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總 說

Conifer Somatic Embryogenesis : New Knowledge in Plant Biology and Breakthrough in Tree Biotechnology*[1]

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針葉樹 體細胞 胚發生을 利用한 林木生物工學*[1]

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ABSTRACT

Clonal forestry and reforestation programmes are especially interested now in development and application of controllable biotechnological systems based on the production of conifer somatic embryos in bioreactors with their following drilling and/or storage in the form of "artificial seeds". Modern achievements in conifer somatic embryogenesis has guided the development not only of biotechnological systems in forestry, but also of basic research in conifer embryology, cell and molecular biology. At the present time, the level of development of applied research on conifer somatic embryogenesis is well ahead our understanding of this complex phenomenon. The "bottleneck" situation in relation between basic and applied sciences will eventually lead to the appearance of "weak points" in biotechnological systems. In the present review, the major advances and the most pressing problems in the application of conifer somatic embryogenesis both to forest biotechnology and to basic research are in the focus of attention.

Key words : forest biotechnology, coniferous species, somatic embryogenesis, embryonal-suspensor mass

적 요

최근에 임목의 영양번식체계나 우량묘목을 양성하기 위하여 임목의 체세포배를 이용해서 "인공종자" 형태로 보관하거나 연속배양기를 사용하여 계속적으로 체세포배를 생산하는 방법은 매우 중요한 기법에 속한다. 이러한 체세포 배양기법은 임목의 생물공학 뿐만아니라 체세포배 발생학, 세포 및 분자생물학 연구에 있어서 중요한 역할을 한다. 현재 침엽수 체세포 배양에 대한 복잡한 기작이 많이 밝혀지고 있으나 이러한 기초적 지식이 현실적으로 잘 응용되지 못하는 점이 임목생물공학의 발전을 저해하는 요소로 지적되고 있다. 본 총설에서는 이러한 기초적 지식을 침엽수 체세포배 배양의 응용적 연구에 이용하는데 초점을 맞추었다.

INTRODUCTION

Coniferous species, as gymnosperms at all, always attracted considerable interest of the plant biologist, because they are intermediate in taxonomic position between cryptogams and flowering plants ; and their high phylogenetic stability,

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which is manifested in the ability to determine many types of forest ecosystems at both hemispheres. The latter, together with inestimable economic value of softwood, make conifers critically important for forestry and horticulture.

Being evolutionary established as early as the late Carboniferous/the early Permian Period of the Palaeozoic Era(290 million years ago) (Gifford & Foster, 1989), conifers possess a number of fundamental differences in reproductive biology compared to angiosperm plants. They do not have double fertilization ; megagametophyte is haploid, and it undergoes development prior to syngamy. Being a rule for angiosperm species, maternal inheritance of chloroplast and mitochondrial DNA, however, does not usually hold for conifers, which may display paternal pathway of inheritance of organelle genomes(for review, see Kriebel, 1993).

There are two interesting features of conifer embryogeny, which are in marked contrast to the angiosperms. First, the initial divisions of zygote nucleus are not accompained by cell wall formation and resulting proembryos are free-nuclear. Among other plant groups, the presence of free-nuclear stage is characteristic only for dicotyledonous genus *Paeonia*(Yakovlev & Yoffe, 1957). In conifers, more advanced the plant phylogenetically, less free nuclei form in proembryogenesis (minimum four for *Picea* and *Pinus*)(Singh, 1978). Secondly, two forms of polyembryony, simple and cleavage, have been evolutionary established in most conifers as a mechanism of adaptation to fluctuating and stress environmental milieu. Although simple polyembryony is generally considered as more primitive type(Chowdhury, 1962), it appears to be yielding more genetic diversity compared to cleavage polyembryony, because of independent fertilization of several eggs belonging to one gametophyte. So, the group of embryos being produced is of fraternal type, rather than clone. Simple polyembryony is a common feature of all genera, but in the absense of cleavage polyembryony, is the only rule common to *Larix*, *Picea*, *Pseudolarix* and *Pseudotsuga*(Chowdhury, 1962). Irrespective of type of polyembryony, ordinarily only one embryo in seed develops to maturity and survives in modern conifers, whereas others(subordinate) are aborted or absorbed by gametophytic tissue(Raghavan, 1976).

All the coniferous species reproduce only sexually. Numerous forms of apomixis(agamospermy) and vegetative propagation peculiar to angiosperms(see Asker & Jerling, 1992) have been lost and/or were merely not fixed in the evolution of conifers. The latter point is the main reason for the difficulties encountered when developing clonal propagation and breeding programmes for conifers. Numerous examples of low rooting potential of cuttings and pronounced grafting incompatibility(*e.g.*, Greenwood & Hutchison, 1993) are the adequate examples of recalcitrant nature of conifers.

The cell and tissue culture methods for propagation of conifers, though been shown to have more capacities to promote and control cell dedifferentiation, morphogenesis and plant regeneration in comparison with traditional silvicultural practice, developed slowly until recent years. We propose three points, all related to above mentioned natural conservatism of conifer reproduction, may constitute the basis for the problem. These are :

(1) high degree of determination of many cell and tissue types, and even shoot meristems ;
(2) rapid transition from juvenile to mature phase during ontogenesis, and strongly determined mature phase(*e.g.*, Bonga & von Aderkas, 1993) ;
(3) high developmental stability of dedifferentiated cells in callus cultures(*e.g.*, Mehra-Palta & Thompson, 1987).

What is why plant regeneration from conifer callus cells, growing either in suspension or on solidified media, has not still been clearly demonstrated. In vitro propagation *via* adventitious or axillary budding(*i.e.*, micropropagation), though achieved for several coniferous species, also can not rely on its large-scale commercial application (Bozhkov & Park, 1996).

Clonal forestry and reforestation programmes are especially interested in development and application of controllable biotechnological systems based on the production of conifer somatic em-

bryos in bioreactors with their following drilling and/or storage in the form of "artificial seeds". As recently as in the early 80s such a strategy would, in the best case, be considered as a success of our progeny. However, breakthrough in plant and tissue culture of conifers and in conifer biotechnology has come before long, in 1985. Somatic embryogenesis has become practicable. *Picea abies*(Hakman *et al.*, 1985 ; Chalupa, 1985) and *Larix decidua*(Nagmani & Bonga, 1985) were the first "embryogenic species".

We can say with reasonable confidence that this achievement has guided the development not only of modern conifer biotechnology, but also of basic research in conifer embryology, cell and molecular biology.

Within last few years, there have been several excellent reviews on conifer somatic embryogenesis directed both to overall survey of the extensive literature(*e.g.*, Attree & Fowke, 1993 ; Becwar, 1993) and to consideration of certain basic (*e.g.*, Durzan, 1988) or applied(*e.g.*, Bornman, 1993 ; Roberts *et al.*, 1993) points. By the present review, we have tried to discuss the major advances and the most pressing problems in the application of conifer somatic embryogenesis both to forest biotechnology and to basic research.

CONIFER SOMATIC EMBRYOGENESIS : NOVEL KNOWLEDGE IN PLANT BIOLOGY

For the real understanding of biological processes at the cellular and molecular levels, plant biologists require reliable cell and tissue culture systems, where exogenously applied certain stimuli result in expected developmental response. So, such systems should be both developmentally stable under the temporally fixed conditions and, a the same time, plastic under some stimuli are applied.

The best examples of such model systems in angiospems are cell and tissue cultures of *Arabidopsis thiliana*, *Catharanthus roseus* and *Daucus carota*, which are routinly being used for basic research in molecular biology, secondary metabolite biosynthesis and cell developmental biology, respectively.

Coniferous species did not develope such model system until 1985. Currently embryogenic cultures of several species(mainly from genus *Picea*) with "true-to-type" characteristics for basic research are available.

It has been clearly demonstrated, that the ontogeny of conifer somatic embryos *in vitro* is very closely related to normal embryogeny of gymnosperms(for three embryogenic types of gymnosperms *in situ*, see Norstog, 1982), and involves the reactivation of much of the developmental programme occuring in zygote(Attree & Fowke, 1993 ; Durzan, 1988 ; Durzan & Gupta, 1988). Somatic embryos follow all the steps of basal plan of zygotic embryogeny, including sometimes even free-nuclear stage(*e.g.*, von Aderkas & Bonga, 1988).

It is particularly remarkable that, irrespective of taxonomic position of the species, all the conifers display cleavage polyembryony *in vitro*, even though the species are normally of noncleavage type(for example, *Larix* spp., *Picea* spp. and *Pseudotsuga* spp.). Consequently, the cleavage is most probably a latent feature of noncleavage type species, and the "cleavage genes" may be activated or repressed by exogenous factors applied *in vitro*. Furthemore, it has been proposed that we must not rule out the possibility to identify naturally growing individuals of spruce, larch or redwood retaining cleavage polyembryony *in situ*(Bozhkov, 1995a). These rare organisms could occur at the margins of habitats as well as being grown under specific microclimate.

The process of cleavage of early conifer somatic embryos *in vitro* is theoretically perpetual and it is promoted by auxin and/or cytokinin(*e.g.*, Attree & Fowke, 1993), and amide nitrogen (*e.g.*, Bozhkov *et al.*, 1993). Under appropriate conditions, embryogenic cultures growing both on semi-solidified medium and in liquid contain no or very small number of true callus cells. They consist entirely of cleaving early somatic embryos and/or long embryonal tube cells, *i.e.*, the secondary suspensor cells, producing proembryonal cells through free nuclear divisions(Becwar, 1993 ; Durzan, 1991 ; von Aderkas *et al.*, 1991). Exogenous abscisic acid(ABA) switches developmental

programme from multiplication of early somatic embryos to their individual development *viz.* maturation(*e.g.*, Bozhkov *et al.*, 1992 ; Dunstan *et al.*, 1988).

Strictly speaking, in the case of conifer somatic embryogenesis we deal with quite a different morphogenic systems comparing with angiosperm embryogenic callus. The major points of difference involve morphology of embryogenic tissue and mechanism of somatic embryo multiplication (Table 1). In order to stress unique features of proliferating conifer embryogenic tissue, namely, the absence of true callus cells and its polyembryonic nature, when early somatic embryos multiply by cleavage process, and suspensor cells "makes" proembryonal cells(or *vice versa*), the term "embryonal-suspensor mass"(ESM) has been deeply embedded in the literature. Another term "somatic polyembryogenesis" has been frequently used to describe the overall process of *in vitro* somatic embryo formation in conifers(Bozhkov, 1995a,b ; Durzan, 1988).

One of the most intriguing questions from the developmental point of view is what are the "step-by-step" algorithms underlying *in vitro* formation of the ESM in different taxonomic groups of conifers, and how these algorithms are related to the tendency to cleavage polyembryony *in situ*. Nowdays, the consensus of opinion on this point is reached only for genera *Picea*(noncleavage type species) and *Pinus*(cleavage type species). In cultured zygotic embryos of *Picea*, ESM forms as a result of dedifferentiation of cells in the hypocotyl-cotyledon region(callus phase), induction and subsequent embryogenic differentiation of callus cells (Fig. 1). This developmental route is an example of reproductive regeneration(Durzan, 1988 ; Sinnott, 1960). In *Pinus* spp., ESM initiates from sus-

Table 1. Comparative characterization of conifer embryonal-suspensor mass(ESM) and angiosperm embryogenic callus.

Characteristics	ESM	Embryogenic callus
1. Presence of true callus cells	very rare or absent	numerous
2. Level of organization	early somatic embryos	single embryogenic cells or proembryonal cell complexes
3. Mechanism of somatic embryo multiplication	cleavage of early somatic embryos	differentiation of callus cells
4. Genetic stability under prolonged culture	high	low
5. Similarity to zygotic embryo formation plan	reactivation of much of the developmental programme occuring in zygote	different situations

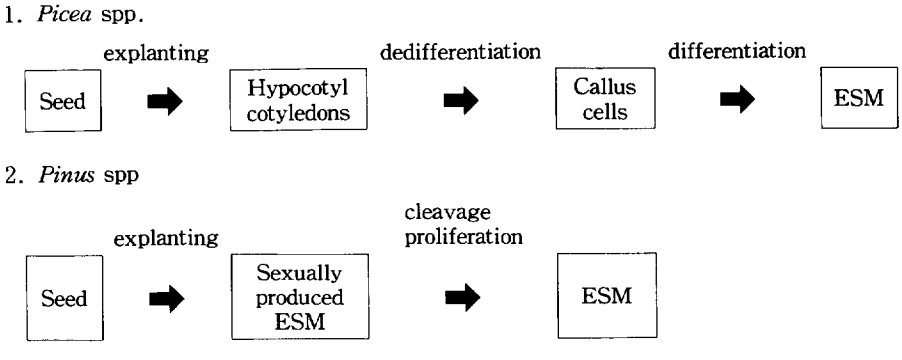

Fig. 1. Different algorithms of steps leading to formation of embryonal-suspensor mass(ESM) in *Picea* spp. and *Pinus* spp.

pensor region of zygotic embryo directly ; callus phase is omitted(Fig. 1). This phenomenon involves merely continuation of cleavage polyembryonic process induced in zygote, and represents a reconstitutive form of regeneration according to Sinnott(1960)(Durzan, 1988).

In other genera situation is not so clear. Both the above-mentioned algorithms appear operational in *Abies*(Guevin *et al.*, 1994 ; Salajova *et al.*, 1996) and *Larix*(von Aderkas *et al.*, 1995), which represent cleavage type species. In this case, expression of one or another algorithm of ESM formation depends on the degree of maturity of cultured zygotic embryos. The direct formation of the ESM from suspensor region may occur only within a brief stage of polyembryonic development during early embryogenesis. As soon as the zygotic embryo cleavage process ceases *in situ*, the polyembryonic complex becomes unresponsive to embryogenic stimuli, and ESM forms *in vitro* from the hypocotyl-cotyledon region employing the "dedifferentiation-induction-differentiation" algorithm.

Irrespective of how conifer ESM initiates *in vitro*, it maintains its highly stable developmental state for as long as auxin and/or cytokinin remain in the culture medium, and until ABA is applied. Moreover, this initial(but, in the same time, theoretically perpetual) state represents already organized multicellular system corresponding developmentally to proembryogenesis and/or early embryogenesis *in situ*. There are no examples analogous to conifer ESM among angiosperm morphogenic systems. The surprising thing is that the ESM combines simultaneously active proliferation and organized development. Really, it is the generally recognized fact, that reprogramming of the plant cell from proliferation to differentiation occurs only when factor(s) promoting proliferation(*e.g.*, auxin) are removed, and other factor (s) supressing proliferation(*e.g.*, ABA) are applied(*e.g.*, Nuti Ronchi & Giorgetti, 1995). Is conifer ESM the only exception from this rule?

The foregoing points to particular value of the ESM, as a versitile tool for research in experimental embryology, and cell and molecular biology of conifers. Today, this line of investigations has already been opened while being still in its infancy.

We propose the following problems of special interest could be explored using conifer embryogenic cultures. First, the unique nature of ESM *per se* undoubtedly attracts for our attention. There are three of questions that need to be answered :

1) what is a cascade of physical-chemical events in cell signal transduction leading to proliferation of early somatic embryos(characterization of plant growth regulator-binding proteins and their genes) ;
2) isolation of "developmental" genes expressed during consecutive steps of embryogenesis, their cloning and analysis of respective proteins ; comparison of conifer and angiosperm proteins regulating embryogenesis ;
3) what is a physiological role of mucilage(*i.e.*, extracellular matrix) of the ESM in conifer somatic embryogenesis(see Bozhkov, 1995a ; Durzan, 1989 ; Egertsdotter *et al.*, 1993).

Second, embryogenic suspension and their morphogenic protoplasts provide adequate material to study the processes of endocytosis and cell division in conifers. Fowke & Attree(1993) investigated endocytosis in white spruce embryogenic protoplasts and have revealed a coated vesicle-mediated pathway very similar to that described in angiosperms. Study on arrangement of microtubules and actin microfilaments during division of conifer embryogenic protoplasts(Tautorus *et al.*, 1992b ; Wang *et al.*, 1991) combined with analysis of microtubule-associated proteins(MAPs) (*e.g.*, Fosket *et al.*, 1993) will give us great insight into the association of cell "mechanics" with gene expression in cytokinesis and differentiation in conifers.

At the present time, the level of development of applied research on conifer somatic embryogenesis is well ahead our understanding of this complex phenomenon. The "bottleneck situation" in relation between basic and applied sciences will eventually lead to the appearance of "weak points" in biotechnological systems. It should be alarming both for scientists and for top executives of R & D organizations and companies fo-

cussing on conifer biotechnology.

GAINS IN CONIFER BIOTECHNOLOGY

With progressive increase in population and shortages of forest resources, we face the problems : how to get enough from a small, how to reduce the risk of forest exploitation, and how to find the optimal approach to the retention of balance between consumption and restoration of forest resources. The biotechnological cloning systems and germplasm preservation technologies are considered the main attributes of a rational politics to be developed in the coming years in the areas of tree improvement and reforestation.

In conifer biotechnology, that developed slowly until 1985, many great strides have been made within the last few years through the implementation of somatic embryogenesis procedures(Table 2).

Let us sketch the broad outline of nowdays' situation in conifer biotechnology. Unlimited number of somatic embryos can be produced within relatively short period of time. Most of somatic embryos undergo true-to-type and synchronous development to plants(*e.g.*, Gupta *et al.*, 1993 ; Webster *et al.*, 1990). So, the real possibilities exist to control and automatically operate cloning process at the level of bioreactor (see Gupta *et al.*, 1991 ; Tautorus *et al.*, 1992a).

Haploid larch has been regenerated through gynogenesis from female gametophyte(Nagmani & Bonga, 1985), what could be the basis for production of homozygous dihaploid clones with superior growth characteristics. Recovery of plantlets from embryogenic protoplasts of *Larix* x *eurolepis*(Klimaszewska, 1989) and *Picea glauca* (Attree *et al.*, 1989) has been recently demonstrated. Conifer ESM, somatic embryos and artificial seeds have been shown to be amenable to long-term storage under sub-zero temperuture and in liquid nitrogen(*e.g.*, Attree & Fowke, 1993 ; Gupta *et al.*, 1987 ; Klimaszewska *et al.*, 1992 ; Laine *et al.*, 1992). Techniques for production of transgenic conifers based on microprojectile bombardment of embryogenic cells and protoplasts are now in the course of development(Ellis *et al.*, 1993 ; Robertson *et al.*, 1992).

Very important question from genetical point of view, is what type of explant tissue is used for *in vitro* clonal propagation. There may be three levels of "cloning success" in tree breeding programmes. The low level is related to propagation of half-sib seeds, each cell line from which is of unproven genotype. At the medium level, full-sib seeds from controlled crosses are used. The progeny prediction can be reliable in that case if only genetics of both parental trees has been examined. And the high level of "cloning success" is associated with the propagation through non-embryonic, *viz.* vegetative, tissues of mature trees, when clonal progeny is genotypically and phenotypically similar to maternal tree.

In relation to conifer somatic embryogenesis, this points to particular importance of recovery of somatic embryos from vegetative tissues of mature trees. However, the level of embryogenic activity in conifers dramatically decreases when

Table 2. Situation in conifer biotechnology prior and after the year 1985.

Goal	Prior	After
Multiplication rate	low	unlimited
True-to type and synchronous development	problematic	achieved
Clonal process control and automation	pessimism	optimism
Long-term germplasm storage with subsequent clonal propagation	rare cases, low efficiency	routine practice
Haploid plant production	impossible	possible in *Larix* spp.
Plant regeneration from protoplasts	no examples	several examples
Regeneration of trangenic plants	impossible	in the course of development

turning even from immature to mature zygotic embryos(Fig. 2). Hence the "window of embryogenic competence" of the most coniferous species, except the spruces, is rather narrow. Very recently Unilever Co. has filed the patent application for the initiation of somatic embryogenesis from vegetative tissues(*e.g.*, needles) taken from mature trees of Norway spruce(see Westcott, 1994), but the fine details of that procedure and the evidence for the presence of normal somatic embryos have not been presented. For all the other conifers, production of somatic embryos is as yet possible, in the best case, from mature zygotic embryos only(*e.g.*, in *Abies* spp.). The genus *Pinus* is known as the most recalcitrant conifer group with the "window of embryogenic competence" involving immature precotyledonary zygotic embryos only(Fig. 2).

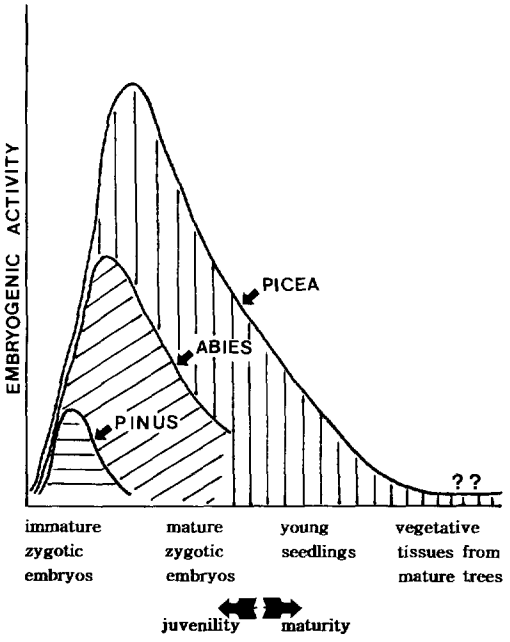

Fig. 2. The "window of embryogenic competence" in *Abies*, *Picea* and *Pinus*.

About ten years of active research on conifer somatic embryogenesis have resulted in development of efficient culture protocols for plant regeneration. The most sophisticated protocols sharing much common traits and the potentialities for their scaling-up have been elaborated for spruces (Fig. 3). They consist of five consecutive steps requiring different nutrient media and physical conditions. The first step includes initiation and maintenance of ESM in the presence of auxin, cytokinin, amide nitrogen, and with specific

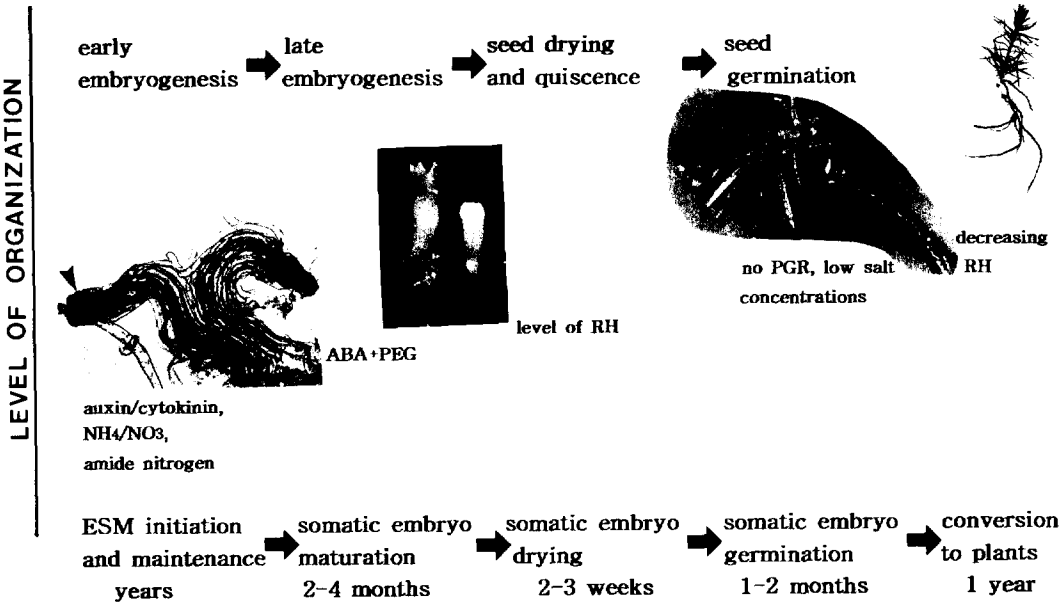

Fig. 3. Spruce plant development through somatic embryogenesis.

ammonium/nitrate molar ratio(*e.g.*, Bozhkov et al., 1993). These events correspond developmentally to the early embryogenesis *in situ*. At the second step, ABA and polyethylene glycol(PEG) are applied simultaneously for somatic embryo maturation(Attree *et al.*, 1991 ; Cornu & Geoffrion, 1990 ; Gupta & Pullman, 1991). This step is similar to the late embryogenesis phase *in situ*. The mature somatic embryos are then subjected to a drying under the fixed level of relative humidity(the third step), what mimics natural seed drying and quiscence(Roberts *et al.*, 1990, 1991 ; Bozhkov, 1994). The fourth step involves somatic embryo germination under the absence of plant growth regulators and low salt concentrations in nutrient medium(analogous to seed germination). Somatic plantlets with well-developed roots and shoots are eventually transferred *ex vitro* under gradually decreasing relative humidity(the fifth step ; Fig. 3).

Such a type of culture protocol takes into account much of the physical-chemical situation during *in situ* embryogenesis and seed germination. So, biotechnologists should always refer to data of basic research on physiology and biochemistry of embryogenesis and seed development, and, in certain instances, fill some gaps in basic knowledge themselves.

A gain from that approach was especially pronounced when elaborating procedures for development of somatic embryos to plants. As recently as five years ago, it was very difficult or even impossible to obtain mature somatic embryos and plantlets of a high quality in conifers. Today this process may already be scaled-up for several species due to the combined application of ABA and PEG with following full or partial drying of somatic embryos.

It has been clearly demonstrated, that ABA and PEG inhibit cleavage polyembryony, and promote both synchronous maturation and acquisition of desiccation tolerance(see Attree & Fowke, 1993). The mechanism of combined action of ABA and PEG on embryo development is likely as following. Abscisic acid activates genes responsive to ABA(*rab*-genes), that leads to the accumulation of mRNA controlling biosynthesis of storage products(Skriver & Mundy, 1990). Polyethylene glycol induces non-plasmolysing osmotic stress(see Attree *et al.*, 1991) resulted in translation of complete set of storage products, namely : storage proteins, late-embryogenesis abandant proteins (LEA), lipids and starch(Attree *et al.*, 1992 ; Misra *et al.*, 1993). Consequently, ABA acts at transcriptional level, and PEG activates following biosynthesis at posttransciptional level through moisture stress(Fig. 4). It is an elegant example of additive effect of two compounds on developmental process.

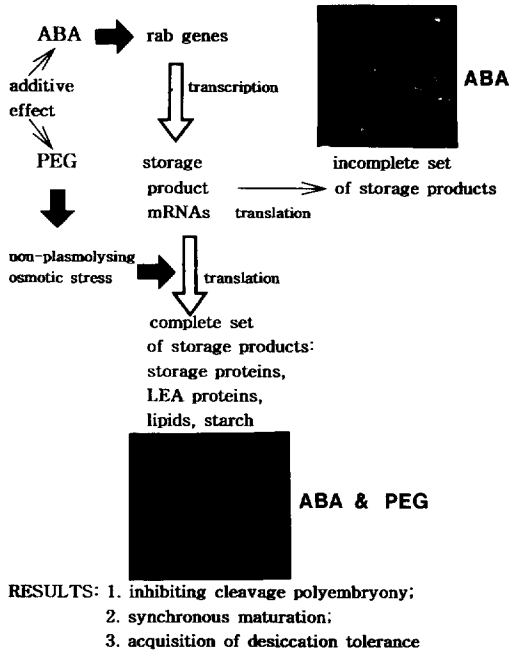

Fig. 4. Physiological basis of the combined effect of ABA and PEG on somatic embryo development in conifers.

Seed drying is necessary to switch developmental programme from maturation to a normal germination(Kermode & Bewley, 1985 ; Kermode *et al.*, 1989). Similarly, partial or full drying of mature somatic embryos of conifers increases germination frequency, decreases germination time and synchronizes root and shoot elongation(Roberts *et al.*, 1990, 1991 ; Bozhkov, 1994). It is proposed that these responses may be due : (1)activation of translation of proteins required for germination (Galau *et al.*, 1991 ; Leal & Misra, 1993) ; (2)re-

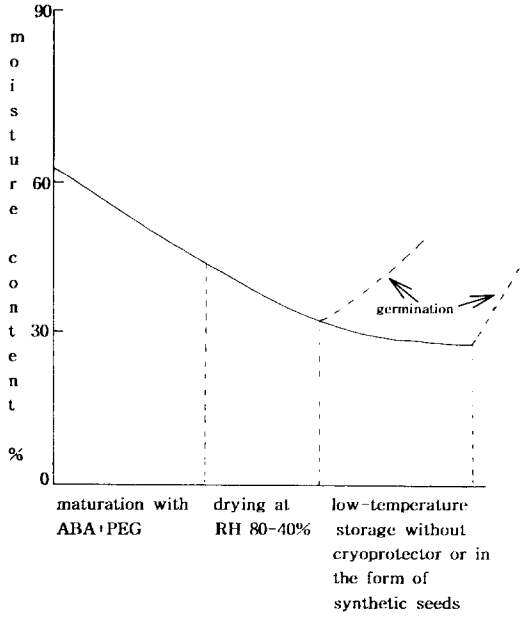

Fig. 5. Pattern of changes in moisture content of spruce somatic embryos in the course of maturation, drying, storage and germination(compiled from data of Attree *et al.*, 1991, 1992 ; Attree & Fowke, 1993).

ducing endogenous level of ABA(Bozhkov, 1994 ; Kermode, 1990), and (3)organization of normally developed shoot meristems(likely through the effect of dehydrotation on auxin metabolism)(Kong & Young, 1992).

Fig. 5 illustrates the pattern of changes in moisture content of spruce somatic embryos in the course of maturation, drying in environments with relative humidity decreasing from 80% to about 40%, storage and germination(see Attree *et al.*, 1991, 1992). Loss in moisture content from about 60% to about 45-50% as a result of maturation treatment with ABA and PEG, and then to less than 32% through drying of mature somatic embryos allows long-term storage of dried somatic embryos or synthetic seeds without cryoprotector and/or true-to-type and rapid germination(Fig. 5 ; see Attree & Fowke, 1993).

In several cases these advances in manipulations with conifer somatic embryos have resulted in large-scale production of high-quality plantlets for nursery growth evaluation tests. It has been conducted with *Picea abies*(Gupta *et al.*, 1993), *Picea glauca* x *engelmanii* complex(Webster *et al.*, 1990), and *Pseudotsuga menziesii*(Gupta *et al.*, 1993). These tests have proved a good growth rate(similar to seedlings) and clonal fidelity of propagules originated through somatic embryogenesis. Within a few years, randomly amplified polymorphic DNA(RAPD) analysis is proposed will be an efficient tool in certification of quality of conifer clonal stock produced by biotechnological cloning systems (*e.g.*, Isabel *et al.*, 1993).

CONCLUSIONS

Because conifers are outbreeders, and each embryogenic cell line is of individual and unproved genotype, the induction of somatic embryo development from non-embryonic tissues of mature trees is desirable. If it will be really achieved, the basic question of whether the cleavage polyembryony or some other type of morphogenetic process is involved in embryogenesis from vegetative cells will be answered. In other words, does the vegetative cell memorize the developmental programme of zygote?

In order to solve this problem, we need much more fundamental information. There are at least three points of special interest. At first, the mucilage from ESM contains some stimuli inducing and/or promoting morphogenesis in the cells of foreign explants(Bozhkov, 1995a ; Durzan, 1989 ; Westcott, 1994). Consequently, investigation on chemical composition of mucilage appears very promising. Secondly, detection and cloning of "embryogenic" and "cleavage capacity" genes will enable us to transform callus- or primary vegetative tissue-originated protoplasts by microprojectiles carrying these genes. And at third, the study on cell signal transduction, involved in embryogenic response of synchronously dividing callus cells, has much potential for yielding information about embryogenic redifferentiation of vegetative cells of mature conifers growing *in vitro*.

The future of conifer biotechnology may probably lie with so-called model-reference adaptive control system(MRAC)(see Durzan, 1988). The

main unit of the MRAC system, adaptive controller, should coordinate the current conditions in embryogenic suspension bioreactor with physical-chemical parameters of zygotic embryogenesis model. In other words, the response of cells in suspension culture should mimic the zygotic model system, *i.e.* the response is "biomimetic". Unquestionably, the development of MRAC system requires not only intimate knowledge of physical, biochemical and molecular processes of conifer embryo development, but also their mathematical interpretation.

In this connection, we would like to cite the words of Professor Don Durzan. "This is indeed a major challenge that will take years of study, but one which will be very worthwhile in scientific returns and principles that will ensure superior products for certification and commercialization"(Durzan, 1988).

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