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EXPRESSION OF *WUSCHEL*-GENE PROMOTING SOMATIC EMBRYOGENESIS IN PLANTS

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Somatic cells are induced to form embryos by the application of stress and high doses of auxin treatments. Hence, embryogenic cells can be considered totipotent cells based on their aptitude to regenerate or develop into an embryo under certain conditions. During conversion of somatic to embryogenic transition, cells have to differentiate, activate their cell division cycle and reorganize their physiology, metabolism and gene expression patterns. Cell differentiation depends on the proper and sequential expression of key genes required for conversion of somatic cells into embryogenic pattern. The homeobox transcription factor *WUSCHEL* (*WUS*) has been shown to cause dedifferentiation when expressed on somatic cells that can lead to somatic embryogenesis or organogenesis. The expression of *WUS* gene in the embryogenic tissue derived from the mature trees of *Pinus roxburghii* revealed that *WUS* might be influencing the molecular mechanism that mediates the vegetative-to-embryogenic transition. *WUS/PGA6* also plays a key role during embryogenesis, presumably by promoting the vegetative-to-embryogenic transition in conifers too.

Keywords: Cell; Cloning; Differentiation; Transcriptional factor.

Introduction

A *WUSCHEL*-like homeobox (*WOX*) gene have been shown to play important role in lateral organ formation and meristem function. *WUS* was originally identified as a central regulator of shoot and floral meristems in *Arabidopsis* where it is expressed in a small group of cells, and is required to maintain the overlying stem cells undifferentiated. Recent studies indicate that *WUS* can act both as transcriptional activator of the floral gene *AGAMOUS* and as a transcriptional repressor of cytokinin response genes, suggesting that its molecular function is modified upon the developmental context. *WUS* has been found to be sufficient to ectopically induce stem cells. Most plant organs are formed during the post embryonic stages from the meristems[1-3]. The shoot apical meristems (*SAMs*) are organized pools of undifferentiated or embryonic cells maintained by a dynamic balance between cell division and differentiation. The balance between the rate of cell division and differentiation is crucial for the ordered transition zones in the meristem. The meristematic identity of cells in the *SAM* is correlated with the expression of specific regulatory genes[1-2]. Cell differentiation depends on the proper and sequential expression of key genes required for morphogenesis. Several aspects of control are required for this which include: chromatin modification, DNA methylation, correct amount of particular transcriptional factors, proper nuclear arrangement recruitment of cells into differentiationnt etc[2-3]. This review paper highlights about the expression of *WUS* gene inducing somatic embryogenesis in plants. It also reports about the molecular mechanism that mediates the vegetative-to-embryogenic transition.

WUSCHEL-gene expression in plants - In *Arabidopsis thaliana*, *WUS*, *CLV*, and *SHOOT MERISTEMS* (STM) have been implicated in the maintenance of undifferentiated cells in the meristem. *STM* is tough to prevent premature recruitment of cells into differentiation pathways, while *WUS* is required to keep the pool of stem cells in the central zone. The combined effect of ectopic *WUS* and *STM* have an additive effect on cell division, expression of the central zone and initiation of organ primordial in *Arabidopsis*. During last few years the homeobox transcription factor *WUSCHEL* (*WUS*) has been shown to cause dedifferentiation when expressed on

somatic cells followed by a production of new cells that can lead to somatic embryogenesis or organogenesis[2-3]. During the past 3 decades, considerable efforts have been made to identify genes with altered expression pattern during somatic embryogenesis. Most of these genes however, are up regulated only in the developmental stages, suggesting that they do not play a direct role in the vegetative-to-embryogenic transition. So far, the only exception is the carrot somatic embryogenesis receptor kinase (*SERK*) gene, whose expression appears to mark vegetative-to-embryogenic transition; however its function remains unclear[2-3]. Overexpression of the *Plant Growth Activator 6 (PGA6)* gene promotes the formation of somatic embryos from various vegetative tissues, as well as from zygotic embryos independent of any external plant hormones[2-3]. These somatic embryos following a developmental process remarkably similar to that of zygotic embryogenesis were able to germinate and grow healthy, fertile plants, suggesting that *PGA6* is involved in the maintenance of embryonic cell identity. *PGA6* was found to be identical to *WUS*, a homeodomain protein previously characterized as a key regulator for the specification of meristem cell fate [2-3]. *WUS* in addition to its role in regulating meristem development as previously suggested also plays a critical role in the maintenance of embryonic cell identity. *WUS* also promotes the vegetative-to-embryonic transition and/or maintaining. This is the first line of genetic evidence on the mechanism of somatic embryogenesis in plants[2-3]. Further expression of *WUS* in *Coffea canephora* plants can induce calli formation as well as a 400% increase in somatic embryo production. Histological analysis showed the development of meristem like structures, from which some produce somatic embryos after 4 weeks. Moreover, a 400% increase in the induction of somatic embryogenesis was observed from the transform *WUS* plants in *Coffea canephora*[2-3]. These results showed that transgenic expression of the transcription factor *WUS* can be useful to increase somatic embryogenesis in heterologus systems[2]. *WUS* has been shown to be a part of *WOX* family of proteins. The *WOX* proteins maintain a common regulatory sequence between them. The function still unknown in the majority of the *WOX* members however it is known that they are expressed asymmetrically and may be involved in the differentiation process [2-3]. Loss of *WUS* function leads to differentiation of stem cells and meristem termination. By contrast, ectopic *WUS* has been shown to induce somatic embryogenesis in *Arabidopsis thaliana* and in others to induce ectopic stem cell identity. During wild type development, *WUS* expression is initiated in four apical cells of the 16 cell embryo after several asymmetric divisions *WUS* expression is confined to the centre of the developing shoot meristem.

It was noticed that *WUS* is capable of promoting the vegetative-to-embryonic transition, and eventually somatic embryo formation in *Arabidopsis thaliana* and *Coffea canephora*, which suggests that the homeodomain protein can play a critical role during embryogenesis, in addition to its function in meristem development. Therefore, it was presumed that the highly restrictive expression of *WUS* hallmarks the putative embryonic organizing centre which in turn, may give rise to stem cells during embryogenesis and later development. This is mainly because of *WUS* expressing cells have not been morphologically and functionally characterized, and it remains interesting to determine wheather this cluster of cells indeed represents a functional organizing centre similar to Spemann's organizer, discovered nearly 80 years ago in *Xenopus* embryos. The mechanism by which *WUS* prevents the differentiation of stem cells is unknown. Furthermore, its activity in dedifferentiating cells may prove unconventional as a way to reorganize heterochromatin to the level of stem cells when expressed in selected tissues. By contrast, *WUS* appears to be a key player in maintaining and /or inducing embryonic potential, as its activity does not appear to require any developmentally specific factors. Therefore, *WUS* plays a predominant role in maintaining embryonic potential, wheareas *LEC1* is probably involved in promoting differentiation of embryonic cells at later developmental stages. A reasonable assumption would be that *LEC1* activity, a driving force for embryo cell differentiation, must be excluded in order to fully maintain the embryonic potential in the putative organizing centre. Additionally the regulatory pathway in which *WUS* may be able to act promoting somatic embryo formation in *Arabidopsis* seems to be conserved as it functions in a similar way in *Coffea*. However, unlike *Arabidopsis* that an induction of *WUS* was all that was necessary for somatic embryo formation or organogenesis.

Somatic embryogenesis is a developmental restructuring of somatic cells towards the embryogenic pathway. This developmental switching involves differential gene expression conferring to somatic cells the ability to manifest the embryogenic potential. In all the in vitro systems phytohormones, particularly auxin or 2,4-D are essential for the induction of somatic embryogenesis, although auxin has to be promptly removed from the medium for somatic embryos to form. This is because appropriate auxin transport and distribution

are needed for embryo development and pattern formation. *WUS* transient overexpression causes highly embryogenic callus formation in the presence of auxin, whereas it directly induces somatic embryo formation from different plant organs in the absence of any exogenous auxin[4]. Therefore, it appears that *WUS* can reprogram cell fate, bypassing the auxin requirement or simply taking advantage for the endogenous auxin flux. Moreover, no callus phase or at least only few cell-division cycles, are sufficient to induce cells to restart a totally new embryogenic pathway in tissues of plants that overexpress *WUS*. However, there is at least one part of the plant that *WUS* can not reprogram to form embryos: the shoot apical meristm. Overexpression of WUS under the control of meristem specific promoters such as *CLV1, ANT, LFY, AP3*, and *AG* did not result in any somatic embryogenesis phenotype. The presence or absence of some factors in the shoot apex could favor one (a shoot meristem organizer) or the other *WUS* function (an embryo organizer). The finding of *PGA6* gain-of-function mutations or overexpression of *WUS* result in somatic embryo formation from vegetative tissues at high frequency should have a significant impact on plant biotechnology, and provides a convenient and attractive model system for many aspects of plant biology research. PGA6 was found to be identical to *WUS*, a homeodomain protein previously characterized as a key regulator for the specification of meristem cell fate. Somatic embryogenesis thus involves several molecular events including not only differential gene expression, but also various signal transduction pathways for activating/repressing numerous gene sets, many of which are yet to be identified and characterized. Mature embryos express *WUS* in a small cell group underneath the two outermost cell layers. *CLV3* functions as a mobile intercellular signal in the shoot apical meristem that spreads laterally from the stem cells and acts both on their neighbors and on the stem cells themselves to repress *WUS* transcription. *CLV* genes promote the progression of meristem cells toward organ initiation. Mutation in any of these genes result in delayed organ initiation, leading to an accumulation of meristem cells and increase in the size of the shoot meristem dome. Mutation in *WUS* results in the misspecification of stem cells and premature termination of shoot and floral meristems after a few organs have been formed. The analysis of most abundant mRNAs representative of different stages of development is a simple way of developing a preliminary understanding of gene expression related to morphogenesis. The advent of molecular techniques has been crucial for the identification of genes that exhibit differential activity, e.g. construction of cDNA libraries, differential display analysis, subtracted probe analysis, and PCR analysis etc. *LEA*-like genes that have been characterized in conifers show developmental regulation during embryo morphogenesis. As an initial global molecular analysis of conifer embryogenesis, a cDNA library was constructed from mature somatic embryos in *Picea glauca* and isolated 28 cDNAs for further analyses[5-7]. The genes were found to encode storage proteins, heat shock proteins, glycine-rich cell wall proteins, metallothioneinlike protein and some other metabolic enzymes or structure proteins. The majority of these genes were expressed during the later stages of embryogenesis although a few were also expressed during the early stages as well. An initial genetic characterization of early embryo development in *Pinus radiata* led to the identification of six gene families preferentially expressed during conifer embryogenesis. Comparative studies of gene expression patterns have also been reported. Comparison of transcript profiles between normal and developmental arrested embryogenic lines has also been described[8-10]. More recently, by using micro array analysis it was shown that 35 genes out of 373 were differentially expressed specifically during normal somatic embryogenesis in Norway spruce[11]. Recently it was described a 500 transcript profile in zygotic and somatic embryogenesis of loblolly pine[12]. In *P. roxburghii* for the first time the expression of cDNA clones of genes involved in programming the apical meristem cells towards somatic embryogenic pathway influenced by external environmental stimulus like cold –pretreatment has been studied[13-16]. Differential display was used to isolate the genes which were expressed specifically in embryogenic tissue induced by cold-pretreatment of thin sections of vegetative shoot apices of mature trees of *Pinus roxburghii*. Of the 56 cold-enhanced embryogenic-associated cDNAs identified, 20 were cloned. Nine of the 20 fragments which generated single bands on re-amplification were selected for cloning and further analysis. During reverse northern hybridization, all the 20 clones selected generated a positive signal when probed with labeled cDNA from cold-enhanced embryogenic tissue, but no signal when probed with cDNA from the non-embryogenic tissue (control treatment). All the 9 clones thus contained inserts that were specific to cold-enhanced somatic embryogenesis[13]. The identification of genes revealed the expression of *WUSCHEL* (*WUS*) in the embryogenic tissue derived from the apical meristematic tissue from mature trees of *P. roxburghii* (Malabadi and co-workers-unpublished work). This

clearly indicates the involment of *WUS* in the induction of embryogenic tissue from apical meristem. Therefore, homeobox transcription factor *WUSCHEL* (*WUS*) might be involved in the molecular mechanism that mediates the vegetative-to-embryogenic transition during cloning of mature trees of *P. roxburghii* (Malabadi and co-workers-unpublished work).

Conclusion - In the plant shoot meristem, the stem cells are specified by *WUS*-dependent signals from underlying organizing centre cells, and transcriptional control of the *WUS* gene within the proliferating shoot apex is a key regulatory switch in stem cell regulation. *WUS* has been shown to have a dominant role in meristem development which also acts to maintain embryonic competence. A major future challenge will be to integrate the roles of other players such as *LEC1, LEC2, PTI/AMP* and *AtSERK1* into the already complicated *WUS-CLV* network. The *WUSCHEL* (*WUS*) gene expression in *Arabidopsis, Coffee* and in the embryogenic tissue derived from mature trees of *P. roxburghii* played an important role by promoting the vegetative-to-embryogenic transition. The diverse regulatory pathways that the stem cells in the shoot meristem converge at these two short sequence elements of the *WUS* promoter, suggesting that the integration of regulatory signals takes place at the level of a central transactivating complex. The finding of *PGA6* gain-of-function mutations or overexpression of *WUS* result in somatic embryo formation from vegetative tissues at high frequency should have a significant impact on plant biotechnology, and provides a convenient and attractive model system for many aspects of plant biology research.

References

1. Leibfried A, To JPC, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ and Lohman JU 2005, WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* **438** (22) 1172-1175.
2. Arroyo-Herrera A, Gonzalez AK, Moo RC, Quiroz-Figueroa FR, Loyola-Vargas VM, Rodriguez-Zapata LC, Burgeff D' Hondt C, Suarez-Solis VM and Castano E 2008, Expression of WUSCHEL in *Coffee canephora* causes ectopic morphogenesis and increases somatic embryogenesis. *Plant Cell Tiss. and Org. Cult.* **94** 171-180.
3. Zuo J, Niu QW, Frugis G and Chua NH 2002, The *WUSCHEL* gene promotes vegetative-to embryonic transition in *Arabidopsis*. *The Plant Journal* **30**(3) 349-359.
4. Baurle I and Laux T 2005, Regulation of *WUSCHEL* transcription in the stem cell niche of the *Arabidopsis* shoot meristem. *The Plant Cell* **17** 2271-2280.
5. Dong JZ and Dunstan DI 1996, Expression of abundant mRNAs during somatic embryogenesis of white spruce *[Picea glauca* (Moench) Voss]. *Planta* **199** 459-66.
6. Dong JZ and Dunstan DI 1997, Characterization of cDNA representing five abscisic acid responsive genes associated whith somatic embryogenesis in *Picea glauca,* and their responses to abscisic acid stereo structure. *Planta* **203** 448-453.
7. Dong JZ and Dunstan DI 2000, Molecular biology of somatic embryogenesis in conifers. *In:* Jain S, Gupta P, Newton R (eds.) *Somatic embryogenesis in woody plants*: 51-87. Kluwer Academic Publishers, Dordrecht.
8. Stasolla C, Belmonte MF, van Zyl L, Craig DL, Liu W, Yeung EC and Sederoff RR 2004, The effect of reduced glutathione on morphology and gene expression of white spruce *(Picea glauca)* somatic embryos. *J. Exp. Bot.* **55** 695-709.
9. Stasolla C, Bozhkov PV, Chu TM, Van Zyl L, Egertsdotter U, Suarez MF, Craig D, Wolfinger RD, Von Arnold S and Sederoff RR 2004, Variation in transcript abundance during somatic embryogenesis in gymnosperms. *Tree Physiology* **24** 1073-85.
10. Stasolla C, van Zyl L, Egertsdotter U, Craig D, Liu W and Sederoff RR 2003, The effects of polyethylene glycol on gene expression of developing white spruce somatic embryos. *Plant Physiology* **131** 49-60
11. van Zyl L, Bozhkov PV, Clapham DH, Sederoff RR and von Arnold S 2003, Up, down and up again is a signature global gene expression pattern at the beginning of gymnosperm embryogenesis. *Gene Expression Patterns* **3** 83-91.
12. Cairney J, Xu N, Mackay J and Pullman J 2000, Transcript profiling: a tool to assess the development of conifer embryos. *In Vitro Cellular and Developmental Biology-Plant* **36** 155-162.
13. Malabadi RB and Nataraja K 2007, Plant regeneration *via* somatic embryogenesis using secondary needles of mature trees of *Pinus roxburghii* Sarg. *Int. J. Bot.* **3** 40-47.
14. Malabadi RB and Nataraja K 2007, Isolation of cDNA clones of genes differentially expressed during somatic embryogenesis of *P. roxburghii. Amer. J. Plant Physiol.* **2** 333-343.
15. Malabadi RB, Teixeira da Silva J and Nataraja K 2008, A new approach involving salicyclic acid and thin cell layers for cloning mature trees of *Pinus roxburghii* (Chir Pine). *The Americas J. Plant Sci. and Biotech.* **2**(2) 56-59.
16. Malabadi RB, Teixeira da Silva J and Nataraja K 2008, Salicylic acid induces somatic embryogenesis from mature trees of *Pinus roxburghii* (Chir pine) using TCL Technology. *Tree and Forestry Sci. and Biotech.*. **2**(1) 34-39.