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Plant morphogenesis: theorical bases

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Abstract
Comprehension of plant morphogenesis is essential for understanding organogenesis and somatic embryogenesis processes, i.e., stages of tissue and organ development of a multicellular organism, which can lead to partial or total plant regeneration. Morphogenesis comprises the integration of growth and differentiation, mediated by cell division and specialization as a result of a complex spatial and temporal hormonal control, which occurs through regulation and expression of multiple gene systems, correlative action of meristems and their derivatives and environmental variations. However, in plant tissue culture, this endogenous links are disrupted. Tissues are exposed to exogenous conditions, represented by plant growth regulators, nutrients from the culture medium and controlled conditions of temperature and light. Therefore, morphogenesis seems to be modulated by the interaction of these factors, and also by other signaling agents, that act directly or indirectly on genetic level, triggering specific processes of synthesis that interfere with various biochemical pathways. Considering that complete elucidation of all the processes involved in morphogenesis has not been established yet, is essential to do a comprehensive study, particularly of the main factors implicated in these processes. In this context, this review aims to discuss, in general, the factors involved in the acquisition of competence, determination and cellular differentiation of morphogenesis processes, which may contribute to a better understanding and provide a basis for new research.
Key words: Morphogenesis; Cellular competence; Cellular determination; Gene expression; Chemical modulators.

Morfogênese vegetal: bases teóricas

Resumo
A compreensão dos processos de organogênese e embriogênese somática é fundamental para o entendimento da morfogênese vegetal, ou seja, das etapas de desenvolvimento de tecidos e órgãos de um organismo multicelular, as quais podem ocasionar a regeneração total ou parcial da planta. A morfogênese compreende a integração entre crescimento e diferenciação, mediada por divisão e especialização celular, resultado de um complexo controle hormonal, espacial e temporal, que ocorre por meio da regulação e expressão de sistemas gênicos múltiplos, da ação correlativa dos meristemas e seus derivados e das variações ambientais. Entretanto, na cultura de tecidos vegetais, ao se romper as relações endógenas, os tecidos ficam sujeitos às condições exógenas, representadas pelos reguladores de crescimento, nutrientes do meio de cultura, e condições controladas de temperatura e luminosidade. Sendo assim, a morfogênese passa a ser modulada pelo balanço destes fatores, e também por outros agentes sinalizadores que, atuando direta ou indiretamente em nível gênico, desencadeiam processos específicos de síntese que interferem em rotas bioquímicas diversas. Considerando que a completa elucidação de todos os processos envolvidos na morfogênese ainda não tenha sido estabelecida, é imprescindível o estudo pormenorizado, particularmente em relação aos principais fatores atuantes nestes processos. Neste contexto, esta revisão pretende discorrer, de maneira geral, os fatores envolvidos na aquisição de competência, determinação e diferenciação celular nos processos de morfogênese *in vitro* e *in vivo*, que poderão contribuir para sua melhor compreensão e fornecer subsídios para novas pesquisas.
Palavras-chave: Morfogênese; Competência celular; Determinação celular; Expressão gênica; Moduladores químicos.

Introduction

Plant morphogenesis corresponds to a biological process in which the vegetal assumes its specific form during their development in relation to its external form and to its internal organization, thus encompassing all levels from the cellular components until the complete plant (Gilbert 2000).

In the literature, initial researches were focused on plant growth regulators and mineral nutrients requirement on the morphogenic processes (Lakshmanan et al. 1997). Subsequently, studies aiming a comprehension of physiological basis of various cellular processes involved in morphogenesis were conducted (Phillips 2004; Dupuy et al. 2008; Papp and Plath 2011; Blervacq et al. 2012).

Plants, both at the cellular level such in tissues, pass through three stages of development, i.e., morphogenic competence, determination of development and morphological differentiation (Christianson and Warnick 1983). The morphogenic competence is defined as the cell's ability to recognize a specific signal that leads to a particular development (Hicks 1994). Competent cells become determined by induction, a process by which a morphogenic signal acts on these, redirecting its development. Subsequently, some cells enter a state of differentiation, assuming a new organization of tissues.

The morphogenic process is modulated not only by a series of cell intrinsic factors, but also by extrinsic factors, whether biotic or abiotic. These factors will act by modulating cellular activity to a particular development into a specific direction, or by cell reprogramming with the restoration of its totipotency characteristics. Therefore, is understood that research on factors involved in the morphogenic processes are essential for the understanding of morphogenesis; thus, his control. The morphogenic process comprises a series of other processes, involving not

only chemical modulators such as plant growth regulators, but also the competence levels of cell, polarity, habituation, and the performance of gene control.

This review aimed to describe, in general, the levels of cellular competence and determination, the genic expression, the influence of chemical modulators, cellular positioning and habituation on the morphogenesis process.

Levels of cellular competence

The morphogenesis process, e.g., the formation of new cellular structures, is intimately related to competence of the cell in answering signs of extrinsic and intrinsic factors, which begins by the breaking of cell determination and with the first cell divisions that originate the meristematic centers or meristemoids (Dhaliwal et al. 2003). The competence acquisition, corresponds particularly, to the ability of a particular target cell has in to respond of defined form to a specific hormonal signal (Cedzich et al. 2008; Thompson 2008; Silveira et al. 2013) (Fig. 1).

In this context, the ability of meristems to develop a new organism from an explant, depend on distinct stages, including acquisition of competence, induction or morphogenic determination to an specific route, the differentiation and finally the development (Christianson and Warnick 1983; Christianson 1985). There is a direct relationship between the cell's ability in originating distinct cell types and degrees of dedifferentiation and competence morphogenic of the same. According to the degree of dedifferentiation, the cells can be characterized as multipotent, totipotent or pluripotent.

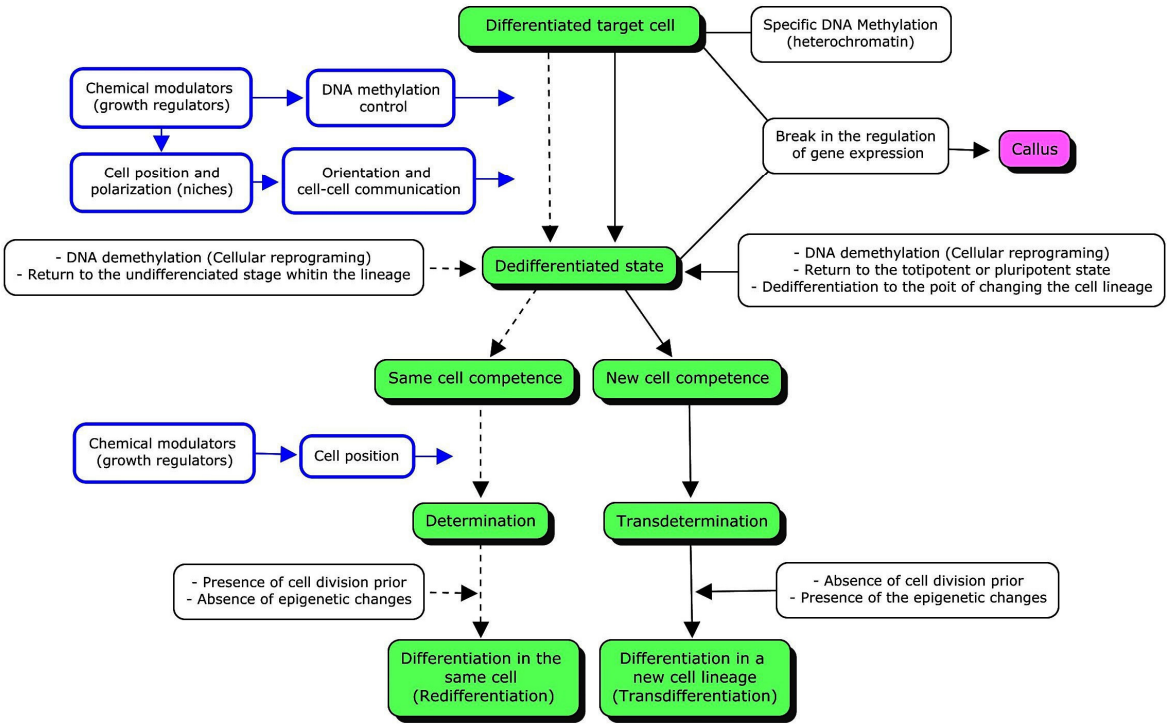

Figure 1. Flowchart representing the main determinants in plant *in vitro* morphogenesis (*boxes with blue arrows*). Boxes with dotted black arrows correspond to the regeneration process that result in a single cellular lineage (redifferentiation) and the boxes with solid black arrows indicate the regeneration process that involves the origin of different cellular types (transdifferentiation). Calluses (*pink box*) are from breaks in the regulation of gene expression of the target cell

The cellular multipotency corresponds to the ability of a single cell to produce different kinds of cell within a particular cell lineage (Hochedlinger and Plath 2009) (Figs. 2a-c), while the pluripotency corresponds to the ability of the cell to differentiate in the majority of cell types, but not in their entirety of the types required for the formation of the plant body, having as an example the formation of a bud or root (Komatsu et al. 2011) (Figs. 2d-f). Totipotent cells in turn can cause all cell types constituting the plant body (Verdeil et al. 2007) (Figs. 2g-i). Therefore, stem cells or target cells are examples of totipotent cells, that after renew themselves, can activate one or more programs of cellular differentiation.

According with Verdeil et al. (2007), totipotent cells have a large nucleus, centralized with a single nucleolus, with irregularly shaped invaginations of the nuclear envelope and a high nuclear cytoplasmic ratio. The cytoplasm is dense, containing high amount of amyloplasts and small vacuoles fragmented. Plasmodesmata are rarely observed in the cell wall, modified by deposition of callose, giving in this way the isolation of its immediate neighboring cells. This physical isolation favors the reprogramming of genomic and cellular functions, essential for the acquisition of totipotentiality and competency to morphogenetic routes.

The pluripotent stem cells are located together to the cells derived from the region of differentiation of the shoot and root meristems, having high nuclear cytoplasmic ratio, with typically spherical nucleus, isodiametric and containing one or more nucleoli (Verdeil et al. 2007) (Figs. 2d-f). The cytoplasm is dense with many fragments of small vacuoles and without the presence of an amyloplast. It presents many plasmodesmata, due to the strong dependence and interaction with neighboring cells, creating a niche that maintains its cellular identity.

The morphogenic competence of a target cell increases with the increase of euchromatin and therefore the property

to develop an adult individual complete. Larger quantities of euchromatin in relation to heterochromatin characterize the totipotency, whereas the increase in genetic material silenced (heterochromatin) characterize the pluripotency (Verdeil et al. 2007). Most probably, the multipotency is accompanied by the presence of considerably greater amounts of heterochromatin in relation to the euchromatin; however, more studies are needed to prove this hypothesis (Fig. 3). A simplified proposal for the description of these events is represented in Figures 2 and 3.

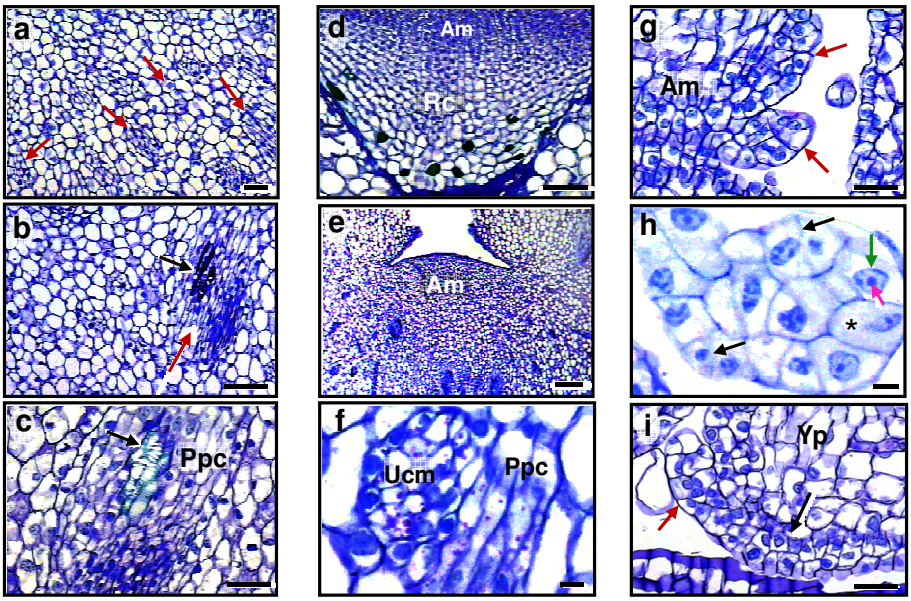

Figure 2. Multipotency, pluripotency and totipotency in *Bactris gasipaes* Kunth. explants *in vitro* cultured. **a**, **b** and **c** Traces of pre-procambials cells (*red arrows* and *Ppc*) acting as multipotent cells with ability to give rise to vascular bundles by the direct organogenic way in culture medium in the presence or absence of plant growth regulators (NAA, BAP, TDZ or 2iP) isolated in the culture medium or the cytokinins combined with NAA Apical meristem (*Am*); protoxylem (*arrows black*). **d** and **e** Apical meristems (*Am*) root (**d**) and stem (**e**) containing pluripotent cells to regenerate of primary and secondary tissues of roots and shoots, respectively. Root Cap (*Rc*). **f** Traces of pre-procambials cells (*Ppc*) acting as pluripotent cells with ability to give rise to adventitious bud by the direct organogenic originating from the unipolarization of meristematic center (*Ucm*) after cultivation in culture medium supplemented with TDZ. **g** and **h** Meristematic cells of apical stem acting as totipotent cells with ability to give rise to somatic embryos by thet direct somatic embryogenesis with multicellular origin after cultivation in culture medium containing the presence of TDZ. In **h**, detail of high nuclear cytoplasmic ratio of the cells of an loose pro-embryo originated via this morphogenic route and the presence of a protoderm (*black asterisk*) well defined and derived from anticlinal division (*black arrows*) of the peripheral layer. Nucleus (*green arrow*); nucleolus (*pink arrows*). **i** Epidermal and subepidermal meristematic cells (*black arrow*) of young pinnae (*Yp*) acting as totipotent cells with ability to give rise to somatic embryos by the direct somatic embryogenesis with multicellular origin after cultivation in culture medium containing the presence of TDZ. Pro-embryo (*red arrow*). *Bars*: **a**, **c** and **i** = 50 µm; **b** and **f** = 10 µm; **d**, **e**, **g** and **h** = 100 µm. Photomicrographs E-J: Graner (2009).

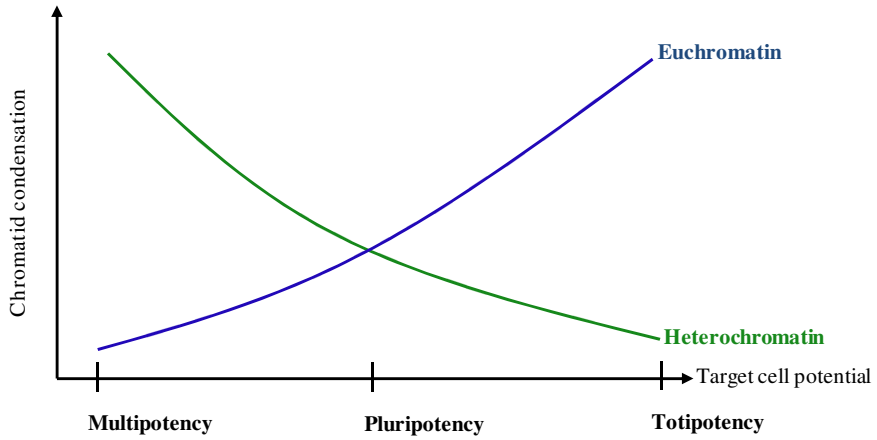

Figure 3. Graphic representative of the morphogenetic potentiality of the target cell as a function of chromatin condensation.

Cellular determination

Shoot and root meristematic cells have competence to develop into initial cells, maintaining their meristematic condition. However, a change of programming or reprogramming at cellular level, induces the production of derived cells, which are able to differentiate, resulting in different cells which will constitute the primary meristematic tissues of the plant body (i.e., protoderm, fundamental meristematic tissue and procambium), cellular specialization that is known as cellular determination.

Cellular determination is the process in which the development competence of a cell becomes limited to a specific route (Christianson 1985) which is in dependence previous to its acquisition (Church and Galston 1988), i.e., is the ability of a particular target cell in respond to specific developmental signals, e.g., metabolic, molecular or hormonal signaling and cellular positioning (Peres 2002; Dolan 2006; Cedzich et al. 2008; Thompson 2008; Almeida et al. 2012; Chupeau et al. 2013; Knauer et al. 2013) (Fig. 1).

Kerbauy (1999) affirms that the determined state is stable and can be transmitted at intact form for several cellular generations, similar to what occurs with the leaf primordia, which after initial differentiation, maintains an irreversible stage of determination, preventing them of originate vegetative buds (Byrne 2012). Christianson and Warnick (1983) and Tucker et al. (1986) reported that the process of determining a cell occurs by the restriction or 'channeling' on their potential to differentiate along of the developing paths, resulting in a more stable involvement to a single route.

In vegetable organisms, through the influence of extrinsic factors from adjacent cells, the differentiation is dependent on the establishment of cell polarity; asymmetric division and also of the positioning of cell in the plant body (Sussex and Kerk 2001; ten Hove and Heidstra 2008; Smolarkiewicz and Dhonukshe 2013). However, in tissue culture, the degree of determination can be altered, inducing the cells to achieve a less differentiated stage. This occurs when cells become free from the control that are being subjected in the body integrity, and when exposed to a new condition in the culture medium, which leads to dedifferentiate and to express its genome in another way, establishing new patterns of differentiation, to form new organized structures (Handro and Floh 1990). This effect is due to the fact that cells or groups of cells acquire competence to the stimulating effects of culture medium (Kerbauy 1999) (Fig. 1).

Although cell division, as everything indicates, is part of dedifferentiation resulting in new cell types, there is a process by which the conversion occurs in a specific cell in another distinct cell type, phenomenon known as transdifferentiation, which can occur without cellular division predicted, which is usually induced by endogenous hormones (McManus et al. 1998; Pang et al. 2008), which provide positional information (McManus et al. 1998) (Fig. 1). As examples of transdifferentiation, there are reports on the conversion of parenchyma cells into tracheary elements (Sugiyama and Komamine 1990); cells of petiole of bean (*Phaseolus vulgaris* L.) into abscission cells responsive to ethylene (McManus et al. 1998), immature cells of the xylem in cells of the phloem (Pang et al. 2008) and further, subepidermal cells in pro-embryonic cells (Almeida et al. 2012). Understands, therefore, that morphogenesis includes all processes of differentiation, development and growth, both *in vitro* and *in vivo* conditions.

A change in the course of differentiation is known for transdifferentiation and entails a change of the competence at differentiation, without cause mutations in the DNA (i.e., somaclonal variation) (Wei et al. 2000) in which only occur epigenetic changes (Meins and Foster 1986) (Fig. 1). Cells can return to a less differentiated stage within their own lineage, proliferate and redifferentiate, replacing the lost cells, while in transdifferentiation, the cells dedifferentiate to a specific point where is possible to alter their lineage (Jopling et al. 2011; Eguizabal et al. 2013) (Fig. 1).

During the dedifferentiation and subsequent differentiation and obtaining of new cellular lineages, there is a direct influence of the age of the cell and its degree of differentiation, determination and/or residual memory retention of the original somatic cell (Kim et al. 2010). Classical studies such as Moore (1979) define the cellular differentiation as the transformation of genetically identical cells, derived from a zygote or any other cell, in biochemical, physiological and structurally specialized cells. Ultimately, cellular differentiation is a process that reflects the effect of at least three factors: genetic, established at fertilization and incorporation of "stock" of potential, which may be expressed during the development; characteristics originated in ontogeny, initially as a response to environmental stimuli but once established tend to remain in a stable or permanent basis, and finally, the characteristics whose expression depends only on the environment (Kerbauy 1999).

Gene expression

The transition from differentiated to undifferentiated stages of cells requires abrupt changes in their interior, such as changes in chromatin structure by means of changes in the portions that are accessible to transcription (e.g., euchromatin) versus the portion that is repressed (e.g., heterochromatin), which occurs in two distinct phases during chromatin decondensation. The first is a phase of transition that provides competence to change the cell's fate, which under suitable conditions is followed by a second phase, proteasome-dependent, which represents a compromise with the mitotic cycle (Zhao et al. 2001).

The regions of DNA methylation, corresponding to regions of heterochromatin (Valledor et al. 2007) prevents gene transcription and characterize the different cell types and differentiation states of the action of messenger RNA (mRNA), which in turn, modulates the levels of gene products transcription (Xu et al. 2009; Li et al. 2011) (Fig. 1). Therefore, the level of methylation allows monitoring the feasibility of cloning by *in vitro* rejuvenation, since increasing the level of methylation reflects in decreased organogenic capacity (Valledor et al. 2007). The pattern of DNA methylation varies between the cell types and tissues, and is a key to differentiation and plant development (Ikegami et al. 2009; Chupeau et al. 2013).

The change in the pattern of DNA methylation depends on the silencing of somatic expression of some genes by cellular reprogramming (Fig. 1) and of embryonic stem cells genes super-regulation of, with the concomitant elimination of chromatin structure (Papp and Plath 2011). The heritability of DNA methylation acts as a 'cellular memory' from the tissue of origin (Ohgane et al. 2008; Kim et al. 2010), which may represent an impediment to achieve a pluripotent state, limiting the new cell lines (Kim et al. 2010). Furthermore, changes in DNA methylation may occur when plants are exposed to *in vitro* culture conditions (Phillips et al. 1994; Chupeau et al. 2013) (Fig. 1), as the expression of silenced genes by extensive phenotypic selection in field conditions (Graner et al. 2013).

The selective activation and differential gene (i.e., cellular reprogramming), known for epigenesis, is also directly related to the presence and number of receivers of plant growth regulators involved in the direct control of

gene activity at transcription and transduction level (Guerra et al. 1999).

According to Jopling et al. (2011) the cell reprogramming is a very stressful process for the cell, which breaks the regulation of epigenetic information in stem cells, potentially pluripotent, can change its dedifferentiation and differentiation properties. Thus, resulting in the initiation and progression of an undifferentiated cell mass and without defined organization, referred as callus (Fig. 1). Therefore, a direct relationship between the reprogramming, induction and development of callus can be evidenced.

The regulation of cellular determination is possible by optimal balance between endogenous and exogenous factors where the cells will be submitted, which will unleash morphogenetic routes for the development of specific cellular structures (Fig. 1). Moreover, in some species, there are differences in the ability of *in vitro* regeneration which are controlled by a few genes (i.e., genes regeneration), which possibly are "masters genes" which can be related to the presence of receptors for hormones of plant and/or can encode some key enzyme in the plant metabolism (Peres 2002; Duclercq et al. 2011). Therefore, the targeting to cellular determination is regulated mainly by chemical modulators, which unleash and define all the routes morphogenetic (Fig. 1).

Chemicals modulators

Morphogenic stimuli that direct cells to an organized growth are mediated by plant growth regulators such as auxins, cytokinins and gibberellins, which interfere in the cellular differentiation, acting as signaling chemical modulators of this process (Fig. 1). The competence of plant cells (i.e., refers to the ability to react to specific signals) is directly related to the production of these plant growth regulators by the plant itself or by its exogenous availability.

Plant growth regulators have a direct influence on the process of morphogenesis in different species and different ways (Lakshmanan et al. 1997). The specific effects of plant growth regulators have not been fully elucidated yet and further investigations regarding auxin, cytokinins, ethylene, polyamines and other regulators in terms of activity in morphogenic process are needed.

The dynamic and differential distribution of the auxin in plant tissues controls a variety of developmental processes, which adjusts the growth and morphology of plants to environmental conditions (Vanneste and Friml 2009), the example of apical dominance, defined by inhibiting the growth of axillary buds due to the growth of the apical bud. Moreover, the development of flower buds, the induction of vascular differentiation and the retardation of leaf abscission and fruit development are also controlled by the levels of auxin (Moore 1979).

Cytokinins in the presence of optimal levels of auxin are able to induce cellular divisions, but its effects are not just limited to the induction of divisions. These plant growth regulators has shown important role in other stages of growth and development of plants such as senescence, apical dominance, cell elongation, differentiation, assimilates flux and nutrients by the plant (Mok and Mok 2001).

During the *in vitro* culture, endogenous hormone levels can be strongly altered by exogenous application of plant growth regulators and exert significant effects on morphogenetic responses (Mok et al. 1987; Moncaleán et al. 2005). The role of hormones, particularly the auxin/cytokinin effect in development depends on the establishment of spatial and temporal gradients, whose main responsibility would be of the peculiarities of synthesis and transport, and cytokinins (Pino-Nunes 2005) and auxins (Wareing and Phillips 1981) inactivation enzymes. Rather than to conceive the system of plant growth regulators as a matrix of parallel pathways to signal processing, this system is more aptly described as a network of interactions in which changes in a particular segment promote adjustments in other areas (Müller et al. 2002). Both the plant hormones, as other regulators, including toxins, light and other elicitors, mediate their effects through the transduction and amplification pathways, and there is evidence that a particular hormone may perform different roles, and not necessarily in the same way in sequence of events for a specific process (Gaspar et al. 2003).

The same way the auxin/cytokinin influences the morphogenesis, has already been reported that exogenous application of indolbutiric acid (IBA) in combination with gibberellic acid (GA) stimulates the exchange activity, through the intensification of the process of cell division this tissue (Wareing 1958; Wareing et al. 1964).

Polyamines are also considered plant growth regulators that act in a number of processes of plant development (Kaur-Sawhney et al. 2003; Silveira et al. 2013): can promote or inhibit the process of adventitious rooting (Geiss et al. 2009), are involved in glycerol-mediated promotion of somatic embryogenesis (Wu et al. 2009) or cause epigenetic disruptions (Konan et al. 2010). These substances are required to induce a biological response such as to control the frequency of cellular divisions, DNA synthesis, RNA and proteins with a consequent growth control and development of plants (Gaspar et al. 2003). Therefore, several authors emphasized that without the synthesis of polyamines would be impossible to cell survival.

The elevation in the concentrations of polyamines in the explants was also associated with high levels of DNA methylation and the consequent loss of embryogenic capacity of cell cultures in *Pinus nigra* (Noceda et al. 2009). Couée et al. (2004) suggests that the elucidation related at polyamines and the control of gene regulation would allow determine its involvement in cell division and differentiation.

Ethylene is a gaseous hormone that is involved in responses to various biotic and abiotic stresses, in addition to inducing a rapid decrease in DNA methylation, assigning a probable increase of the morphogenic potential (Galaud et al. 1993), as founded by Lu et al. (2011) in *Pinus sylvestris* somatic embryogenesis.

Considering that plant growth regulators are involved in the direct control of gene activity at the level of transcription and transduction, through the selective activation and differential of genes (e.g.; cellular reprogramming) (Lambé et al. 1997; Guerra; et al. 1999) and through control of DNA methylation (Vlasova et al. 1995; Lambé et al. 1997) the "return" to the stadium totipotent or pluripotent (i.e., dedifferentiation), most probably, is related to the its action, as previously described (Fig. 1).

Cellular positioning

Physiological and genetic analyzes have proved the importance of the position and polarity of cell in tissue for the differentiation and subsequent development (Smet et al. 2009; Almeida et al. 2012; Smolarkiewicz and Dhonukshe 2013). The maintenance of the identity of certain cells in strong interaction and dependence of neighboring cells receives the designation of cellular niche, e.g., cells of apical meristem of shoot and root (Verdeil et al. 2007). Furthermore, the use of plant growth regulators can lead to different morphogenic pathways leading to potential niche establishment, depending on the positioning of the competent cells and their interaction with neighboring cells (Almeida et al. 2012) (Fig. 1).

The cellular polarization has certain dependence on the extrinsic factors from the adjacent cells, as the polarization of the egg-cell after fertilization (e.g., zygote), which occurs through specific signals from the maternal tissue cells. Other examples would the polarization of the hypophysis cell, as mentioned previously, which occurs through the basipetal flux of auxin or signals induced by this hormone (ten Hove and Heidstra 2008) and the polarization of the pericycle cell, which probably occurs after receiving the transcription factors (De Smet and Beeckman 2011) from the adjacent cells at protoxylem poles by the presence of auxin (De Smet et al. 2007).

Although the molecular processes which regulate the polarization of the nucleus is not know, has been reported that microtubules are involved in this process (De Smet and Beeckman 2011). Once the asymmetric cell division is established, besides the different intrinsic factors present in each daughter-cell, such as transcription factors and differential expression of genes, both are under the action of extrinsic factors, such as auxin or other transcription factors (ten Hove and Heidstra 2008).

In this context, and according to the positioning of daughter-cells, a transcriptional network in conjunction with plant growth regulators, act in the signals communication of cell-to-cell, promoting the cellular determination and differentiation (De Smet et al. 2009; Papp and Plath 2011; Smet and Beeckman 2011) (Fig. 1). Furthermore, the high activity of cyclin dependent kinases (CDKs), which is directly related to the entry or not of the cell in mitotic cycle, can be detected in the apical meristems. The more the cells move away from the meristem, the level of activity of CDKs reduces considerably, along with the mitogenic factors, such as plant growth regulators and carbohydrates, inducing differentiation. Inversely, in proximity of cells to meristematic centers, where are concentrated the mitogenic compounds, the activity of CDKs is high while maintaining therefore the undifferentiated stage of division. However, the physiological events that interfere with the activity of CDKs with cellular differentiation are not yet completely understood (De Veylder et al. 2007).

Cellular habituation

The habituation is defined by Meins (1989) as a stable and hereditary loss of growth factors requirements by cells of cultivated plants. According to the author, the cellular habituation of auxin and cytokinin, results from reversible changes in cellular heredity, known as epigenetic changes. In contrast to mutations, epigenetic changes are reversible and directed, i.e., occur in response to a specific inductor (Demarly 1976; Meins and Seldran 1994) and imply changes in DNA that influence the gene expression (Kim et al. 2010). The developmental stage of the cells has a strong influence in relation to the tendency to habituation (Meins and Lutz 1980; Turgeon 1982), as well as different tissues also differ regarding the competence for cytokinin habituation (Meins and Lutz 1979).

Although there is evidence that habituation results from the accumulation of hormones by cells to which are habituated, it is unknown whether this accumulation occurs by increasing of synthesis, by the decrease in degradation or by a combination of both factors, may exist a mechanism of synthesis/degradation of metabolites, characterizing a feedback process (Meins 1989).

The habituation not only causes cell sensitivity to endogenous hormones, but also the accumulation of metabolites that may substitute the control of cytokines in cell division, alterations in the metabolism of polyamines and ethylene, an increase in the content of diacylglycerol as well as an increase in levels and conversion of inositol phosphates (Gaspar et al. 2000; 2003).

Although there are reports that the independence of the cells to cytokinins and auxins, as well as the synthesis of polyamines during the process of habituation is directly integrated with primary biochemical pathways (Gaspar et al. 2000; 2003), it seems that habituation does not imply increased production of cytokinins, but promote an increase in the sensitivity through an increase in the synthesis of CRE1 cytokinin receptors (Pischke et al. 2006). Indeed, the increase in the production of auxin and cytokinin in habituated cells has not been confirmed (Kevers et al. 1999; Gaspar et al. 1999).

The cellular habituation occasioned by prolonged periods of *in vitro* subcultures may be the limiting factor for commercial production, due to result of the progressive decline of plant vigor (Akin-Idowu et al. 2009), e.g., tobacco callus habituated to cytokinin, where was shown that the higher the level of these endogenous substances, the lower the ability the development of adventitious buds, indicating an inverse correlation for both processes (Kerbauy 1981). Furthermore, the presence of achlorophyllous cells have also been observed in habituated callus of *Beta vulgaris*, due to the deviation of α-ketoglutarate for the synthesis of polyamines, rather than the metabolic pathway "Beale" for the synthesis of chlorophyll (Gaspar et al. 1998, 1999; Häsler et al. 2003).

Considering that both natural and synthetic plant growth regulators acts on the control in DNA methylation of plant cells (Vlasova et al. 1995), the increase in sensitivity of cells to cytokines may be due to overexpression of genes for CRE1 receptors (Loidl 2003), as mentioned before, resulting in DNA hypomethylation in the expression of these genes (Pischke et al. 2006), in turn, the DNA hypermethylation in the heterochromatic regions (Lambé et al. 1997; Valledor et al. 2007) which promotes considerable loss of organogenic potential (Fraga et al. 2002) and somatic embryogenesis (Salajova et al. 1999) due to the age of the tissue or prolonged periods of *in vitro* cultivation.

Stem cells have the unique ability to self-renew and also activate one or more differentiation programs. Although these cells express transcription factors that are associated with totipotency and/or pluripotecy, there are substantial differences in the characteristics of the transcriptional regulatory networks that characterize them. Decipher these networks is the way to elucidate new mechanisms of understanding to regulate the morphogenesis stages, and thus enable control of organogenesis and somatic embryogenesis.

The transcription factors are modulators of pluripotent stage, which can induce the transition between different stages. Many of the available methods to convert or induce a dedifferentiated stage, involve the use of chemical inhibitors of specific targets of signaling pathways, emphasizing the importance of understanding the roles of signaling of extrinsic factors.

The gene expression associated with chemical modulators may enable the development of new approaches to control cellular stages. The induction of less differentiated states (i.e., multipotent, pluripotent and totipotent), with less determination and acquisition of competences for new morphogenetic routes implies a reduction of DNA methylation, memory loss and cellular reprogramming, which consequently enable the rejuvenation of tissues to obtain plant regeneration and cloning. Despite the great number of studies performed, the influence of endogenous and exogenous factors that involve the plant morphogenesis has not been fully elucidated.

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