

# Strategies for spatiotemporal regeneration



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## INTRODUCTION OF SPATIOTEMPORAL REGENERATION

*De novo* shoot organogenesis in tissue culture provide an excellent opportunity in order to study the factors that elicit the totipotency of cells which lead to shoot regeneration in higher plants. Auxins and cytokinin plays an important role for this. In addition to this histological study of the plant tissue at different time interval provides an important tool to reveal the various factors responsible for shoot development. It has been found that the ratio of auxin to cytokinin is critical for plant to induce cell reprogramming and in directing plant tissue regeneration (Skoog and Miller, 1957). Higher concentration of auxin to cytokinin favours root formation whereas higher cytokinin concentration leads to shoot formation. When both hormones were used in equal amount callus is formed. However, the basic mechanism responsible for regeneration is poorly understood.

Christianson and Warnick (1983, 1985) by using *Convolvulus arvensis* as regenerating system divided the process of *in vitro* organogenesis into three phases. In the initial phase, the explant which is incompetent state acquires competence to initiate division in stem cells. After acquiring competence, the explants undergoes inductive phase in which organ formation starts. At the end of the inductive phase, morphogenesis takes place in which either shoot or root are formed. Recently it has been observed that callus bears milieu of pluripotent cell which consists of root pericycle cell, lateral root progenitor and cell of root meristem. These act as stem cell and give rise to either root or shoots or somatic embryos (Atta et al, 2009).

Using *Arabidopsis* as a regenerating system, it has become easy to genetically dissect developmental processes and understand the histological changes, molecular genetics and events underlying *in vitro* shoot regeneration in plants (Cary et al, 2002; Lall et al., 2004; Che et al., 2006).

When root explants were incubation on CIM, it caused pericycle cells to divide and form roots similar to lateral root formation from pericycle (Casmiro *et al.*, 2001). Further by transferring it into SIM, the pericycle cells continue to proliferate by disrupting the radial organization of the vascular bundle. When shoot buds are visible, new centers of radial organization are formed that appear to be meristematic. A number of genes that get activated in response to external stimulus of growth regulators and lead to transformation of cultured explants into callus and de novo regeneration of shoot buds (Hwang and Sheen, 2001; Cary *et al.*, 2002; Daimon *et al.*, 2003; Hibara *et al.*, 2003; Reddy and Meyerowitz, 2005; Verdeil *et al.*, 2007; Gordon *et al.*, 2007; Atta *et al.* 2009; Sugimoto *et al.*, 2010).

In summit of shoot apical meristem, a central zone (CZ) is located which contains population of undifferentiated, slowly dividing pluripotent cells. The cells in its periphery zone are usually smaller and divide more rapidly and directs the differentiation both in spatio and temporal manner in order to produce leaf primordial, lateral organs, auxillary meristem and outer tissue of the stem. Below the CZ, rib meristem is present which give rise to pith of the stem. The cells arising in the CZ either maintain the integrity of the stem cell population or are displaced into the peripheral zone or rib meristem which give rise to new organ. The function of the rib meristem is to control the internal stem structure and the vasculature. As all the cell of the central

zone remain undifferentiated until they may be incorporated into organ primordial. Despite cells being continuously synthesized and diverted to differentiation pathways, the cell in each functional domain of SAMs remains largely constant, (Steeves and Sussex, 1989). The peripheral zone

Homeostatic balance between different functional domains is due to coordination between gene expression and cell proliferation (Meyterowitz, 1997). These pluripotent cells are located in three tiers at the very apex and coincide with the domain where the *CLAVATA 3 ( CLV3 )* gene is expressed (Fletcher et al, 1999).

The genes which are expressed for the development of shoot apical meristem are those of *WUSCHEL ( WUS )*, *CLAVATA ( CLV )*, *CUP SHAPED COTLYEDONS ( CUC 1 & 2 )* and *SHOOT MERISTEMLESS*. The expression of *WUS* is required for the activation of stem cells that stimulate SAM (Gallois et al, 2004).

The expression of *WUS* domain is limited to deep central cell that constitute the SAM organizing centre (Mayer et al., 1998). *WUS* encodes a homeodomain protein of a novel class which is expressed in a group of cells located beneath the stem cells of SAM. The initiation of *WUS* expression is independent of *STM* activity as *WUS* is expressed in small subdomain of the SAM (Mayer et al., 1998).

On the basis of its levels and place of expression, *WUS* perform multiple functions. In the central zone elevated level of *WUS* induce expansion of the central zone and but also result in increased division rate in the cells of peripheral zone. However, decrease in *WUS* levels lead to decrease in cell

division rate of peripheral zone and also smaller central zone (Yadav et al. 2010). Thus confinement of *WUS* expression to central zone is important for maintaining constant number of stem cell which is partly negatively controlled by class of *CLAVATA* (*CLV*) genes. *CLV3* expressed in the stem cells of the CZ encodes a small secreted peptide that binds and activates the *CLV1* transmembrane receptor kinase (Clark et al., 1997; Jeong et al, 1999 Schoof et al, 2000).

By inducing *CLV3*, the *CLV* complex regulates the size of stem cells (Schoof et al, 2000). In the central zone *CLV3* gene encodes a small peptide which that activates *CLV1* transmembrane protein receptor kinase. In addition another transmembrane protein receptor kinase, *CORYNE* along with *CLV1* regulates the *WUS* expression and therefore SAM size (Muller et al., 2008; Bleckmann et al, 2010). However, the *CLV3* expression is auto-activated by *WUS* expression (Yadav et al. 2010).

A constant number of stem cells in central zone are maintaining by *CLV3* thereby preventing differentiation of daughter stem cell that has been displaced by division in central zone into the surrounding peripheral zone. Thus, *CLV3* maintains the overall size of SAM by repressing cell division rates in the cell of peripheral zone (Reddy and Meyerowitz, 2005).

Over expression of *CLV3* results in decrease mitotic activity in central zone. The increase cell division rate in cells of the peripheral zone leads to transient down-regulation of *CLV3* level which in turn causes expansion of central zone and Rib meristem (Reddy and Meyerowitz, 2005).

However, the establishment and maintenance of SAM is carried out by *SHOOT MERISTEMLESS*, (Lenhgard et al, 2002). In the functional SAM, both *STM* and *CLV* may act antagonistically on common *WUS* downstream targets. The *STM* gene encodes Class 1 Knotted ( *KNOX* ), a homeodomain containing protein family which acts as transcription factor. It is expressed at top of central cell which prevent the incorporation of central cell in lateral primordial (Endrizzi et al, 1996; Daimon et al, 2003). The overexpression of *KNOX* gene leads to growth in ectotropic buds on leaves thereby increasing capacity to leaf explants to form *in vitro* shoots (Chuck et al, 1996).

Another gene *CUC 1* and *CUC 2* controls shoot apical meristem formation via transcription activation of *STM* . *CUC 1* encodes a NAC-domain protein highly homologous to *CUC 2* and *NAM* expression. In *Arabidopsis* , *CUC 1* functions upstream of *STM* and regulates SAM formation during embryogenesis (Takada et al. 2001). *CUC 1* and *CUC 2* are required for embryonic SAM development and for keeping cotyledons and floral organs from fusing together. Although genes that influenced *in vitro* shoot regeneration are being identified but still the precise developmental events and their regulation which lead to shoot or root regeneration is yet to be unravelled fully. Therefore, the precise and intricate mechanisms that regulate meristematic activity its timing, location and extent of cell division, cellular differentiation and expansion that contribute to shoot morphogenesis and its regeneration in *Piper longum* .