Plant Cell Monogr (2)
A. Mujib · J. Šamaj: Somatic Embryogenesis
DOI 10.1007/7089_034/Published online: 30 November 2005

Participation of Plant Hormones in Determination and Progression of Somatic Embryogenesis

Víctor M. Jiménez[1] (✉) · Clément Thomas[2]

[1]CIGRAS, Universidad de Costa Rica, 2060 San Pedro, Costa Rica
vjimenez@cariari.ucr.ac.cr

[2]Plant Molecular Biology, CRP-Santé, Bâtiment modulaire,
84 Val Fleuri, 1526 Luxembourg, Luxembourg
clement.thomas@crp-sante.lu

Abstract In vitro culture protocols have been developed for many species, mainly using empirical approaches, to induce somatic embryogenesis from various explant types. However, the underlying biochemical mechanisms governing induction, expression and maturation during somatic embryogenesis are still poorly understood. Among the signals that participate directly in the regulation of the different phases of this process, plant hormones emerged as candidates of choice. In this chapter, studies concerning the role of exogenously added plant growth regulators in somatic embryogenesis are reviewed. In addition, we discuss possible relationships between hormonal contents in starting explants and in cultures derived from them with their embryogenic competence. Moreover, information on evolution of endogenous plant hormone levels during induction and progression of somatic embryogenesis is presented. Finally, an overview of interactions between exogenous plant growth regulators and endogenous hormones in embryogenic systems is also included.

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
CK	Cytokinin
E	Embryogenic
GA	Gibberellin
GA_3	Gibberellic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
NAA	Naphthalene acetic acid
NE	Nonembryogenic
PGR	Plant growth regulator
PAT	Polar auxin transport
SE	Somatic embryogenesis
TIBA	2,3,4-Triiodobenzoic acid

1 Introduction

Plant hormones play a determinant role in practically every developmental process studied to date in plants, somatic embryogenesis (SE) being no exception. Substances classified as plant hormones are of organic nature and act at very low concentrations. Whether these compounds should be named hormones or not, taking into consideration the properties of the corresponding compounds in animal physiology, has been a topic of some debate during the past few years. The term plant growth regulator (PGR) has been proposed as an alternative that matches more precisely the characteristics of these substances, but has the disadvantage that it has been used to name synthetic substances of this class (for details refer to Davis 1995). In this chapter, the term plant hormone will be used to define the endogenous and naturally occurring substances in the tissues, while the expression PGR will refer to those exogenously added compounds, usually of synthetic origin.

Most studies on regulation of SE have focused on one or another of the several stages in which this process has been divided. The first one involves the induction stage, in which somatic tissues acquire, directly (without a dedifferentiation step) or indirectly (by dedifferentiating tissues already differentiated, usually involving a callus phase), embryogenic (E) competence. This stage is followed by the expression of SE, in which the competent cells or proembryos start developing, after receiving the proper stimulus, passing through the phases characteristic of zygotic embryo development, i.e., globular, heart-shaped and torpedo-shaped stages in dicots, globular, scutellar (transition) and coleoptilar stages in monocots, and globular, early cotyledonary and late cotyledonary embryos in conifers (Jiménez 2001). Finally, during maturation, somatic embryos prepare themselves for germination, by desiccating and accumulating reserves.

SE is a very complex developmental process that shares similar characteristics, mainly in morphology and anatomy, within the same group of plants (monocots, dicots, gymnosperms), but which, at the same time, differs in the requirements needed to induce and govern its determination and progression. This complexity has impeded fully understanding the biochemistry and physiology of SE, the role of plant hormones and PGRs included. Therefore, in spite of the large amount of research conducted on the involvement of plant hormones, but especially of PGRs during SE, the way they interact with the cells and tissues to render an observed response is still not clear. Therefore, for specific genotypes, trial-and-error experiments to establish the proper culture conditions and media, especially the type and level of PGRs to be used, are nowadays still common practice (Huang et al. 2004; Zhang et al. 2005).

The aim of this review is to summarize relevant and recent findings related to the involvement of plant hormones and PGRs in the determination and progression of SE. Whenever possible, review works will be cited to avoid

mentioning very large amounts of literature, although specific references, not always of recent publication, related to relevant findings will be also employed.

2
Effect of PGRs on SE

2.1
PGRs as Inducers of SE

The PGR composition of the culture medium is of prime importance to achieve the desired morphogenic reaction. In several culture systems, such as in *Arachis hypogaea* seedlings (Victor et al. 1999), *Juglans regia* embryonic axes (Fernández et al. 2000) and sunflower zygotic embryos (Thomas et al. 2004), the morphogenic pathway can be oriented through either shoot organogenesis or SE, only by modifying the PGR composition of the culture medium.

Among individual groups of PGRs, auxins are the most routinely used agents to mediate the transition from somatic to E cells. In more than 80% of 124 recently published protocols, induction of SE required the presence of auxins alone, or in combination with cytokinins (CKs) (Gaj 2004). The auxin most frequently used to initiate in vitro SE is the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), also known for its herbicide activity. Naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), Picloram and Dicamba are used to a lesser extent (Raemakers et al. 1995).

The mode by which 2,4-D efficiently induces E competence remains unclear. On the one hand, 2,4-D could regulate SE through its strong auxinic activity, either directly or indirectly, by influencing the endogenous metabolism of other phytohormones (Sect. 5). On the other hand, 2,4-D could act as a strong stressor leading to SE, considered by some authors as an extreme stress response of cultured plant cells (reviewed by Fehér et al. 2003). This hypothesis is supported by the fact that several stress treatments can trigger SE (reviewed by Gaj 2004).

In most instances in which CKs induced SE, they were added to the culture medium together with auxins (Gaj et al. 2004). However, in some cases, the addition of CKs as the sole source of PGR is sufficient to generate somatic embryos (Bronner et al. 1994; Iantcheva et al. 1999). The most commonly used CKs in culture are N^6-benzylaminopurine, kinetin, zeatin and, more recently, thidiazuron.

There are only a few reports of abscisic acid (ABA) acting as an effective inducer of SE, in most cases by producing somatic embryos directly on the surface of the explants (Bell et al. 1993; Charrière and Hahne 1998). In a relatively recent work, Nishiwaki et al. (2000) reported the formation of somatic embryos from carrot seedlings cultured on a medium containing ABA as the

sole source of PGRs. In this work, the number of embryos induced per number of seedlings was dependent on the seedling age as well as on the ABA concentration in the medium.

The effect of exogenously applied gibberellins (GAs) on induction of SE is highly variable in different species or tissues. For example, exogenous gibberellic acid (GA_3), the most commonly used synthetic GA, stimulated embryogenesis in both chickpea immature cotyledon cultures (Hita et al. 1997) and *Medicago sativa* tissue cultures (Ruduś et al. 2002), whereas it was detrimental to somatic embryo formation in geranium hypocotyl cultures (Hutchinson et al. 1997) and in *Citrus* ovule callus (Kochba et al. 1978). In carrot, the effect of GA on SE is also controversial. Tokuji and Kuriyama (2003) reported that GA_3 inhibited embryogenesis at the globular stage, while uniconazole, a GA biosynthesis inhibitor, promoted secondary embryogenesis when embryos were induced directly from carrot hypocotyl segments. In contrast, Mitsuhashi et al. (2003) observed that exogenous treatment with uniconazole caused a reduction of both the number of the developed embryos and the size of the torpedo-shaped embryos. These abnormalities in the latter case were prevented by GA_1 or GA_4 application.

There are several reports that support the inhibiting effect of external application of ethylene on induction of SE (reviewed by Minocha and Minocha 1995; Nomura and Komamine 1995; Thorpe 2000), while others indicate a neutral role (Roustan et al. 1994).

2.2 PGRs on Progression of SE

In most protocols in which auxins act as an efficient inducer of SE, development of somatic embryos is achieved by reducing or removing auxin from the culture medium. To explain this result, it was proposed that continuous exposition of explants to high exogenous auxin levels interferes with the polar auxin gradient that is normally established during embryogenesis, preventing the correct apical–basal embryo patterning (Schiavone and Cooke 1987; Liu et al. 1993).

The importance of polar auxin transport (PAT) in embryo morphogenesis was demonstrated by treating different stages of carrot somatic embryos with the PAT inhibitors 2,3,4-triiodobenzoic acid (TIBA) and *N*-(1-naphthyl)phthalamic acid (Schiavone and Cooke 1987; Cooke et al. 1993). Both inhibitors blocked the ability of somatic embryos to undergo morphogenic transitions to the subsequent stages. In a more recent experiment, Tokuji and Kuriyama (2003) treated carrot hypocotyls, in which SE was directly induced with a 24-h pulse of 2,4-D, with TIBA and 2,4,6-trichlorophenoxyacetic acid, another inhibitor of PAT, and found inhibition in the development of the somatic embryos, but not in the frequency of SE.

As will be pointed out later in this review (Sect. 3.2), formation of an auxin gradient appears to be necessary to establish bilateral symmetry dur-

ing the initial steps of embryogenesis, a requisite for further development of the embryos (Schiavone and Cooke 1987; Liu et al. 1993; Fischer and Neuhaus 1996).

An exogenous supply of CKs during the expression phase has produced ambiguous results. While some reports indicate an inductive role of CKs during progression of SE (e.g., Fujimura and Komamine 1975; Sagare et al. 2000), the contrary has also been described (Li and Demarly 1996). As mentioned later, CKs seem to play an important role in cell division, rather than in embryo differentiation (Danin et al. 1993).

Addition of GAs during progression of SE has also shown confusing outcomes. On one hand, it stimulated embryo development in chickpea, *Iris germanica* and *M. sativa*, while on the other hand, it inhibited this event in carrot, mandarin, orange and anise (Ruduś et al. 2002 and references therein). Later in the progress of SE, it was observed that in some species normally showing dormancy, adding GA_3 promotes germination and conversion of somatic embryos into plants (reviewed by Gaj 2004).

Maybe the most relevant effect of ABA during progression of SE has been reported for conifers. In this plant group, development of somatic embryos has to be stimulated by exogenous addition of ABA (reviewed by Dong and Dunstan 2000; Stasolla et al. 2002). A more general effect of ABA has been observed during maturation of somatic embryos in numerous species, especially, but not restricted to, conifers (Mauri and Manzanera 2004; Sharma et al. 2004). Indeed, similarly to the effect produced by the natural increase of endogenous ABA in zygotic embryos, the addition of ABA into the culture medium induces a reduction in precocious germination and an increase in the number of mature somatic embryos. However, an extensive duration of the treatment could influence negatively conversion of mature embryos into plantlets (von Arnold et al. 2002).

Again, there is a limited number of reports on the effect of addition of ethylene on the progression of SE. In one of them, Roustan et al. (1994) observed an arrest in embryo development only when ethylene was applied during the first 7 days in the expression stage of carrot SE. When this compound was included after that moment, no effect was evident.

3
Is E Competence of Explants Determined by Endogenous Hormones?

3.1
The Situation in Donor Explants

Some attempts have been made to associate the endogenous hormone contents of donor plant tissues, on one hand, and of callus or cell suspension cultures derived from them, on the other, with their E competence.

Concerning the donor tissues, there are several works that report differences among responsive and unresponsive explants, and thus support the participation of endogenous plant hormones on E competence. In this way, a correlation between higher endogenous IAA concentrations and an increased E response was reported in leaves of alfalfa (Ivanova et al. 1994), *Pennisetum purpureum* (Rajasekaran et al. 1987a) and *Dactylis glomerata* (Wenck et al. 1988), as well as in immature zygotic embryos of wheat (Kopertekh and Butenko 1995) and sunflower (Charrière et al. 1999; Thomas et al. 2002).

A relationship between the levels of ABA and the E competence of the initial explants was reported in the aforementioned work of Kopertekh and Butenko (1995) and also by Jiménez and Bangerth (2001a), both in wheat. The latter authors suggest that the effect of ABA on competence might occur by reducing precocious germination and then indirectly favoring callus formation. Similarly, Rajasekaran et al. (1987b) found higher concentrations of ABA in E than in nonembryogenic (NE) leaf sections of *P. purpureum*, while Ivanova et al. (1994) found the opposite in equivalent tissues of *M. falcata*. Additional support for the positive role of endogenous ABA in determining E competence of the donor tissues derives from the results of Senger et al. (2001), working with *Nicotiana plumbaginifolia*. They reported that both transgenic plants that overexpress an anti-ABA single-chain variable fragment antibody and mutants that have a defect in the ABA synthesis rate exhibit abnormal morphogenesis at preglobular embryoid formation. This phenotype could be reversed by simple exogenous ABA application.

Concerning CKs, lower levels of total CKs were observed in competent tissues in leaves of *P. purpureum* (Rajasekaran et al. 1987a) and *D. glomerata* (Wenck et al. 1988), as well as in immature zygotic embryos of wheat (Kopertekh and Butenko 1995), than in their noncompetent ones. Sometimes the factor to be considered is not the pattern of total CKs, but the levels of individual members of this group of plant hormones. For example, even though Centeno et al. (1997) did not find differences in the total amounts of CKs between competent and noncompetent genotypes of *Coryllus avellana*, they reported differences in the contents of the individual CKs evaluated.

Supporting the positive role of CKs during induction of SE, Tokuji and Kuriyama (2003) reported that purine riboside, an anti-CK, severely inhibited SE from epidermal cells of carrot. This effect was counteracted by the simultaneous application of zeatin riboside, suggesting that CKs are involved in the very early stages of SE, such as the formation of E cell clumps. These findings support the concept, mentioned before, that CKs have a role in cell division rather than in embryo differentiation (Danin et al. 1993).

Regarding GAs, there are contrasting reports about the role played by endogenous levels of this hormone in donor explants. There are several publications that do not show differences in GA levels in genotypes differing in their E competence (Jiménez and Bangerth 2001a, b), an indication of the minor role these compounds might play during this phase. Supporting these

findings, Rajasekaran et al. (1987a) observed that paclobutrazol, an inhibitor of GA synthesis, did not alter E nature of *P. purpureum* explants. However, other works suggest a negative role of endogenous GAs on E competence (Hutchinson et al. 1997).

Further evidence against a relationship between endogenous plant hormones in donor explants and their E competence comes from studies in maize (Jiménez and Bangerth 2001b) and asparagus (Limanton-Grevet et al. 2000) genotypes in which it was not possible to identify differences in the endogenous hormone contents in genotypes having different E capacity. Additionally, immature zygotic embryos of barley that contained variable IAA and GA levels displayed a similar degree of competence (Jimenez and Bangerth 2001c).

3.2
The Situation in Cultures with Distinct E Capacity

Some divergences have also been reported when endogenous hormone contents were evaluated in callus and cell suspension cultures varying in their degree of E capacity. Most works conducted with this purpose support the occurrence of higher auxin levels in E than in NE cultures (reviewed by Jiménez 2001). However, in other works, no differences in the endogenous auxin contents could be established between E and NE cultures (Besse et al. 1992; Michalczuk et al. 1992a). It was postulated that high endogenous auxin contents help to set up the auxin gradient necessary to establish bilateral symmetry during zygotic and SE (Schiavone and Cooke 1987; Liu et al. 1993; Fischer and Neuhaus 1996).

Higher levels of total CKs have been reported in NE than in E callus of *P. purpureum* (Rajasekaran et al. 1987a) and of *M. arborea* (Pintos et al. 2002). In addition, similarly to the aforementioned report of Centeno et al. (1997), Guiderdoni et al. (1995) found differences in the contents of individual CKs between E and NE callus cultures, in sugarcane. However, in spite of the previous reports, some researchers argue that CK levels are probably more related to the growth of the callus cultures than to the E competence (reviewed by Jiménez 2001).

A similar scene to the one described for auxins is found in ABA: even though the majority of publications support higher levels of this plant hormone in E than in NE cultures (reviewed by Jimenez 2001; Nakagawa et al. 2001), the contrary was reported for *Hevea brasiliensis* (Etienne et al. 1993) and alfalfa (Ivanova et al. 1994) cultures. A completely ambiguous situation is found in GAs, where higher levels of this hormone were found in E than in NE cultures in some works, while the contrary was found in others, whereas no differences were reported in some other publications (reviewed by Jimenez 2001).

Since ethylene quantification within the tissues is a very difficult task, indirect information about the role endogenous contents of this plant hor-

mone might play in determination of the E potential of cultures comes from experiments using inhibitors of ethylene synthesis and action. Most works on this subject indicate that ethylene plays a negative role on induction of SE (reviewed by Thorpe 2000). However, in a carrot line in which ethylene promoted SE, the use of inhibitors of biosynthesis also slightly inhibited SE (Nissen 1994).

Because spatial information is lost during global quantification of endogenous hormones, researchers have tried to obtain a precise localization of plant hormones within the tissues. This can only be achieved with the help of in situ techniques, such as immunolocalization. Whereas analytical methods for quantifying plant hormones have been strongly improved during recent years, in situ specific detection of these compounds has been more difficult. As an example, despite auxin having proven to be a difficult molecule to localize in tissues, being highly diffusible and occurring in both active and inactive (conjugated) forms (Normanly and Bartel 1999), successful immunohistochemical localization of IAA has been recently reported (Moctezuma 1999; Moctezuma and Feldman 1999; Aloni et al. 2003), including a report during early phases of SE (Thomas et al. 2002).

4
How Do Endogenous Hormone Contents Evolve in the Progress of SE?

Several studies aiming to evaluate the way endogenous hormone concentrations change during development of SE, specifically after expression has been induced, have been carried out. In some of them, the initial stages of embryo development have been analyzed, i.e., before the first morphological changes had occurred, but when biochemical and physiological determination of embryo development has already started (Dodeman and Ducreux 1996). The other group of studies focused on the later phases of embryo development, when it is possible to synchronize and separate the different embryo stages through a series of steps of sieving and centrifugation (reviewed by Osuga et al. 1999; Sharma 1999). Synchronization of E cultures allows a more accurate estimation of the hormone status in each phase of embryo development.

Endogenous contents of most hormones remained steady or showed only minor changes during the first 7 days after 2,4-D had been eliminated from the medium in carrot E cultures (Fujimura and Komamine 1979; Michalczuk et al. 1992a; Jiménez et al. 2005); only increased contents of the polyamines putrescine, spermidine and spermine have been, to the best of our knowledge, reported (Feinberg et al. 1984). In citrus E cultures, in which expression of SE was triggered by a stimulus other than reducing the auxin content in the medium, auxin and CKs accumulated within the first 5 days after sucrose had been replaced by glycerol in the culture medium, the triggering factor, while the levels of ABA and GAs remained stable (Jiménez et al. 2001).

When studying evolution of endogenous hormones in SE after the first morphological changes had occurred, Michalczuk et al. (1992a) reported that auxin levels decline steadily after the globular stage in all subsequent stages of embryo development. Additional information, in their case for ABA, was provided by Kamada and Harada (1981). They found that, after remaining low during the first 7 days of culture in the absence of 2,4-D, the concentrations of ABA increased during further development of carrot somatic embryos until day 10, and then decreased. Similarly, Rajasekaran et al. (1982) found that ABA levels in hybrid grapevine somatic embryos decreased from the globular to the mature stage. The role of endogenous ABA has been more evident during the latter stages of embryo development, especially during maturation and germination. In this sense, Kermode et al. (1989) and, recently, Prewein et al. (2004) related an increase in germination to a reduction in ABA content in the tissues, while Finkelstein et al. (1985) related the beginning of germination to a change in the sensitivity of the tissues to this plant hormone.

Information regarding GA content during the final phases of somatic embryo development originates from two early works (Noma et al. 1982; Takeno et al. 1983). In the first one, polar and less polar GA contents were compared during this phase and lower levels of polar and higher levels of less polar GAs were found, while in the second, a reduction in the levels of free and highly soluble GA-like substances on a dry weight basis was observed during embryo development.

Endogenous ethylene increased at day 1 after transferring somatic embryos of white spruce into the maturation medium, and then declined transiently and increased again gradually, in the second half of the culture period (Kong and Yeung 1994). Concerning polyamines, very recently, Minocha et al. (2004) found a correlation in the relationship of several members of this plant hormone group with the developmental stage of red spruce somatic embryos.

5
PGRs Acting on Endogenous Hormones During SE

The mode of action of PGRs involves modulation of endogenous plant hormone concentrations, among other effects, a process that may occur directly, through synthesis of enzymes, or indirectly, with the intervention of effectors (Thorpe 2000; Gaspar et al. 2003; Gazzarrini and McCourt 2003). An exogenous PGR can, positively or negatively, modulate internal concentrations of plant hormones belonging to the same as well as to other groups.

Examples of exogenous PGRs modulating levels of endogenous hormones of the same group in SE include the accumulation of endogenous IAA in soybean hypocotyl explants after treatment with the synthetic auxins NAA and IBA (Liu et al. 1998). Also, using gas chromatography/mass spectrometry, Michalczuk et al. (1992a, b) showed that carrot cells treated with 2,4-D accu-

mulate large amounts of endogenous IAA during SE. Further evidence in this sense is provided by the increase in the IAA levels observed in alfalfa leaf protoplasts cultured in the presence of 2,4-D (Pasternak et al. 2002). Moreover, Ceccarelli et al. (2002) found that two variant cell lines of carrot, capable of growing in high concentrations of 2,4-D and that showed disturbances in embryogenesis, raised the level of free IAA in response to the high exogenous auxin concentration.

Modulation of endogenous hormone levels by exogenous PGRs belonging to a different group has also been documented during SE. For example, application of ABA to immature zygotic sunflower embryos increased levels of endogenous IAA (Charrière et al. 1999). Moreover, high levels of exogenous ABA decreased ethylene contents during maturation of somatic embryos of white spruce (reviewed by Stasolla et al. 2002). There is also evidence, from a very early work, for 2,4-D regulating the rate of polar and less polar GAs (Noma et al. 1982).

It is suggested that the mechanism by which thidiazuron induces SE in peanut involves modulation of endogenous levels of auxin and CKs (Murthy et al. 1995). Moreover, the impairment in progression of embryo development caused by 2,4-D in carrot might be related to the increase in ethylene synthesis caused by the high levels of exogenous auxin (Minocha and Minocha 1995). Concerning polyamines, it has been observed that exogenous auxins suppressed the activity of two polyamine biosynthetic enzymes in carrot cultures, the effect of 2,4-D and IAA being distinct (Feinberg et al. 1984). Interaction of polyamines with other hormones has been reviewed by Kakkar and Sawhney (2002).

6 Concluding Remarks

Even though there are several factors that induce and govern SE in plants, the evidence available indicates that plant hormones, in response to the exogenous PGRs applied, or acting independently, in those few systems in which PGRs are not necessary for this process to occur, play a significant role. Together with the concentration of individual hormones, the interaction between members of different groups and the sensitivity/responsiveness of the tissues and cells (a factor not covered in this review) seem to condition the responses observed (Dudits et al. 1995; Thorpe 2000).

More than 10 years ago, when the first review articles involving quantification of endogenous hormones were published, it was postulated that knowing the endogenous hormone contents and their relation to the E competence of the explants would permit the induction and expression of SE in recalcitrant genotypes. That would take place through amendments to the culture medium, with substances that may mimic the inductive condition (supplying

a deficiency or counteracting an excess) (Merkle et al. 1995). However, even though several works characterizing hormone status in responsive genotypes and E cultures have been published, most new publications defining adequate conditions to induce and allow progress of SE are still based on trial and error, as indicated at the beginning of the present review (Sect. 1).

Despite the progress achieved during the last few years in understanding the mechanisms involved in hormonal signaling of SE, there are still many aspects that are not fully understood and need to be studied in more detail. Progress is currently being achieved in comprehending the molecular responses that PGRs and plant hormones generate, mainly in gene expression (Thomas and Jiménez, this volume). It is to be expected that this alternative way to study hormonal regulation of SE will bring new insights on the subject.

References

Aloni R, Schwalm K, Langhans M, Ullrich CI (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in *Arabidopsis*. Planta 216:841–853

Bell LM, Trigiano RN, Conger BV (1993) Relationship of abscisic acid to somatic embryogenesis in *Dactylis glomerata*. Environ Exp Bot 33:495–499

Besse I, Verdeil JL, Duval Y, Sotta B, Maldiney R, Miginiac E (1992) Oil palm (*Elaeis guineensis* Jacq.) clonal fidelity: endogenous cytokinins and indoleacetic acid in embryogenic callus cultures. J Exp Bot 43:983–989

Bronner R, Jeannin G, Hahne G (1994) Early cellular events during organogenesis and somatic embryogenesis induced on immature zygotic embryos of sunflower (*Helianthus annuus*). Can J Bot 72:239–248

Ceccarelli N, Mondin A, Lorenzi R, Picciarelli P, Lo Schiavo F (2002) The metabolic basis for 2,4-D resistance in two variant cell lines of carrot. Funct Plant Biol 29:575–583

Centeno ML, Rodríguez R, Berros B, Rodríguez A (1997) Endogenous hormonal content and somatic embryogenic capacity of *Corylus avellana* L. cotyledons. Plant Cell Rep 17:139–144

Charrière F, Hahne G (1998) Induction of embryogenesis versus caulogenesis on in vitro cultured sunflower (*Helianthus annuus* L.) immature zygotic embryos: role of plant growth regulators. Plant Sci 137:63–71

Charrière F, Sotta B, Miginiac É, Hahne G (1999) Induction of adventitious shoots or somatic embryos on in vitro cultured zygotic embryos of *Helianthus annuus*: variation of endogenous hormone levels. Plant Physiol Biochem 37:751–757

Cooke TJ, Racusen RH, Cohen JD (1993) The role of auxin in plant embryogenesis. Plant Cell 5:1494–1495

Danin M, Upfold SJ, Levin N, Nadel BL, Altman A, van Staden J (1993) Polyamines and cytokinins in celery embryogenic cell cultures. Plant Growth Regul 12:245–254

Davies PJ (1995) The plant hormones: their nature, occurrence, and functions. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology, 2nd edn. Kluwer, Dordrecht, pp 1–12

Dodeman VL, Ducreux G (1996) Total protein pattern expression during induction and development of carrot somatic embryos. Plant Sci 120:57–69

Dong J-Z, Dunstan DI (2000) Molecular biology of somatic embryogenesis in conifers. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants, vol 1. Kluwer, Dordrecht, pp 51–87

Dudits D, Györgyey J, Bögre L, Bakó L (1995) Molecular biology of somatic embryogenesis. In: Thorpe TA (ed) In vitro embryogenesis in plants. Kluwer, Dordrecht, pp 276–308

Etienne H, Sotta B, Montoro P, Miginiac E, Carron MP (1993) Relation between exogenous growth regulators and endogenous indole-3-acetic acid and abscisic acid in the expression of somatic embryogenesis in *Hevea brasiliensis* (Müll. Arg.). Plant Sci 88:91–96

Fehér A, Pasternak TP, Dudits D (2003) Transition of somatic plant cells to an embryogenic state. Plant Cell Tissue Organ Cult 74:201–228

Feinberg AA, Choi JH, Lubich WP, Sung ZR (1984) Developmental regulation of polyamine metabolism in growth and differentiation of carrot culture. Planta 162:532–539

Fernández H, Pérez C, Sánchez-Tamés R (2000) Modulation of the morphogenic potential of the embryonic axis of *Juglans regia* by cultural conditions. Plant Growth Regul 30:125–131

Finkelstein RR, Tenbarge KM, Shumway JE, Crouch ML (1985) Role of ABA in maturation of rapeseed embryos. Plant Physiol 78:630–636

Fischer C, Neuhaus G (1996) Influence of auxin on the establishment of bilateral symmetry in monocots. Plant J 9:659–669

Fujimura T, Komamine A (1975) Effects of various growth regulators on the embryogenesis in a carrot cell suspension culture. Plant Sci Lett 5:359–364

Fujimura T, Komamine A (1979) Involvement of endogenous auxin in somatic embryogenesis in a carrot cell suspension culture. Z Pflanzenphysiol 95:13–19

Gaj MD (2004) Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regul 43:27–47

Gaspar T, Kevers C, Faivre-Rampant O, Crèvecoeur M, Penel C, Greppin H, Dommes J (2003) Changing concepts in plant hormone action. In Vitro Cell Dev Biol Plant 39:85–105

Gazzarrini S, McCourt P (2003) Cross-talk in plant hormone signaling: what arabidopsis mutants are telling us. Ann Bot 91:605–612

Guiderdoni E, Mérot B, Eksomtramage T, Paulet F, Feldmann P, Glaszmann JC (1995) Somatic embryogenesis in sugarcane (*Saccharum* species). In: Bajaj YPS (ed) Somatic embryogenesis and synthetic seed I. Biotechnology in agriculture and forestry, vol 31. Springer, Berlin Heidelberg New York, pp 92–113

Hita O, Lafarga C, Guerra H (1997) Somatic embryogenesis from chickpea (*Cicer arietinum* L.) immature cotyledons: the effect of zeatin, gibberellic acid and indole-3-butyric acid. Acta Physiol Plant 19:333–338

Huang LVT, Takamura T, Tanaka M (2004) Callus formation and plant regeneration from callus through somatic embryo structures in *Cymbidium* orchid. Plant Sci 166:1443–1449

Hutchinson MJ, KrishnaRaj S, Saxena PK (1997) Inhibitory effect of GA_3 on the development of thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium* x *hortorum* Bailey) hypocotyl cultures. Plant Cell Rep 16:435–438

Iantcheva A, Vlahova M, Bakalova E, Kondorosi E, Elliott MC, Atanassov A (1999) Regeneration of diploid annual medics via direct somatic embryogenesis promoted by thidiazuron and benzylaminopurine. Plant Cell Rep 18:904–910

Ivanova A, Velcheva M, Denchev P, Atanassov A, Van Onckelen HA (1994) Endogenous hormone levels during direct somatic embryogenesis in *Medicago falcata*. Physiol Plant 92:85–89

Jiménez VM (2001) Regulation of in vitro somatic embryogenesis with emphasis on the role of endogenous hormones. Rev Bras Fisiol Veg 13:196–223

Jiménez VM, Bangerth F (2001a) Endogenous hormone levels in initial explants and in embryogenic and non-embryogenic callus cultures of competent and non-competent wheat genotypes. Plant Cell Tissue Organ Cult 67:37–46

Jiménez VM, Bangerth F (2001b) Hormonal status of maize initial explants and of the embryogenic and non-embryogenic callus cultures derived from them as related to morphogenesis in vitro. Plant Sci 160:247–257

Jiménez VM, Bangerth F (2001c) In vitro culture and endogenous hormone levels in immature zygotic embryos, endosperm and callus cultures of normal and high-lysine barley genotypes. J Appl Bot 75:1–7

Jiménez VM, Guevara E, Herrera J, Bangerth F (2001) Endogenous hormone levels in habituated nucellar *Citrus* callus during the initial stages of regeneration. Plant Cell Rep 20:92–100

Jiménez VM, Guevara E, Herrera J, Bangerth F (2005) Evolution of endogenous hormone concentration in embryogenic cultures of carrot during early expression of somatic embryogenesis. Plant Cell Rep 23:567–572

Kakkar RK, Sawhney VK (2002) Polyamine research in plants—a changing perspective. Physiol Plant 116:281–292

Kamada H, Harada H (1981) Changes in the endogenous level and effects of abscisic acid during somatic embryogenesis of *Daucus carota* L. Plant Cell Physiol 22:1423–1429

Kermode AR, Dumbroff EB, Bewley JD (1989) The role of maturation drying in the transition from seed development to germination. VII. Effects of partial and complete desiccation on abscisic acid levels and sensitivity in *Ricinus communis* L. seeds. J Exp Bot 40:303–313

Kochba J, Spiegel-Roy P, Neumann H, Saad S (1978) Stimulation of embryogenesis in citrus ovular callus by ABA, Ethephon, CCC and Alar and its suppression by GA_3. Z Pflanzenphysiol 89:427–432

Kong L, Yeung EC (1994) Effects of ethylene and ethylene inhibitors on white spruce somatic embryo maturation. Plant Sci 104:71–80

Kopertekh LG, Butenko RG (1995) Naturally occurring phytohormones in wheat explants as related to wheat morphogenesis in vitro. Russ J Plant Physiol 42:488–491

Li XQ, Demarly Y (1996) Somatic embryogenesis and plant regeneration in *Medicago suffruticosa*. Plant Cell Tissue Organ Cult 44:79–81

Limanton-Grevet A, Sotta B, Brown S, Jullien M (2000) Analysis of habituated embryogenic lines in *Asparagus officinalis* L.: growth characteristics, hormone content and ploidy level of calli and regenerated plants. Plant Sci 160:15–26

Liu C-M, Xu ZH, Chua N-H (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630

Liu Z-H, Wang W-C, Yen Y-S (1998) Effect of hormone treatment on root formation and endogenous indole-3-acetic acid and polyamine levels of *Glycine max* cultivated in vitro. Bot Bull Acad Sin 39:113–118

Mauri PV, Manzanera JA (2004) Effect of abscisic acid and stratification on somatic embryo maturation and germination of holm oak (*Quercus ilex* L.). In Vitro Cell Dev Biol Plant 40:495–498

Merkle SA, Parrott WA, Flinn BS (1995) Morphogenic aspects of somatic embryogenesis. In: Thorpe TA (ed) In vitro embryogenesis in plants. Kluwer, Dordrecht, pp 155–203

Michalczuk L, Cooke TJ, Cohen JD (1992a) Auxin levels at different stages of carrot embryogenesis. Phytochemistry 31:1097–1103

Michalczuk L, Ribnicky DM, Cooke TJ, Cohen JD (1992b) Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures. Plant Physiol 100:1346–1353

Minocha SC, Minocha R (1995) Role of polyamines in somatic embryogenesis. In: Bajaj YPS (ed) Somatic embryogenesis and synthetic seed I. Biotechnology in agriculture and forestry, vol 30. Springer, Berlin Heidelberg New York, pp 53–70

Minocha R, Minocha SC, Long S (2004) Polyamines and their biosynthetic enzymes during somatic embryo development in red spruce (*Picea rubens* Sarg.). In Vitro Cell Dev Biol Plant 40:572–580

Mitsuhashi W, Toyomasu T, Masui H, Katho T, Nakaminami K, Kashiwagi Y, Akutsu M, Kenmoku H, Sassa T, Yamaguchi S, Kamiya Y, Kamada H (2003) Gibberellin is essentially required for carrot (*Daucus carota* L.) somatic embryogenesis: dynamic regulation of gibberellin 3-oxidase gene expression. Biosci Biotechnol Biochem 67:2438–2447

Moctezuma E (1999) Changes in auxin patterns in developing gynophores of the peanut plant (*Arachis hypogaea* L.). Ann Bot 83:235–242

Moctezuma E, Feldman LJ (1999) Auxin redistributes upwards in graviresponding gynophores of the peanut plant. Planta 209:180–186

Murthy BNS, Murch SJ, Saxena PK (1995) Thidiazuron-induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogaea*): endogenous growth regulator levels and significance of cotyledons. Physiol Plant 94:268–276

Nakagawa H, Saijyo T, Yamauchi N, Shigyo M, Kako S, Ito A (2001) Effects of sugars and abscisic acid on somatic embryogenesis from melon (*Cucumis melo* L.) expanded cotyledon. Sci Hortic 90:85–92

Nishiwaki M, Fujino K, Koda Y, Masuda K, Kikuta Y (2000) Somatic embryogenesis induced by the simple application of abscisic acid to carrot (*Daucus carota* L.) seedlings in culture. Planta 211:756–759

Nissen P (1994) Stimulation of somatic embryogenesis in carrot by ethylene: Effects of modulators of ethylene biosynthesis and action. Physiol Plant 92:397–403

Noma M, Huber J, Ernst D, Pharis RP (1982) Quantitation of gibberellins and the metabolism of [^{3}H]gibberellin A_1, during somatic embryogenesis in carrot and anise cell cultures. Planta 155:369–376

Nomura K, Komamine A (1995) Physiological and biochemical aspects of somatic embryogenesis. In: Thorpe TA (ed) In vitro embryogenesis in plants. Kluwer, Dordrecht, pp 249–265

Normanly J, Bartel B (1999) Redundancy as a way of life—IAA metabolism. Curr Opin Plant Biol 2:207–213

Osuga K, Masuda H, Komamine A (1999) Synchronization of somatic embryogenesis at high frequency using carrot suspension cultures: model systems and application in plant development. Methods Cell Sci 21:129–140

Pasternak TP, Prinsen E, Ayaydin F, Miskolczi P, Potters G, Asard H, Van Onckelen HA, Dudits D, Fehér A (2002) The role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of alfalfa. Plant Physiol 129:1807–1819

Pintos B, Martín JP, Centeno ML, Villalobos N, Guerra H, Martín L (2002) Endogenous cytokinin levels in embryogenic and non-embryogenic calli of *Medicago arborea* L. Plant Sci 163:955–960

Prewein C, Vagner M, Wilhelm E (2004) Changes in water status and proline and abscisic acid concentrations in developing somatic embryos of pedunculate oak (*Quercus robur*) during maturation and germination. Tree Physiol 24:1251–1257

Raemakers CJJM, Jacobsen E, Visser RGF (1995) Secondary somatic embryogenesis and applications in plant breeding. Euphytica 81:93–107

Rajasekaran K, Vine J, Mullins MG (1982) Dormancy in somatic embryos and seeds of *Vitis*: changes in endogenous abscisic acid during embryogeny and germination. Planta 154:139–144

Rajasekaran K, Hein MB, Vasil IK (1987a) Endogenous abscisic acid and indole-3-acetic acid and somatic embryogenesis in cultured leaf explants of *Pennisetum purpureum* Schum. Plant Physiol 84:47–51

Rajasekaran K, Hein MB, Davis GC, Carnes MG, Vasil IK (1987b) Endogenous growth regulators in leaves and tissue cultures of *Pennisetum purpureum* Schum. J Plant Physiol 130:12–25

Roustan J-P, Latché A, Fallot J (1994) Role of ethylene on induction and expression of carrot somatic embryogenesis: relationship with polyamine metabolism. Plant Sci 103:223–229

Ruduś I, Kępczyńska E, Kępczyński J (2002) Regulation of *Medicago sativa* L. somatic embryogenesis by gibberellins. Plant Growth Regul 36:91–95

Sagare AP, Lee YL, Lin TC, Chen CC, Tsay HS (2000) Cytokinin-induced somatic embryogenesis and plant regeneration in *Corydalis yanhusuo* (Fumariaceae)—a medicinal plant. Plant Sci 160:139–147

Schiavone FM, Cooke TJ (1987) Unusual patterns of somatic embryogenesis in the domesticated carrot: developmental effects of exogenous auxins and auxin transport inhibitors. Cell Differ 21:53–62

Senger S, Mock H-P, Conrad U, Manteuffel R (2001) Immunomodulation of ABA function affects early events in somatic embryo development. Plant Cell Rep 20:112–120

Sharma AK (1999) Synchronization in plant cells—an introduction. Methods Cell Sci 21:73–78

Sharma P, Pandey S, Bhattacharya A, Nagar PK, Ahuja PS (2004) ABA associated biochemical changes during somatic embryo development in *Camellia sinensis* (L.) O. Kuntze. J Plant Physiol 161:1269–1276

Stasolla C, Kong L, Yeung EC, Thorpe TA (2002) Maturation of somatic embryos in conifers: morphogenesis, physiology, biochemistry, and molecular biology. In Vitro Cell Dev Biol Plant 38:93–105

Takeno K, Koshioka M, Pharis RP, Rajasekaran K, Mullins MG (1983) Endogenous gibberellin-like substances in somatic embryos of grape (*Vitis vinifera* x *Vitis rupestris*) in relation to embryogenesis and the chilling requirement for subsequent development of mature embryos. Plant Physiol 73:803–808

Thomas C, Bronner R, Molinier J, Prinsen E, van Onckelen H, Hahne G (2002) Immunocytochemical localization of indole-3-acetic acid during induction of somatic embryogenesis in cultured sunflower embryos. Planta 215:577–583

Thomas C, Meyer D, Himber C, Steinmetz A (2004) Spatial expression of a sunflower SERK gene during induction of somatic embryogenesis and shoot organogenesis. Plant Physiol Biochem 42:35–42

Thorpe TA (2000) Somatic embryogenesis: morphogenesis, physiology, biochemistry and molecular biology. Korean J Plant Tissue Cult 27:245–258

Tokuji Y, Kuriyama K (2003) Involvement of gibberellin and cytokinin in the formation of embryogenic cell clumps in carrot (*Daucus carota*). J Plant Physiol 160:133–141

Victor JMR, Murch SJ, KrishnaRaj S, Saxena PK (1999) Somatic embryogenesis and organogenesis in peanut: The role of thidiazuron and N^6-benzylaminopurine in the induction of plant morphogenesis. Plant Growth Regul 28:9–15

von Arnold S, Sabala I, Bozhkov P, Kyachok J, Filonova L (2002) Developmental pathways of somatic embryogenesis. Plant Cell Tissue Organ Cult 69:233–249

Wenck AR, Conger BV, Trigiano RN, Sams CE (1988) Inhibition of somatic embryogenesis in orchardgrass by endogenous cytokinins. Plant Physiol 88:990–992

Zhang Q, Chen J, Henny RJ (2005) Direct somatic embryogenesis and plant regeneration from leaf, petiole and stem explants of golden pothos. Plant Cell Rep 23:587–595