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Endogenous Factors that Regulate Plant Embryogenesis: Recent Advances

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ABSTRACT

In seed plants, embryogenesis is an important process to produce a new generation. It comprises three steps: establishment of organization as an embryo, accumulation of storage substances in the embryo, and acquisition of desiccation tolerance and seed dormancy. These steps are accurately regulated by many factors, including phytohormones, proteins, transcription factors, and other substances related to embryogenesis. The embryogenesis mechanism has been analyzed through biochemical, biological, and molecular approaches using embryo-defective mutants or somatic embryogenesis whose traits are similar to zygotic embryogenesis, both morphologically and physiologically. Appropriate auxin transport plays an important role in the formation of cotyledon and meristem during early embryogenesis. Some transcription factors (*LEC1*, *ABI3*, *LEC2*, and *FUS3*) that have been isolated from embryo-defective mutants are characterized as embryo-related genes. Among them, some transcription factors are related to phytohormone signaling. *ABI3* and ABA regulate the expression of the LEA gene, whose proteins are accumulated during late embryogenesis. Also, *LEC2* and *FUS3* negatively regulate bioactive GA synthesis. On the other hand, some regulatory factors have been isolated and identified from culture medium during somatic embryogenesis. The factors are low molecular substances such as the phenolic compounds (4HBA, VBE and 4PMP) that inhibit somatic embryogenesis, or the peptidyl growth factor, PSK, which stimulates somatic embryogenesis. Here, we review recent findings of various factors regulating plant embryogenesis.

Keywords: phenolic compounds, phytohormone, seed plants, somatic embryo, transcription factor, zygotic embryo
Abbreviations: ABA, abscisic acid; **ABI3**, ABA-insensitive3; **ABRE**, ABA responsive element; **AGL15**, AGAMOUS-like 15; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **EC**, embryogenic cells; **FUS3**, FUSCA3; **GA**, gibberellic acid; **4HBA**, 4-hydroxybenzyl alcohol; **LEA proteins**, late-embryogenesis abundant proteins; **LEC**, LEAFY COTYLEDON; **PIN**, PIN-FORMED; **PLT**, PLETHORA; **4PMP**, 4-[(phenylmethoxy)methyl] phenol; **PSK**, phytosulfokine; **SAM**, shoot apical meristem; **VBE**, vanillyl benzyl ether; **VP1**, VIVIPAROUS 1

CONTENTS

INTRODUCTION

In seed plants, embryogenesis is an important morphogenesis involving drastic changes by which an individual is born from a fertilized egg. A zygote divides transversely and asymmetrically to form a small apical cell and a large basal cell. The apical cell develops to the embryo proper, and the basal cell develops to the suspensor, which remains attached to the mother tissue and provides nutrients and growth regulators for development of the embryo proper. Development of the embryo proper is distinguishable as three steps: establishment of organization as an embryo, accumulation of storage substances in the embryo, and acquisition of desiccation tolerance and seed dormancy.

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Invited Mini-Review

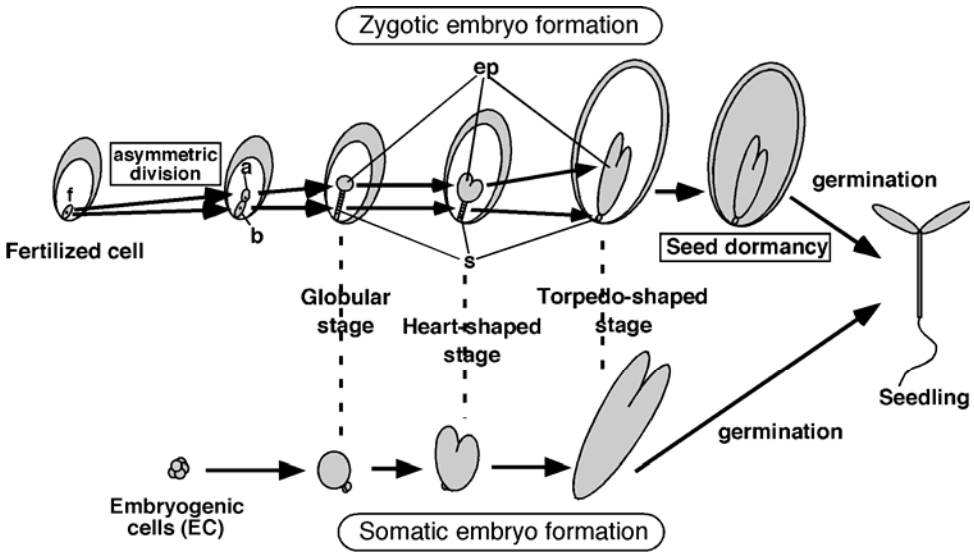

Fig. 1 Model scheme of zygotic and somatic embryo formation in angiosperms. Somatic embryogenesis is morphologically and developmentally similar to zygotic embryogenesis in both spatial and temporal aspects: a, apical cell; b, basal cell; ep, embryo proper; f, fertilized egg; s, suspensor.

These steps are regulated accurately by many factors, including phytohormones, proteins, transcription factors, and other substances related to embryogenesis.

Fertilization and the subsequent development of zygotic embryos occur deep inside both the endosperm and maternal tissue (West and Harada 1993). The physical inaccessibility of zygotic embryos renders biochemical and molecular analyses of zygotic embryogenesis difficult. Therefore, little is known about early events of embryogenesis. However, somatic cells that have embryogenic potential are useful to produce morphologically and developmentally normal mature plants. Since the first reports on somatic embryogenesis in carrot (Stewart *et al*. 1958; Reinert 1959), somatic embryos have been obtained in various plants. The process of plantlet induction from somatic cells is called somatic embryogenesis, in which development of somatic embryos closely resembles that of zygotic embryos. In addition, the spatial and temporal aspects of the programs of gene expression and the accumulation of storage proteins appear to be similar in somatic and zygotic embryos (**Fig. 1**, Zimmerman 1993). For those reasons, somatic embryogenesis is useful as a model system to investigate the plant embryogenesis mechanism. Somatic embryogenesis also plays an important role in clonal propagation (Williams 1987). The system is a valuable tool to advance the genetic improvement of various crops using conventional breeding programs. It is important for many researchers to understand the factors that regulate plant embryogenesis. In this review, we summarize recent works on some important factors affecting the development of plant embryos to elucidate the possibility of somatic embryogenesis.

MAJOR TRANSCRIPTION GENES REGULATING PLANT EMBRYOGENESIS

LEAFY COTYLEDON 1 (*LEC1*)

Four transcription factors (*LEC1*, *LEC2*, *FUS3*, and *ABI3*) that have been isolated from *Arabidopsis* embryo-defective mutants control many aspects of embryo development and maturation during zygotic and somatic embryogenesis (reviewed in Baumbusch 2006). One of them, *LEC1*, encodes a HAP3 subunit of the CCAAT-binding transcription factor (Lotan *et al*. 1998). *LEC1* expression is seed-specific, and the ectopic expression of *LEC1* in transgenic plants induces formation of somatic embryo-like structures. Embryos of *lec1* mutant exhibit abnormal embryos with trichomes on the cotyledons, and loss of desiccation tolerance and seed storage proteins (Vicient *et al*. 2000; Brocard-Gifford *et al*. 2003). In addition to *LEC1*, *LEC1-like* (*L1L*) is known as a *LEC1*-type HAP3 gene. Expression of *L1L* is also observed in developing embryos, and the *lec1* mutation is rescued by *L1L* ectopic expression (Kwong *et al*. 2003). Between the LEC1 and L1L, the B domain is highly conserved and Asp-55 is most important for these protein functions in developing embryos (Lee *et al*. 2003).

FUSCA 3 (*FUS3*)

FUS3 encodes a B3 domain-containing protein, whose gene expression is observed in developing embryos from a very early stage to immediately before germination (Luerßen *et al*. 1998). Embryos of the *fusca3* mutant (*fus3*) produce trichomes on the cotyledons and exhibit increased accumulations of anthocyanin and decreased accumulations of seed storage proteins compared to wild-type embryos (Bäumlein *et al*. 1994; Keith *et al*. 1994). The FUS3 protein binds directly to RY motif, which is conserved in the promoter of many seed-specific genes and which regulates the expression of these genes during embryogenesis (Kroj *et al*. 2003; Mönke *et al*. 2004). Induction of *FUS3* gene expressions in the L1 layer of the shoot apical meristem (SAM) using *AtML1* promoter produces cotyledon-like organs in the transgenic *Arabidopsis* SAM (Gazzarrini *et al*. 2004).

LEAFY COTYLEDON 2 (*LEC2*)

LEC2 encodes B3-domain-contaning protein, which is related closely to FUS3 (Stone *et al*. 2001). Embryos of the *lec2* produce trichomes on the cotyledons and display abnormal suspensor morphology. Expression of *LEC2* is silique-specific, and ectopic expression of the *LEC2* gene induces the formation of somatic embryo-like structures; it often confers embryonic characteristics to seedlings. LEC2 protein binds to RY motif upstream of target genes (Braybrook *et al*. 2006). Expression of *LEC2* gene in leaves regulates the gene expression of *ABI3*, *FUS3*, and *LEC1* genes and seed-specific lipids accumulation (Santos-Mendoza *et al*. 2005). In *lec2* mutant, the expression of *FUS3: GUS* and *ABI3:GUS* is reduced. The seed phenotype is partially restored by introgression of *35S:FUS* or *35S:ABI3* genes (To *et al*. 2006). These results indicate that *LEC2* functions in the upstream of *ABI3* and *FUS3* in some cases.

ABA-Insensitive 3 (*ABI3*) / *Viviparous 1* (*VP1*)

Arabidopsis abi3 and maize *vp1* are seed-specific ABA-insensitive mutants. Seeds of these mutants undergo viviparous germination, have no seed dormancy, acquire no desiccation tolerance, and accumulate few seed storage proteins. *ABI3/VP1* contains some conserved domains (B1, B2, and B3), of which B2 and B3 might be involved in seed-specific gene expression. Expression of *ABI3/VP1* is observed mainly in embryos; *ABI3/VP1* expression during zy-

gotic embryogenesis begins at a very early stage and is detectable continuously until the late stage of embryogenesis. The expression of *ABI3* is also regulated by LEC1, LEC2, FUS3 and ABI3 itself (To *et al*. 2006).

On the other hand, ABI3 protein controls ABA-induced *Late-Embryogenesis Abundant* (*LEA*) gene during the late stage of embryogenesis expression (Parcy *et al*. 1994). Analyses of the mechanisms regulating the expression of seed-specific ABA-inducible genes (*Em* and *Osem*) suggest that the B2 domain of *ABI3/VP1* regulates the expression of ABA-inducible genes via the *cis*-regulatory ABA responsive element (ABRE), which resembles the G-box element (Marcotte *et al*. 1989; Hattori *et al*. 1995). In the regulatory scheme, *ABI3/VP1* does not bind to ABRE directly, but might bind via formation of a complex with bZIP proteins (Nakagawa *et al*. 1996; Lopez-Molina *et al*. 2002; Lara *et al*. 2003). These results suggest that ABI3 is an important factor in various ABA responses during embryo development and germination.

Function of the transcription factors in somatic embryogenesis

Expression of *LEC1* and *LEC1*-homologs is observed with the same pattern during somatic embryogenesis in *Arabidopsis*, maize, and carrot (Ikeda-Iwai *et al*. 2002; Zhang *et al*. 2002; Yazawa *et al*. 2004). *In-situ* hybridization analysis reveals that the expression patterns of *ZmLEC1* and *C-LEC1* are similar in zygotic and somatic embryos (Zhang *et al*. 2002; Yazawa *et al*. 2003). Expression of *FUS3* occurs in somatic embryos of *Arabidopsis* (Ikeda-Iwai *et al*. 2002, 2003), but expression of *LEC2* during somatic embryogenesis remains to be examined. In *lec1*, *fus3*, and *lec2* single and double mutants, the frequency of somatic embryo formation is very low (Gaj *et al*. 2005), which suggests that these factors have an important function in formation of plant somatic embryo.

ABI3 gene expression is also observed in somatic embryos of *Arabidopsis* (Ikeda-Iwai *et al*. 2002, 2003) and carrot (Shiota *et al*. 1998). The data indicate that ABI3 also regulates ABA signal transduction in somatic embryos. However, somatic embryos might be formed. Ikeda-Iwai *et al*. (2002, 2003) showed *ABI3* gene expression in somatic embryos and embryonic cultures of *Arabidopsis*. However, *abi3-6*, a null mutant of *ABI3*, suggests that *ABI3* is not an essential factor for somatic embryo formation (Umehara and Kamada 2004).

LOW MOLECULAR WEIGHT SUBSTANCES

Auxin

Auxin is an important phytohormone in many processes during plant development. In early embryogenesis, auxin transport and distribution mainly affect the embryo axis formation. Here, we review PIN proteins, which serve an important role in auxin polar transport, although many reports describe effects of auxin in embryogenesis. Auxin polar transport in embryogenesis was reported in early studies using *in-vitro* culture of *Brassica juncea* zygotic embryos (Liu *et al*. 1993; Hadfi *et al*. 1998). Embryos, treated with anti-auxin or an inhibitor of auxin transport during early embryogenesis, lose polarity and develop abnormally shaped cotyledons and hypocotyls. In *Arabidopsis*, the *pin-formed* (*pin*) mutant has been isolated as a mutant of flower phenotype, characterized by diminished polar auxin transport (Okada *et al*. 1991). This phenotype can be mimicked by chemical inhibition of polar auxin transport.

The activity of the synthetic auxin-responsive promoter *DR5* has been used to visualize the spatial pattern of the auxin response, which indirectly reflects the distribution of auxin (Sabatini *et al*. 1999). By measuring this activity, Friml *et al*. (2003) investigated auxin distribution using a reporter gene, *DR5rev::GFP*, to monitor the auxin response and to examine the expression and localization of the *AtPIN* family of genes. During early embryogenesis (at the two-cell stage), PIN1 is located in border membranes between cells of the embryo proper, and auxin is provided to the embryo proper by the suspensor via PIN7. After the 32-cell stage, auxin is synthesized within the apical region of the embryo proper. It is accumulated within the hypophysis via PIN1, PIN3, and PIN4, and is transported to suspensor cells via PIN7. In the early stage of embryogenesis, auxin accumulates in the embryo proper and triggers apical pole specification. In later-stage embryos, the direction of auxin transport reverses, and its accumulation within the hypophysis triggers root pole specification. Among eight PIN proteins, four PIN proteins (PIN1, PIN3, PIN4, and PIN7) are active in *Arabidopsis* embryos. These PIN proteins, especially PIN1and PIN4, not only play a central role in the establishment of these auxin gradients: they are essential components in the robust maintenance of the gradients (Weijers *et al*. 2005). In *Arabidopsis* somatic embryogenesis, not only complete somatic embryos but also adventitious and fused shoots are often formed (Bassuner *et al*. 2007). In abnormal shoots, *PIN4* expression is not observed at the base of shoots. For establishment of a root meristem in somatic embryos, maintenance of appropriate auxin levels through PIN proteins is required during the course of their development.

Furthermore, PIN proteins interact with *PLETHORA* (*PLT*) genes, major determinants for root stem cell specification (Blilou *et al*. 2005). Also, PIN proteins restrict *PLT* expression in the basal region of the embryo proper to initiate root primordium formation. Then *PLT* genes maintain PIN transcription, which stabilizes the position of the distal stem cell niche.

In addition to PIN proteins, MONOPTEROS, BODENLOS, and some proteins are involved in auxin transport or response. Interactions between these proteins and PIN proteins should also be considered for auxin transport and response during embryogenesis.

Gibberellic acid (GA)

GA is a tetracyclic diterpenoid that is an essential endogenous regulator of plant growth and development. Earlier studies demonstrated that endogenous GA levels in the suspensor are higher than that in the embryo proper, and that GA might play an important role in early embryo development for *Phaseolus* (Alpi *et al*. 1975), *Tropaeolum*, and *Cytisus* (Picciarelli *et al*. 1984). In pea, GA produced in the embryo is necessary for normal seed growth and survival (Swain *et al*. 1997). In microspore-derived embryos of *Brassica napus*, GA is required for elongation of the cell and embryo axis (Hays *et al*. 2002).

In 2003, the opposite results were proposed by Tokuji *et al*. and Mitsuhashi *et al*. on the effect of gibberellin in carrot somatic embryogenesis. Tokuji *et al*. (2003) demonstrated that GA inhibits somatic embryogenesis, but Mitsuhashi *et al*. (2003) showed that GA is required for somatic embryogenesis. We would like to specifically address the explants that they used as the plant material. To induce somatic embryogenesis, the former used hypocotyls (somatic cells) as the explant, the latter used embryogenic cells (EC) which had acquired embryogenic potential.

On the other hand, postembryonic expression of *LEC2* induces the formation of somatic embryos on the cotyledon (Stone *et al*. 2001). The gene expression of *AtGA3ox2*, which encodes the key enzyme AtGA3ox2, which catalyzes the ultimate step of bioactive GA synthesis, is negatively regulated by transcriptional factor, *LEC2* and *FUS3* in *Arabidopsis* (Curaba *et al*. 2004; Gazzarrini *et al*. 2004). *FUS3* represses *AtGA3ox2* expression mainly in epidermal cells of the embryo axis. *AtGA2ox6*, which encodes AtGA2ox6, which converts active GA to inactive GA, is regulated by AGL15, a member of the MADS domain family of DNA binding transcriptional regulators (Wang *et al*. 2004). Although somatic embryos are formed when *AGL15* constitutively expresses, the frequency of somatic embryo forma-

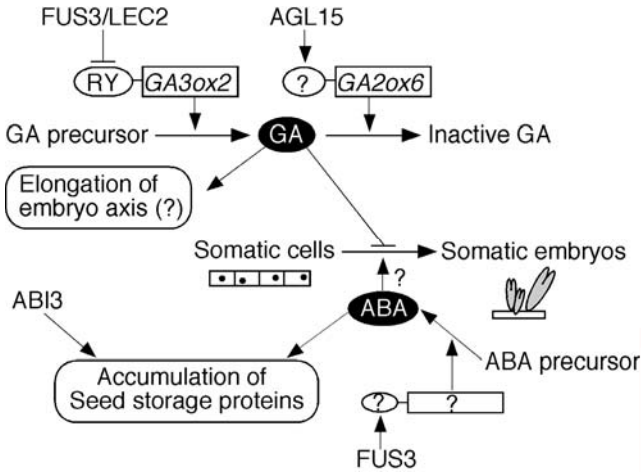

Fig. 2 A hypothetical model of the roles of GA and ABA during embryogenesis. GA, whose production is regulated by some transcription factors, might maintain the identity of somatic cells (e.g. epidermal cells). ABA, whose production is also regulated by the transcription factor, might stimulate somatic embryo formation in somatic cells. Both GA and ABA might act competitively and thereby affect determination of the cell fate directly or indirectly.

tion, which is reduced in plants, decreases expression of *AtGA2ox6*. Addition of uniconazole or paclobutrazol, which are inhibitors of GA synthesis, promotes secondary embryos from the primary embryo (Tokuji *et al*. 2003) or increases the percentage of seedlings that produce somatic embryos (Wang *et al*. 2004).

These results indicate that GA is necessary for normal development of the embryo proper, but maintains the identity of somatic cells, not to differentiate to the embryos (**Fig. 2**). It is considered that GA affects not only elongation of the embryo axis: GA synthesis is related to acquisition of embryonic potential in somatic cells.

Abscisic acid (ABA)

In plant embryogenesis, ABA is synthesized at the late stage of embryogenesis and controls the acquisition of desiccation tolerance and seed dormancy. In *Arabidopsis* ABA-deficient mutants, embryogenesis progresses normally but the mutants have no desiccation tolerance or seed dormancy. It is not considered that ABA is directly related to zygotic embryo development.

However, somatic embryogenesis can be induced by treatment with various stresses such as high osmotic stress and high temperature under a 2,4-D-free condition in carrot (Kikuchi *et al*. 2006). After various stresses, ABA is known to increase. Therefore, it has been proposed that increased endogenous ABA levels might induce somatic embryogenesis. Some reports suggest that ABA is involved in somatic embryogenesis. Treatment with 10^{-4} M ABA induces somatic embryo formation in carrot apical tip explants (Nishiwaki *et al*. 2000). Somatic embryos (primary embryos) form from seed coats without auxin treatment (Ogata *et al*. 2005). Subsequently, many secondary embryos are produced on the hypoctyl and root region of the primary embryos. The primary embryos contain higher levels of ABA than secondary embryos. Formation of secondary embryos is suppressed by treatment with fluridone, an ABA-synthesis inhibitor. Kikuchi *et al*. (2006) reported that ABA is required for acquisition of embryogenic competence of somatic cells using carrot stress-inducible somatic embryogenesis. These results indicate the possibility of mutually competitive roles of ABA and GA in embryogenesis.

On the other hand, different results are shown in *Arabidopsis*. Using stress-inducible somatic embryogenesis in *Arabidopsis* (Ikeda-Iwai *et al*. 2003), the effect of ABA was investigated (Umehara and Kamada 2004). Somatic embryogenesis is inhibited by treatment with fluridone, and is recovered by addition of ABA. However, somatic embryos are induced from ABA deficient mutants and transformants; they are rather inhibited by addition of ABA (Umehara and Kamada 2004). Gaj *et al*. (2006) also shows that the efficiency of somatic embryo induction in ABA deficient mutants is comparable to that of the wild type in *Arabidopsis* somatic embryogenesis. In *Arabidopsis*, addition of 2,4-D is required for stress-inducible somatic embryogenesis, different from that of carrot. Although endogenous ABA content increases during seed maturation, no somatic embryo forms within the seed. Other factor(s) might be considered to act during somatic embryogenesis to connect these inconsistent results.

Phytosulfokine

Somatic embryogenesis depends on several modulatory substances, some of which accumulate in culture medium. From culture medium of *Asparagus officinalis* L., Matsubayashi and Sakagami (1996) isolated phytosulfokine (PSK), the sulfated pentapeptide H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH, which stimulates mitogenic activity in mesophyll cells of *Asparagus*. The effects of PSK on cell division and morphogenesis have also been examined in various species of angiosperms, including *Oryza sativa*, *Arabidopsis thaliana*, *Zinnia elegans*, and asparagus (Matsubayashi personal communications). These facts suggest that PSK is a common peptidyl substance in seed plants and plays a basic role in plant growth and development.

In carrot, exogenously applied PSK stimulates somatic embryogenesis by activating cell division of embryogenic cells (EC; Kobayashi *et al*. 1999; Hanai *et al*. 2000). The stimulatory effect of PSK in somatic embryogenesis has been confirmed in gymnosperms: *Cryptomeria japonica* (Igasaki *et al*. 2003) and *Larix leptolepis* (Umehara *et al*. 2005b).

PSK stimulates cell division under the presence of auxin and cytokinin in asparagus (Matsubayashi *et al*. 1999) and under the presence of auxin in carrot (Eun *et al*. 2003). Matsubayashi *et al*. (2002) identified PSK receptor gene, which encodes leucine-rich repeat, a single transmembrane domain, and a cytoplasmic kinase domain. PSK binds the membrane-localized receptor PSKR1, which is a leucine-rich repeat receptor kinase (Matsubayashi *et al*. 2002). A loss-of-function mutant of PSK receptor gene, *pskr1-1*, exhibits morphologically normal growth until 3 weeks after germination (Matsubayashi *et al*. 2006). Therefore, PSK might be a supporting factor, rather than a critical factor, for cell proliferation and differentiation in embryogenesis.

Phenolic compounds

In carrot, EC, which have embryonic competence, are induced when explants are cultured on medium containing 2,4-D (Kamada and Harada 1979). After transfer of EC to medium without 2,4-D, somatic embryos form from EC. However, when EC are cultured at high cell density, somatic embryogenesis is strongly inhibited, even with the use of 2,4-D-free medium (Fridborg *et al*. 1978). The efficiency of somatic embryo formation can be improved by adding activated charcoal, which absorbs some inhibitory factors. Various phenolic compounds are accumulated in culture medium when charcoal is not present. For this reason, phenolic compounds have long been thought to inhibit somatic embryogenesis. An inhibitory factor isolated from the carrot culture medium was first characterized as a 4-hydroxybenzyl alcohol (4HBA; Kobayashi *et al*. 2000a). 4HBA specifically inhibits rapid cell division during the early globular stage of somatic embryogenesis (Kobayashi *et al*. 2000b) and accumulates in the medium during the early days of culture (Kobayashi *et al*. 2001). The production and inhibitory effects of 4HBA have been found not only in somatic embryogenesis but also in zygotic embryo formation of carrot (Kobayashi *et al*. 2003).

In some conifers, somatic embryogenesis is strongly inhibited when cell density is high. In somatic embryogenesis

of Japanese larch, some conditioning factors suppress somatic embryogenesis by blocking the development of the suspensor in high-cell-density culture (Umehara *et al*. 2004a). Somatic embryogenesis occurs under high-cell-density conditions in the medium with activated charcoal. An inhibitory factor that accumulates in the medium can be purified; vanillyl benzyl ether (VBE) has been identified from such a culture medium (Umehara *et al*. 2005a). VBE is accumulated at high concentrations (greater than 10^{-5} M) in high-cell-density cultures. However, this inhibitory effect is smaller than that of the addition of medium cultured at high cell density. Therefore, VBE is a main inhibitory conditioning factor that regulates suspensor development, but other inhibitory factors remain to be discovered. Recently, another complementary inhibitory factor was identified as 4-[(phenylmethoxy)methyl] phenol (4PMP), which had a similar chemical structure to VBE (Umehara *et al*. 2007). However, the physiological meaning of these phenolic compounds in seed development remains unknown. At least, these phenolic compounds should be eliminated to improve the conifer tissue culture system.

PERSPECTIVES

Embryogenesis is a complex process that is regulated by various factors including phytohormones, proteins, and transcription factors. Many chemical substances act in gene expression as signals, and the correct expression is required for normal and rapid development of the embryos. Each factor controlling embryogenesis has been investigated using somatic and zygotic embryos in various plants. Recently, novel factors affecting plant embryogenesis have been identified; cross-linking between phytohormone and transcription factors is likely to display a part of embryogenesis. However, the mechanism of plant embryogenesis remains partially unclear. Recently, results of epigenetic approaches suggest that DNA methylation and chromatin modification are also important factors for plant embryogenesis (Reyes 2006; Xiao *et al*. 2006). Future studies will clarify and connect the interactions of these factors, thereby revealing the entire embryogenesis regulatory mechanism.

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JAPANESE ABSTRACT

種子植物において、胚発生は次世代を作る重要な形態形成である。それには胚としての基本構造の確立、貯蔵タンパク質の蓄積、乾燥耐性獲得と種子休眠という大きく３つの過程が含まれ、植物ホルモン、タンパク質、転写因子など様々な物質によって精密に制御されている。これまで、異常な胚発生を示す変異体や種子胚に似た形態変化を示す不定胚形成系を用いて、胚発生のメカニズムについて調べられてきた。例えば、植物ホルモンでは、オーキシンの極性輸送が初期胚の子葉や分裂組織の発達に重要である。また、胚発生に関与する転写因子（*LEC1*, *ABI3*, *LEC2*, *FUS3*）のうち、*LEC2*, *FUS3*はジベレリンの生合成を負に制御し、*ABI3*はABAとともに後期胚発生中に作られるLEAタンパク質の合成を制御している。一方、不定胚の培養培地から胚発生を制御している物質が新たに単離されている。この総説では、植物の胚発生を制御する因子の最近の知見について紹介する。