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SOMATIC EMBRYOGENESIS IN HIGHER PLANTS

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ABSTRACT

Somatic embryogenesis is an illustration of plant totipotency. There are many factors involved in causing development switching during somatic embryogenesis. These include combination of plant growth regulators, media, pretreatments and culture environments, which relate to various molecular events encompassing gene expression and signal transduction pathways. The present review collates information on various aspects of somatic embryogenesis focusing on genes involved, proteins and metabolites that have been identified during the last few years. Future work on integrating various data on somatic embryogenesis using the computational or systems biology approach is suggested.

Key words: Somatic embryogenesis, genes, proteins, metabolites

INTRODUCTION

There are three ways to induce embryo development from *in vitro* cultured plant cells; i.e. *in vitro* fertilization, from microspores and *in vitro* somatic embryogenesis (SE) (Féher *et al.,* 2003). *In vitro* SE can develop either indirectly, that is through callus (indirect SE) or directly from the explant without any intermediary callus formation (direct SE) (Solis-Ramos *et al.,* 2012). The direct or indirect embryogenesis depends on culture conditions, explant source, tissue and stage of development of the explant materials (Carman, 1990). The direct or indirect embryogenesis is plant specific. Some species easily go through the direct somatic embryogenesis while others need more treatment to be embryogenic (Von Arnold *et al.,* 2002).

Somatic embryogenesis (SE) is the process by which somatic cells, under induction, generate embryogenic cells via a series of morphological and biochemical changes (Quiróz-Figueroa *et al.,* 2006). Plant cellular totipotency where individual somatic cells can regenerate into a whole plant makes SE possible. SE has been reported in many plant species since the first report on carrot by Steward *et al.,* (1958a, 1958b). The requirements for SE *e.g.* combination of plant growth regulators, media, pretreatments and culture environments, are according to species, genotype and culture environment (Féher, 2008; Jalil *et al.,* 2008). The process of somatic embryogenesis is expected to follow the stages in the zygotic embryogenesis process, where the globular, heart and torpedo shape stages are successively observed (Zimmerman, 1993).

Somatic embryos originate by two pathways; i.e. unicellular or multicellular (Quiroz-Figueroa *et al.,* 2006). When embryos have a unicellular origin, coordinated cell divisions are observed and the embryo is sometimes connected to the maternal tissue by a suspensor-like structure (Williams and Maheswaran, 1986). In contrast, multicellular-origin embryos are initially observed as a protuberance, with no coordinated cell divisions observable, and those embryos in contact with the basal area are typically fused to the maternal tissue (Quiroz-Figueroa *et al.,* 2006).

SE produces a higher number of regenerates compared to organogenesis. Organogenesis is a process of cell differentiation to form organs such as leaves, stem or roots. A group of differentiated cells are required to form these organs. In contrast, only a single cell from embryogenic callus is needed for induction of a somatic embryo. SE also contains a low frequency of chimeras, a high number of regenerates and a limited level of somaclonal variation (Ahloowalia, 1991; Henry, 1998). Hence, it is a more preferred system for genetic transformation, *in vitro* mutagenesis and selection. Somatic embryos are also being used for management and conservation of genetic resources using cryopreservation techniques for they give an

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appreciably higher rate of multiplication compared to any other clonal propagation systems (Sharma, 2005).

FACTORS INFLUENCING SOMATIC EMBRYOGENESIS

Gaj (2004) reviewed factors that influence the initiation of somatic embryogenesis in plants. These include genotype, type of plant, age and developmental stage of an explant, physiological state of an explant-donor plant, and the external environment that includes composition of media and physical culture conditions (light, temperature). The selection of medium is also an important issue, however MS (Murashige and Skoog, 1962) medium is widely used for the induction of embryogenic callus in many species (Singh *et al.,* 2004). Endogenous hormone levels can be considered as major factors in determining specificity of cellular responses to rather general stimuli such as wounding, high salt concentration, heavy metal ions or osmotic stress (Fehér *et al.,* 2003; Jime'nez, 2005). Exogenous application of plant growth regulators in particular auxins and cytokinins has shown that they play an integral role in dediffentiation process during SE (Dudits *et al.,* 1995; Elhiti *et al.,* 2013).

External stimuli such as plant growth regulators have been most frequently considered to generate somatic embryos. This is particularly so with 2,4-dichlorophenoxy acetic acid (2,4-D) for induction of embryogenic response. It was suggested by Gaj (2004) that this synthetic growth regulator appear to act not only as an exogenous auxin analogue but also as an effective stressor. 2,4-D plays an important role in cell division and differentiation (Fehér *et al.,* 2003) which has been demonstrated in many experiments for examples, *Arecha catachu* (Wang *et al.,* 2006)*, Psidium guajava* cv Banarasi local (Rai *et al.,* 2007), oil palm (Scherwinski-Pereira *et al.,* 2010) and *Arabidopsis* (Elhiti *et al.,* 2010). Other auxin, for example indole acetic acid (IAA) and α-naphthaleneacetic acid (NAA) are also able to induce somatic embryogenesis. NAA alone induces somatic embryogenesis in *Solanum melongena* (Swamynathan *et al.,* 2010). According to Dudits *et al.* (1995), the mechanism of action of auxin in physiological and regulatory processes is related to the presence of protein receptors located in the membrane, cytoplasm and nucleus. There is, in the latter the activation of RNA-polymerase, which is specific to the transcription of genes involved in the regulation of cell division. In other words, auxin is necessary for "competent" cells to express totipotency. However, the removal of auxin is necessary in the obtaining somatic embryogenesis of litchi after callus induction in the medium of high auxin (Yu *et al.,* 2000). Auxin is proven to inhibit the differentiation process when not removed from the culture medium of *Lilium longiforum* (Nhut *et al.,* 2006).

Although cytokinin normally promotes cell division, combinations with auxin are suitable for some species to induce somatic embryogenesis (Mashayekhi *et al.,* 2008; Mahendran and Bai, 2012; Sane *et al.,* 2012). Although in a very rare case, combination of thidiazuron (TDZ) and BAP are possible options in inducing somatic embryogenesis. This can be seen in the case of somatic embryogenesis in mangosteen (Rohani *et al.,* 2012). Meanwhile, a combination of 2,4-D with dicamba (3,6-dichloro-2-methoxybenzoic acid) has been shown to be successful in *Areca catachu* (Wang *et al.,* 2006).

Generally, SE involves induction, maturation and germination/conversion. Low efficiency of embryo maturation, germination and conversion to plantlets is a major problem in the completion of somatic embryogenesis (Vahdati *et al.,* 2008). The maturation step is a process in which the embryogenic callus will transform and differentiate, usually indicated by differentiation of callus into a heart shape structure, at which step, abscisic acid (ABA) has been found to be effective to promote maturation of the embryogenic callus in many plants (Misra, 1994). Exogenous ABA has been used in a wide range of 1-100 μM during the maturation process (Stasolla *et al.,* 2002). The temporary culture of *Podophyllum peltatum* L. embryogenic callus in 11.35 μM ABA followed by transfer to the MS free medium has successfully stimulated the development of somatic embryos (Kim *et al.,* 2007). In Persian walnut, the addition of 2 mgL^{-1} ABA in MS medium has been observed to be suitable in promoting the maturation and germination compared to another combination of hormones, though the percentage was low (Vahdati *et al.,* 2008). ABA has also been recognized as a factor for promotion of normal development and maturation of somatic embryos and according to Misra (1994) ABA is essential for the accumulation of storage reserves and to synchronize maturation of somatic embryos. Tian and Brown (2000) and Vahdati *et al.* (2006) suggested that among the successive developmental stages of somatic embryos, the globular stage is the best stage for the application of ABA as it is only at the globular stage that embryos will respond to ABA. There are other treatments that are used to stimulate the maturation process, namely coconut water, Kinetin, IAA and sucrose (Das and Rahman, 2013; Lara-Chavez *et al.,* 2011; Balaraju *et al.,* 2011).

Carbohydrate types and concentrations have been found to play important roles in different stages of the somatic embryogenesis process. Generally, saccharides such as sucrose, maltose and glucose serve as carbon and energy sources, osmotic agents, stress protectants, and signal molecules in plants (Lipavska and Konradova, 2004). The carbohydrate source has been shown to be an important factor for *in vitro* growth, affecting both somatic embryogenesis and embryo maturation (Hassan & Taha 2012; Businge *et al.,* 2013). The application of sugars in inducing process is species and genotype specific (Yancheva and Roichev, 2005). For example, Kulkarni and Bapat (2013) reported that maltose is the best carbohydrate source for maintenance of embryonic cell suspension of banana (Rajeli AAB cultivar), but in grape culture, sucrose is used in the induction and development of embryogenic callus (Yancheva and Roichev, 2005).

Amino acids are another effective factor in inducing maturation in somatic embryogenesis. A study on strawberry somatic embryogenesis showed that the best amino acid is proline (Gerdakaneh *et al.,* 2011). They also reported cultures grown on amino-acid free medium attained lower percentage of somatic embryos than cultures grown on amino acid-treated medium. The frequently used amino acid is glutamine, which was reported to enhance maturation on *Cajanus cajan* (Aboshama, 2011), *Macrotyloma uniflorum* (Varisai Mohamed *et al.,* 2004), soybean (Schmidt *et al.,* 2005) and *Psoralea corylifolia* (Sahrawat and Chand, 2001). A study also reported the use of polyethylene glycol (PEG), for example in *Leucojum aestivum* (Ptak *et al.,* 2013).

The process of obtaining somatic embryogenesis sometimes requires very specific treatment, such as light and temperature. A study carried out on *Agave tequilana* revealed that applying either white or red light during callus induction followed by wide-spectrum light during maturation induced higher percentage of germinated embryos (Rodriguez-Sahagun *et al.,* 2011). A report on *Holvenia dulcis* revealed the sensitivity of that species to temperature for induction of secondary embryo (Yang *et al.,* 2013). At higher temperature (30ºC), the explants were effective in inducing secondary somatic embryos, but lower temperature (20ºC) was found to be more suitable for further embryo development, conversion and transplant survival.

Having competent cells, which are morphologically small, rounded cells with rich cytoplasm and small vacuoles, allows dedifferentiation of somatic cells, which consequently respond to new developmental signals (Fehér, 2005). The developmental switching in somatic embryogenesis also involves differential gene expression conferring on the somatic cells the ability to manifest the embryogenic potential (Ragavan, 1997; 2000).

DEVELOPMENT SWITCHING AND GENE EXPRESSION DURING SOMATIC EMBRYOGENESIS

An excellent review by Fehér *et al.* (2003) on transition of somatic plant cells to an embryonic state is referred to. During this transition, cells need to dedifferentiate, activate their cell division cycle and reorganize their physiology, metabolism and gene expression patterns. It has been suggested that *in vitro* condition exposes the explants to a considerable stress condition such as wounding, high salt concentration, heavy metal ions or osmotic stress which, influence/induce SE (Dudits *et al.,* 1995). Adaptation to this condition include the reprogramming of gene expression as well as changes in the physiology and metabolism of the cells (Fehér *et al.,* 2003; Elhiti *et al*., 2013).

The developmental switching from somatic cells into embryogenic cells involves differential gene expression resulting in activating or suppressing genes which have not been identified (Chugh and Khurana, 2002). Hormones are the most likely candidates in the regulation of developmental switches (Fehér *et al.,* 2003; Elhiti *et al*., 2013). Elucidation of the signaling pathways where plant cells remodel their gene expression programme is central to understanding the regulation of the somatic embryogenesis process (Thomas and Jimenez, 2005). In this respect, the induction phase of somatic embryogenesis is of primary interest as it governs the subsequent stages of the somatic embryogenesis process (Fehér, 2008) and he hypothesized that although plant cells in general have the capability for embryogenesis, the expression trait (the acquisition of embryogenic competence) is mainly determined by the given physiological state of the cell which is determined by its genetic and developmental conditions and by environmental cues.

The advent of molecular techniques has been crucial in identification of genes that exhibit differential activity, which had been categorized based on the gene structure and function (Chugh and Kurana, 2002). Two of the earlier studies using the molecular approach were reported by Franz *et al.* (1989) and Rao *et al.* (1990) who demonstrated the isozyme differences between embryogenic and non embryogenic cultures. Chugh and Kurana (2002), Karami *et al.* (2009) and Yang and Zhang (2010) reviewed gene expression and regulation of SE quite thoroughly. Selected genes from the reviews with current findings are described in this review.

Somatic embryogenesis receptor kinase (SERK)

SERKs are involved in the acquisition of embryogenic competence in plant cells, where in carrot and *Arabidopsis*, SERKs were shown to be characteristic markers of embryogenic cell cultures and somatic embryogenesis (Schmidt *et al.,* 1997; Hecht *et al.,* 2001). The SERK is now assumed to be the marker for somatic embryogenesis (Thomas and Jimenez, 2005). *SERK* gene, first isolated from carrot somatic embryos (Schmidt *et al.,* 1997) was shown to be a specific marker, as it is able to distinguish individual embryo-forming masses in induced carrot suspension cultures and may also serve as a characteristic molecular marker for differentiating between competent and non-competent cells. *SERK* belongs to a small gene family with different number of family members reported in different species. At least five members of SERK family reported in *Arabidopsis* (*AtSERK1-5*) (Hecht *et al.,* 2001), six in *Medicago trunctulata* (*MtSERK1-6*) (Nolan *et al.,* 2003; 2011), four in *Helianthus annuus* (Thomas *et al.,* 2004), two in rice (*OsSERK1-2*) (Ito *et al.,* 2005), three in maize (*ZmSERK1-3*) (Baudino *et al.,* 2001), three in *Triticum aestivum* (*TaSERK1-3*) (Singla *et al.,* 2008), and one in *Cocos nucifera* (*CnSERK*) (Pérez-Nuñez *et al.,* 2009).

The ectopic expression of *AtSERK1* (*Arabidopsis*) gene enhanced embryogenic cells in developing ovules, early embryos and in vascular tissues. The *AtSERK* family is divided into two subfamilies, comprises *AtSERK1* and *AtSERK2*, while the second comprises *AtSERK 3-5* (He *et al.,* 2007; Albrecht *et al.,* 2008). The expression pattern of the *ZmSERK*s revealed that a strong correlation exists between the developing stages of the immature embryo and *ZmSERK* expression in maize. *ZmSERK1* and *ZmSERK2* appear to play an important role in maintaining embryogenesis, while *ZmSERK3* appears to have a dual role in embryogenesis by modulating its expression level (Zhang *et al.,* 2011). *In situ* hybridization analysis revealed *CitSERK1-like* gene was mainly located in the embryogenic callus and vascular cells of different embryos or tissues of *Citrus sinensis* cv. 'Valencia', showing that the gene played critical roles throughout the process of somatic embryogenesis (Ge *et al.,* 2010). Expression of *AaSERK1* during somatic embryogenesis of a gymnosperm *Araucaria angustifolia* was reported by Steiner *et al.* (2011). Rohani *et al.* (2012) detected *SERK1* in *Garcinia mangostana* in the globular structure during somatic embryogenesis. From these reports it can be summarized that *SERK1* expression is important in acquisition of SE.

Other related/significant genes

Several genes encoding transcription factors have been isolated and identiûed in somatic embryogenesis. These include *BABY BOOM* (*BBM*), *LEAFY COTYLEDON1* (*LEC1*), *LEAFY COTYLEDON2* (*LEC2*), *WUSCHEL* (*WUS*) and *AGAMOUS like-15* (*AGL15*) that play a role in promoting somatic embryogenesis (Lotan *et al.,* 1998; Hecht *et al.,* 2001; Stone *et al.,* 2001; Boutilier *et al.,* 2002; Zuo *et al.,* 2002; Arroyo-Herrera *et al.,* 2008; Thakare *et al.,* 2008, Karami *et al*., 2009). The findings suggest that a large number of transcription factors may play important roles in the process of somatic embryogenesis, especially in the transition from somatic to embryonic cells (Zhao *et al.,* 2011).

It was suggested that *BBM* gene is likely to promote cell proliferation and morphogenesis during embryogenesis (Boutilier *et al.,* 2002; Kulinska-Lukaszek *et al.,* 2012). Zheng *et al.* (2013) reported that *AGL15* related to *Medicago truncatula* somatic embryogenesis gene *MtSERF1* in *Arabidopsis* and soybean. Zheng *et al.* (2013) again reported that in soybean, two orthologs are expressed in response to induction of somatic embryogenesis in culture. Increased in *GmAGL15* leads to increased ethylene production and may involve in induction of somatic embryogenesis (Zheng *et al.,* 2013).

The *CsSCARECROW* (*CsSCR*) was identified after the induction of somatic embryogenesis in cucumber (*Cucumis sativus*). Localization by *in situ* hybridization of *CsSCR* gene was reported in undifferentiated cells in the globular and heart stages of somatic embryogenesis of cucumber (Wisniewska *et al.,* 2013). Wisniewska *et al.* (2013) reported expression of this gene in the endodermis of torpedo and cotyledonary stage somatic embryos. They also reported the presence of *CsSCR* gene in developing primary and lateral roots, which suggest that *CsSCR* is likely to play a role in tissue radial organization during somatic embryogenesis and root development.

Late embryogenesis abundant (LEA) protein genes are expressed in the later stages of embryo maturation, are in abundance and are capable of surviving the period of desiccation. While late in embryogenesis, the lectin and storage protein-coding genes genes are required for initiating and/or maintaining maturation phase and repressing precocious germination (Lotan *et al.,* 1998; Stone *et al.,* 2001). It has been indicated that heat shock protein *hsps* may have a specific role during developmental switching in plant cells (Györgyey *et al.,* 1991).

Germins and germin-like proteins (GLPs) are known to play a wide variety of roles as enzymes, structural proteins, or receptors during somatic embryogenesis, salt stress and pathogen responses (Dunwell *et al.,* 2008; Bernier and Berna, 2001; Lane, 2002, Neutelings *et al*., 1998). Yang and Zhang (2010) reviewed the extracellular protein such as the arabinogalactan proteins, non-specific lipid transfer proteins and germin and germin-like proteins as markers for SE. Most of the plant shoot originates from a small group of stem cells are specified by *WUSCHEL* (*WUS*) (Gallois *et al.,* 2004), expressed during leaf development. However, ectopic *WUS* expression in induced somatic embryogenesis suggests that *WUS* also promotes embryogenic identity (Zuo *et al.,* 2002). The study on the expression of *WUS* in somatic embryogenesis, reported by Herrera *et al.* (2008), found that the expression of *WUS* increased the production somatic embryo significantly.

Oxidative stress might induce somatic embryogenesis, as suggested in gene expression studies by Szechynska-Hebda *et al.* (2012), Zhang *et al*. (2010) and Bossio *et al*. (2013). The interaction between glutathione biosynthesis genes (*GSH*) (genes involved in antioxidant responses) and auxin in controlling somatic embryo development is reported by Bossio *et al*. (2013). They investigated the influence of post-transcriptional silencing (PTGS) of the biosynthesis genes *GSH1* and *GSH2*, and concluded that *GSH* is essential for somatic embryogenesis in wheat (Bossio *et al.,* 2013).

Cell division kinase (CDK) protein is one of the main enzymes of cell cycle regulation. The cell cycle regulator (*cdc2*) gene encodes for the catalytic subunit of this protein kinase. The expression profile of *Picdc2* showed two main phases followed by a final decline in *Prunus incisa* (Ben Mahmoud *et al.,* 2013), where in the first 10 days, the low levels observed were associated with cellular dedifferentiation phase and for the second phase, the increase peak at day 25 was related to the activation of cell proliferation and callus formation observed in wounded sites of leaves followed by embryoid dedifferentiation.

PROTEOMIC AND METABOLOMIC ANALYSIS OF SOMATIC EMBRYOGENESIS

Research in somatic embryo development extends over the past 20 years, but much of this work has been focused on culturing technologies (Marsoni *et al.,* 2008). There are still many aspects of somatic embryogenesis that are not yet understood. Identification of proteins and metabolites associated or involved in somatic embryo development may help to elucidate mechanistic insights into SE. Recent improvements of the high resolution two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) technique have made the large-scale proling and identication of proteins a dynamic area of research in plant biology (Marsoni *et al.,* 2008) as been shown in *Picea glauca* (Stasolla *et al.,* 2004), *Cyclamen persicum* (Winkelmann *et al.,* 2006; Bian *et al.,* 2010); *Eruca sativa* (Chen *et al.,* 2012) and *Zea mays* (Sun *et al.,* 2013). Proteins predominantly expressed in embryogenic calli of *Cyphomandra betacea* included metabolism-related proteins such as enolases or treonine synthases and also heat-shock and ribosomal proteins (Correia *et al.,* 2012). Proteomic analysis of developing somatic embryos of *Coffea arabica* by Tonietto *et al.* (2012) revealed some proteins to be specific to different stages of SE. One of these is enolase, a glycolytic enzyme that catalyses the reversible conversion of 2-phospho-D-glycerate to phosphoenolpyruvate (PEP) and could be a candidate for maturation stage. Enolase was also found at a torpedo stage in *Picea glauca* (Lippert *et al.,* 2005), *Cyclamen persicum* Mill (Rode *et al.,* 2011), and *Eruca sativa* (Chen *et al.,* 2012). Chen *et al.* (2012) found sucrose synthase, (also by Noah *et al.,* 2013) and phospolipase D to be highly expressed in embryogenic calli of *Eruca sativa*. Enzymes for carbohydrate metabolism, such as lactoylglutathione lyase, malate synthase and malate dehydrogenase, were found to be most abundant in cocoa cells undergoing somatic embryogenesis (Noah *et al.,* 2013). As has been suggested/speculated that oxidative stress stimulates cell dedifferentiation and promotes somatic embryo formation (Fehér *et al.,* 2003), Noah *et al*. (2013) also observed high abundance of stress related proteins such as the peroxidases, pathogenesis related proteins and glutathione S-transferase. Marsoni *et al.* (2008) previously identied several stress-related proteins induced in *Vitis vinifera* embryogenic cultures such as two forms of cytosolic ascorbate peroxidase and glutathione-S-transferase. Glutathione metabolism and anti-oxidative stress was also observed in *Citrus sinensis* (Pan *et al.,* 2009). Tonietto *et al.* (2012) found glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to be highly expressed in the globular stage, less in other stages. Hence, the up-regulation of GAPDH may be related to the control of reactive oxygen species (ROS) level.

The accumulation of ferritins in mature citrus somatic embryos was observed and spermidine synthase was found to be up-regulated during the citrus SE (Pan *et al.,* 2009). Early studies showed that spermidine (a polyamine) positively affected the embryogenic capability in several species such as *Panax ginseng* (Kevers *et al.,* 2000), *Picea rubens* Sarg (Minocha *et al.,* 2004) and *Citrus sinensis* (Wu

et al., 2008). Liu *et al.* (2005) reported that, spermidine increased with the development of Valencia somatic embryo and peaked at the globular embryo stage. Some investigators have suggested that polyamines viz. spermidine, spermine and putrescine are either essential as plant growth regulators or secondary messenger in signaling pathways (Lippert *et al.,* 2005; Roberts, 2002). Polyamines have been shown to play a crucial role during somatic embryogenesis in several important plants such as *Momordica charantia* (Paul *et al.,* 2009), *Picea abies* (Mala *et al.,* 2009) and *Vitis vinifera* (Bertoldi *et al.,* 2004). Akhtar (2013), indicated that temporal regulation of somatic embryogenesis of guava (*Psidium guajava* L.) cv. Allahabad safeda by 2,4-D was modulated by polyamine metabolism.

In terms of cell proliferation, Sun *et al.* (2013) reported two forms of tubulins to be up-regulated in embryogenic calli. Tubulin plays an important role in the separation of the daughter chromosomes and tubulin microtubules and actin microtubules are known to constitute the cytoskeleton. Some tubulins were also shown to be up-regulated in embryogenic calli by earlier studies (Marsoni *et al.,* 2008; Pan *et al.,* 2009; Zhang *et al.,* 2009). Storage globulin 11S was observed at torpedo stage while heat shock proteins expressed under stress conditions and essential for cellular recovery and normal functioning, and annexin involved in structural organization, were found at cotyledonary stages (Tonietto *et al.,* 2012).

Metabolomics approach will add to information obtained through the gene expression and proteomics as regulation of developmental events can be further elucidated at the metabolic level. Nevertheless, at present the number of reports on metabolomics/metabolite profiling during somatic embryogenesis is still small compared to the work on proteomics and gene expression.

Predicting regenerative capacity of SE in conifer by metabolomics indicated that limited production of mature viable embryos might be associated with stress-linked mechanism (Robinson *et al.,* 2009). This is in line with the proteomics and the gene expression findings above. Metabolic footprinting study of white spruce somatic embryogenesis using NMR spectroscopy (Dowlatabadi *et al.,* 2009) suggested that endogenous auxin and sugar signaling affects initial stages of somatic embryo development. Businge *et al.* (2012) hypothesized that the presence of tryptophan during proliferation and embryo differentiation is indicative of the essential role auxin has during normal somatic embryo development at these stages. The presence of stress-related metabolites during late embryogeny is consistent with *Pinus taeda* L. showing an association between the capability of cell lines to form mature embryos and their response to stress conditions during maturation (Robinson *et al.,* 2009). This is in line with proteome analysis in *P. glauca,* which revealed a differential expression of stress response proteins during the maturation of somatic embryos (Lippert *et al.,* 2005). Robinson *et al.* (2009) suggested a possible application of metabolomics is to use specic metabolite sets for monitoring the physiological status of cultures, in determining the appropriate timing for a switch from proliferation to maturation media, or in the development of improved culture procedures.

FUTURE PROSPECTS

Successful SE protocols have been established and reported for many species but for many others these have not been achieved. Understanding SE would not only solve the problem of micropropagation but also will assist in crop/plant improvement. In addition to biochemical and molecular approaches to studying embryogenesis, recent advances in technologies such as genomics, proteomics, metabolomics and computational biology has opened more avenues to elucidate SE. For example, the different proteins expressed and metabolites found at different SE stages would be a very interesting basis of studying SE of mangosteen (*Garcinia mangostana*) both naturally and *in vitro*. Mangosteen has a unique structure of seed where there is no differentiated embryos formed, and the seeds are formed apomictically. The formation of somatic embryos in mangosteen also has not been following the normal embryo formation from globular to cotyledonary (Elviana *et al.,* 2011). However, a common characteristic of globular formation during somatic embryogenesis was observed, and histological analysis of sections of globular structures showed accumulation of dense meristematic cells. Molecular analysis detected the gene *somatic embryogenesis receptor-like kinase 1* (*SERK1*) (Rohani *et al.,* 2012).

Hence, understanding the whole process or mechanism of somatic embryogenesis is utmost important and this can be achieved through an integrated systems biology approach. Analysis of plant embryogenesis using the 'omics' technology, for example, studies by Businge *et al.* (2012) on metabolite profiling and Noah *et al.* (2013) on proteomics during SE, together with computational biology will reveal the mechanisms at work in the establishment of the polarity, the differentiation of the tissue systems and the elaboration of the pattern that ultimately carries each species into the next generation. The application of systems biology

experiments on somatic embryos can contribute significantly to elucidate the mysteries of plant development as well as providing an analytical understanding of the totipotency in higher plants.

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