Lamb.). Lesnictvi (Czech. Forestry) 18: 1089–1106.

Clifton, C.E. 1958. Introduction to the bacteria. McGraw-Hill, N.Y. p. 250–252.

Dogra, P.D. 1967. Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae. Studia Forestalia Suecica 45: 1–97.

— 1980. Embryogeny of gymnosperms and taxonomy – an assessment. In: Nair, P.K.K. (ed.). Glimpses in plant research 5. Vikas House Ltd., New Delhi. p. 114–128.

Durzan, D.J. 1988a. Process control in somatic polyembryogenesis. Molecular Genetics of Forest Trees. Franz Kempe Symp., June 14–16, 1988, Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Report 8. p. 147–186.

— 1988b. Somatic polyembryogenesis for the multiplication of tree crops. Biotechnology & Genetic Engineering Reviews 6: 341–377.

— 1988c. Metabolic phenotypes in somatic embryogenesis and polyembryogenesis. In: Hanover, J.W. & Keathley, D.E. (eds.). Genetic manipulation of woody plants. Plenum Press, New York. p. 293–311.

— 1991. Necking in embryonal tube cells and its implications for morphogenic protoplasts and conifer tree improvement. In: Ahuja, R. (ed.). Woody plant biotechnology. Plenum Press, New York. p. 123–137.

— & Chalupa, V. 1968. Free sugars, amino acids, and soluble proteins in the embryo and female gametophyte of jack pine as related to climate at the seed source. Canadian Journal of Botany 46: 417–428.

— & Durzan, P.E. 1991. Future technologies: model-reference control systems for the scale-up of embryogenesis and polyembryogenesis. In: DeBergh, P. & Zimmerman, R.H. (eds.). Micropropagation. Martinus Nijhoff, Dordrecht. p. 389–423.

— , Jokinen, K.J., Guerra, M.P., Santerre, A., Chalupa, V. & Havel, L. 1994. Latent diploid parthenogenesis and parthenote cleavage in egg-equivalents of Norway spruce. International Journal of Plant Sciences. (in press).

Egertsdotter, U. & von Arnold, S. 1993. Classification of embryogenic cell-lines of Picea abies as regards protoplast isolation and culture. Journal of Plant Physiology 141: 222–229.

Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. Longman/John Wiley, N.Y. 27 p.

Gifford, E.M. & Foster, A.S. 1989. Morphology and evolution of vascular plants. W.H. Freeman Co., NY.

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

— , Pullman, G., Timmis, R., Kreitinger, M., Carlson, W.C., Grob, J. & Welty, E. 1993. Forestry in the 21st century. The biotechnology of somatic embryogenesis. Bio/Technology 11: 454–459.

Hakman, I. & von Arnold, S. 1985. Plantlet regeneration through somatic embryogenesis in Picea abies (Norway spruce). Journal of Plant Physiology 121: 149–158.

— , Stabel, P., Engström, P. & Eriksson, T. 1990. Storage protein accumulation during zygotic and somatic embryo development in Picea abies (Norway spruce). Physiologia Plantarum 80: 441–445.

Hämäläinen, J.J. & Jokinen, K.J. 1992. Selection of Norway spruce somatic embryos by computer vision. In: DeShazer, J.A. & Meyer, G.E. (eds.). Optics in agriculture and forestry. SPIE–The International Society for Optical Engineering. Vol. 1836. p. 195–205.

Hong, L., Boulay, M., Gupta, P.K. & Durzan, D.J. 1991. Variations in somatic polyembryogenesis: Induction of adventitious embryonal suspensor masses on developing Douglas fir embryos. In: Ahuja, R. (ed.). Woody plant biotechnology. Plenum Press, N.Y. p. 105–121.

Jain, S.M., Newton, R.J. & Soltes, E.J. 1988. Enhancement of somatic embryogenesis in Norway spruce (Picea abies L.). Theoretical & 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.

Klekowski, E.J., Jr. 1988. Mutation, developmental selection and plant evolution. Columbia University Press, N.Y. p. 228–275.

Lelu, M-A.P., Boulay, M.P. & Bornman, C.H. 1990. Somatic embryogenesis in cotyledons of Picea abies is enhanced by an adventitious bud-inducing treatment. New Forests 4: 125–135.

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

Simak, M. 1973. Polyembryonal seeds of Pinus sylvestris in arctic regions. Research Notes, Dept. of Reforestation, Royal College of Forestry, Stockholm. p. 1–14.

Singh, H. 1978. Embryology of gymnosperms. Encycl. plant development, Vol. 10, Part 2. Gebrüder, Borntraeger, Berlin. p. 203–220.

Sinnott, E.W. 1960. Plant morphogenesis. McGraw-Hill, N.Y. p. 235–257.

Verhagen, S.A. & Wann, S.R. 1989. Norway spruce somatic embryogenesis: high-frequency initiation from light-cultured mature embryos. Plant Cell, Tissue and Organ Culture 16: 103–111.

von Arnold, S. 1987. Improved efficiency of somatic embryogenesis in mature embryos of Picea abies. Journal of Plant Physiology 128: 233–244.

von Arnold, S. & Eriksson, T. 1981. In vitro studies of adventitious shoot formation in Pinus contorta. Canadian Journal of Botany 59: 870–874.

Total of 34 references