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**Anjanasree K. Neelakandan & Kan Wang**

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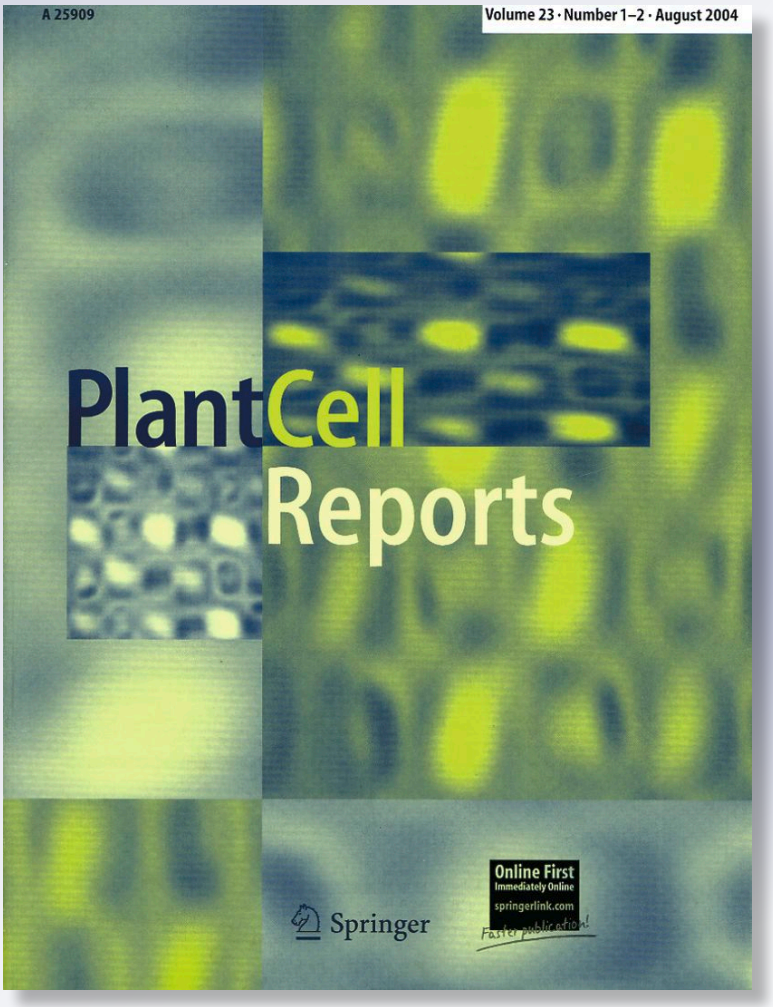


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REVIEW

# Recent progress in the understanding of tissue culture-induced genome level changes in plants and potential applications

**Anjanasree K. Neelakandan · Kan Wang**

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**Abstract** In vitro cell and tissue-based systems have tremendous potential in fundamental research and for commercial applications such as clonal propagation, genetic engineering and production of valuable metabolites. Since the invention of plant cell and tissue culture techniques more than half a century ago, scientists have been trying to understand the morphological, physiological, biochemical and molecular changes associated with tissue culture responses. Establishment of de novo developmental cell fate in vitro is governed by factors such as genetic make-up, stress and plant growth regulators. In vitro culture is believed to destabilize the genetic and epigenetic program of intact plant tissue and can lead to chromosomal and DNA sequence variations, methylation changes, transposon activation, and generation of somaclonal variants. In this review, we discuss the current status of understanding the genomic and epigenomic changes that take place under in vitro conditions. It is hoped that a precise and comprehensive knowledge of the molecular basis of these variations and acquisition of developmental cell fate would help to devise strategies to improve the totipotency and embryogenic capability in recalcitrant species and genotypes, and to address bottlenecks associated with clonal propagation.

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A. K. Neelakandan · K. Wang (✉)
Department of Agronomy, Iowa State University,
G405 Agronomy Hall, Ames, IA 50011-1010, USA
e-mail: kanwang@iastate.edu

A. K. Neelakandan · K. Wang
Center for Plant Transformation, Plant Sciences Institute,
Iowa State University, G405 Agronomy Hall, Ames,
IA 50011-1010, USA

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## Introduction

Plant cells are unique in that they retain totipotency and developmental plasticity in the differentiated state and have the ability to dedifferentiate, proliferate, and subsequently regenerate into mature plants under appropriate culture conditions in a hormone-dependent manner (Skoog and Miller 1957; Steward et al. 1964). As a consequence, plants can initiate cell proliferation and development from diverse tissues in response to hormonal stimuli. The ability of cultured explant tissue to reset its genetic and epigenetic program in order to endure the artificial hormonal environment will ultimately determine its fitness and adaptability to in vitro cultures. As a consequence to these dynamic processes orchestrated at the molecular level, off-types or variants are often identified among these clonally propagated progenies. The term 'somaclonal variation' refers to tissue culture-induced stable genetic, epigenetic or phenotypic variation in clonally propagated plant populations (Larkin and Scowcroft 1981, 1983). This is considered a major problem in commercial micropropagation wherein the regenerant population is expected to be homogenous. However, these somaclonal variations generated in vitro have been efficiently exploited in developing new varieties with superior agronomic traits in diverse species (Jain 2001).

Plant stem cells naturally present in the root and shoot apex of intact plants are considered 'pluripotent', since they are able to form cell and tissue types present in either

root or shoot tissue. In contrast, the term 'totipotent' is applied to somatic cells cultured in vitro to produce embryogenic cells, which give rise to somatic embryos and regenerated whole plants (reviewed in Verdeil et al. 2007). Factors influencing in vitro adaptability and regeneration are varied, ranging from genotype, origin of explant, culture conditions, and hormonal effects. Establishment of stable, efficient in vitro regeneration systems in economically important crops is a prerequisite for biotechnology and molecular breeding applications. Furthermore, plant cell cultures have evolved as in vitro experimental models for studying cell division, differentiation and morphogenesis, which are important in key developmental processes such as meristem formation and embryogenesis (Zimmerman 1993) and stress-related genome plasticity in plants (Madlung and Comai 2004).

The availability of genome sequence information and mutant/tagged collections in select plant species, extensive exploitation of novel genomic tools, use of precise developmental, stage-specific molecular markers, advances in live imaging and microscopy (including laser capture micro-dissection) and application of spectroscopic techniques such as laser-induced fluorescence (LIF) have enabled researchers to accurately monitor the developmental changes that take place in plant cell culture. These high-throughput approaches provide an array of candidate genes whose function can subsequently be verified by reverse genetics strategies. The identification of key genes involved in in vitro adaptation and development is immensely helpful in developing strategies to enhance regeneration and morphogenesis in heterologous, recalcitrant species.

In this review, we discuss the current understanding of genomic and epigenomic changes that take place under in vitro conditions. These changes are believed to facilitate explant adaptation to culture conditions and to aid in subsequent morphogenesis processes. Understanding the molecular basis of these changes and acquisition of developmental cell fate will enable researchers to undertake informed hypothesis-driven strategies aimed at producing true-to-type plants in clonal propagation and to improve the totipotency and embryogenic capability of recalcitrant species. Alternatively, regulatory circuits could be modified to expand the repertoire of somaclonal variations for crop improvement and reverse genetic approaches.

## Molecular changes during in vitro culture

Figure 1 outlines the scheme of plant cells in culture and highlights factors affecting genome stress and molecular regulation of developmental events in vitro. Explant tissue is excised from the intact plant after dissecting that involves wounding, and is incubated under aseptic, artificial conditions with an exogenous nutrient source provided by the media on which the tissue is cultured (Skoog and Miller 1957). Typically, the explant undergoes direct organogenesis or somatic embryogenesis. In the indirect method of regeneration, the explant passes through a 'callus phase' wherein it undergoes dedifferentiation and loss of photosynthetic ability, thereby necessitating the addition of a carbon source, such as sucrose, in the medium. In some cases like protoplast culture, chemical/mechanical treatment is required to eliminate plant cell walls thereby contributing to additional stress. Other physical factors such as the reduction–oxidation (redox) environment, temperature, light quality, photoperiod and presence of specific hormones all influence the ability of the tissue to adjust to these conditions and initiate developmental transitions for survival.

These dynamic changes are facilitated by reprogramming of cellular physiology, metabolism changes, revival of cell division, dedifferentiation, redifferentiation, morphogenesis, etc., all of which are initiated by profound molecular changes. A remodeling of gene expression reflected at the steady state RNA and protein levels corresponding to specific developmental programs has also been documented using subtractive hybridization, gene chip and whole genome microarrays and proteomics approaches (Yin et al. 2007). These proteins belong to diverse families such as receptor kinases, transcription factors, structural proteins and enzymes, and are implicated in cell differentiation and morphogenesis.

In vitro environment is associated with permanent genetic changes such as chromosomal ploidy level, chromosome breakage and rearrangement, base substitution in DNA sequence, and activation and mobility of transposable elements to other genomic locations (Phillips et al. 1994). Epigenetic deregulation, reflected primarily as alteration in methylation levels, also reportedly occur in the in vitro cultured tissues (Kaeppler et al. 2000). Recent insights into epigenetic reprogramming of the genome in terms of variation in chromatin modification and small RNA-mediated regulation are beginning to open up new vistas and provide new tools for desirably manipulating the in vitro response of diverse species and cultivars.

### Gene expression regulation of developmental cell fate in vitro

Plant cells in culture have the unique potential to alter their developmental program in order to adapt to culture conditions. This plasticity is influenced by multiple factors including the genetic makeup of the plant and environmental factors, such as hormones and nutrient molecules. The molecular regulatory mechanisms underpinning

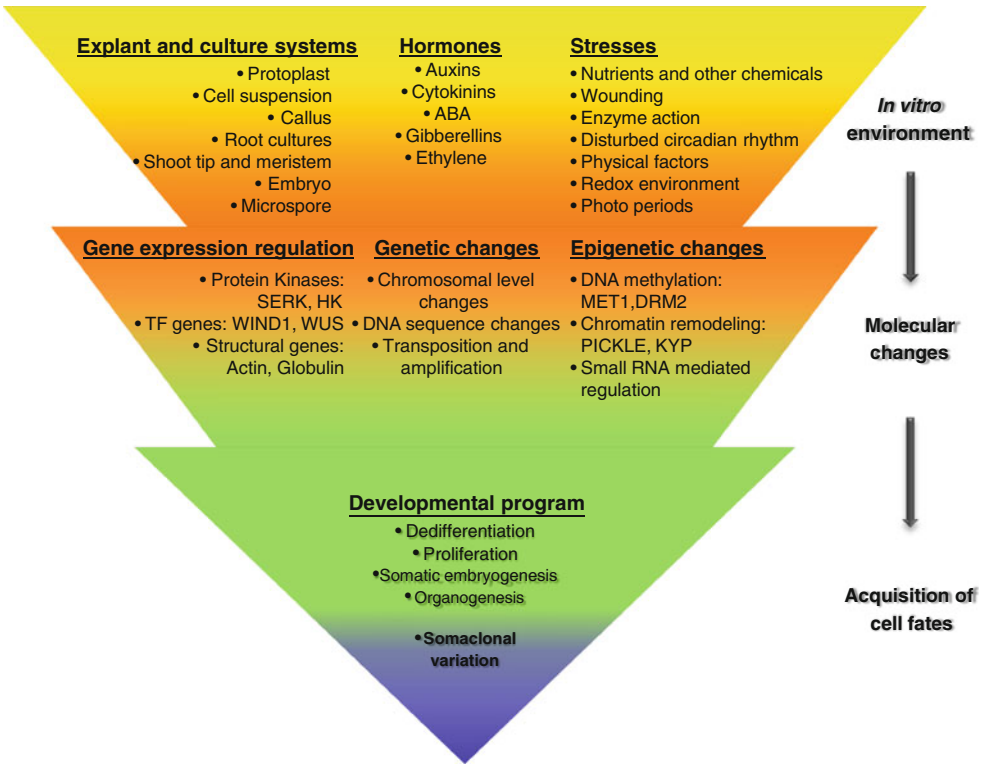


**Fig. 1** In vitro culture and molecular changes caused in the process. Cultured plant cells are believed to generate genomic stress resulting from wounding, physical–chemical factors, presence of hormones and/or enzymes, coupled with the developmental events of dedifferentiation and regeneration. These changes are manifested at the gene expression level in protein kinases, transcription factors (*TF*) and structural genes and contribute to explant adaptation to stress and reorientation of its developmental program. In vitro culture is also associated with genetic changes including chromosomal changes, DNA sequence alterations, amplifications and transpositions. More recent discoveries point to epigenetic changes at the level of DNA methylation, chromatin modification and small RNA-mediated regulation taking place in cultured tissues. The spectrum of genetic and epigenetic changes can potentially give rise to phenotypic changes among the regenerants that are termed somaclonal variations

hormonal crosstalks affecting in vitro adventitious shoot regeneration have been recently reviewed (Duclercq et al. 2011). The use of developmental markers associated with in vivo shoot and root apical meristem formation, hormone synthesis, transport and signaling has also been instrumental in precisely monitoring and dissecting these in vitro developmental events (Atta et al. 2009). Molecular control of developmental switches is orchestrated by profound, but transient changes in gene expression (reflected in RNA and protein levels) involved in hormone synthesis, signaling and response, and transcriptional regulators affecting development.

Several strategies including QTL analysis (Taguchi-Shiobara et al. 2006; Bolibok et al. 2007; Krakowsky et al. 2006; Song et al. 2010), biochemical studies (Tang and Newton 2005), global expression profiling (Che et al. 2006a, b) and candidate gene analysis (Gong et al. 2005) have been used to study molecular mechanisms underlying in vitro responses such as callus initiation, production of somatic embryos, and organogenesis in different species. Quantitative proteomics techniques such as stable isotope labeling by amino acids in cell culture (SILAC) have been refined to suit plant systems and exploited recently in Arabidopsis suspension cell culture systems to analyze relative protein expression levels (Gruhler et al. 2005; Schütz et al. 2011). High-throughput transcriptomic (Singla et al. 2007; Bao et al. 2009) and proteomic techniques (Yin et al. 2007; Marsoni et al. 2008), state-of-the-art protein analysis techniques such as liquid chromatography mass spectrometry (LC–MS) (Jung et al. 2008) and multidimensional protein identification technology (MudPIT) (Chen et al. 2009b; Cho et al. 2009) are extremely powerful tools to simultaneously identify and monitor a large number of RNA/protein and their expression changes in vitro. Some of the techniques used for analyzing the variations generated under in vitro culture conditions are summarized in Table 1.

### *Protein kinases*

Protein kinases are enzymes that catalyze the transfer of phosphate groups from a nucleoside triphosphate to amino acids such as serine and threonine, or histidine residues present in plant proteins thereby modulating the properties,

**Table 1** Techniques used for detecting cell culture-induced genomic changes

| No. | Type of variation | Technique | Example reference(s) |
|---|---|---|---|
| 1 | Chromosome level changes | Flow cytometry | Leal et al. (2006) |
| | | Fluorescent in situ hybridization (FISH) | Gernand et al. (2007) |
| 2 | DNA sequence changes | Restriction fragment length polymorphism (RFLP) | Andreev et al. (2005) |
| | | Inter-simple sequence repeats markers (ISSR) | Sreedhar et al. (2007) |
| | | Random amplified polymorphic DNA (RAPD) | Jin et al. (2008) |
| | | Microsatellites or simple sequence repeat (SSR) | Jin et al. (2008) |
| | | Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) | Kour et al. (2009) |
| | | Amplified fragment length polymorphism (AFLP) | Li et al. (2007) |
| | | Next generation sequencing of genomic DNA libraries | Jiang et al. (2011) |
| 3 | DNA sequence-specific changes | Inter-retrotransposon amplified polymorphism (IRAP) | Smýkal et al. (2007) |
| | | Sequence-specific amplification polymorphism (SSAP) | Kour et al. (2009) |
| | | Transposon display | Ngezahayo et al. (2009) |
| 4 | RNA expression changes | Differential display | Linkiewicz et al. (2004) |
| | | Suppression subtractive hybridization | Zeng et al. (2006) |
| | | EST array or Gene Chip hybridization | Che et al. (2006a, b) |
| | | cDNA macroarray hybridization | Singla et al. (2007) |
| | | Genome-scale microarray hybridization | Bao et al. (2009) |
| 5 | Protein expression changes | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) | Krsnik-Rasol (1991) |
| | | Two-dimensional gel electrophoresis (2-DGE) | Yin et al. (2007) |
| | | Stable isotope labeling of amino acids in cell culture (SILAC) | Schütz et al. (2011) |
| 6 | Isozyme pattern changes | Starch gel electrophoresis and staining | Mangolin et al. (1994) |
| 7 | Metabolite changes | Nuclear magnetic resonance spectroscopy (NMR) | Palama et al. (2010) |
| 8 | DNA methylation changes | Methylation-sensitive Restriction fragment length polymorphism (metRFLP) | Kaeppler and Phillips (1993a, b) and Jaligot et al. 2002 |
| | | Methylation-sensitive restriction fragment length polymorphism (metRFLP) | Bednarek et al. (2007) |
| | | Methylation-sensitive amplification polymorphism (MSAP) | Kour et al. (2009) |
| | | Based on treatment with the restriction enzyme McrBC, which only cleaves DNA at 5-methylcytosine | Tanurdzic et al. (2008) |
| | | DNA degradation followed by reversed-phase high-performance liquid chromatography (RP-HPLC) | Kubis et al. (2003) |
| | | DNA degradation followed by high performance capillary electrophoresis (HPCE) | Berdasco et al. (2008) |
| | | Based on bisulfite treatment, which changes un-methylated cytosines into uracil, and sequencing | Ngezahayo et al. (2009) |
| | | Transposon methylation display (TMD) | Ngezahayo et al. (2009) |
| 9 | Chromatin condensation | Fluorescent in situ hybridization (FISH) | Koukalova et al. (2005) |
| 10 | Chromatin modification | Chromatin IMMUNOPRECIPITATION (ChIP) analysis | Grafi et al. (2007) |
| 11 | Small RNA expression changes | Cloning and northern analysis | Luo et al. (2006) |
| | | Cloning and qRT-PCR | Wu et al. (2011) |
| | | MicroRNA array hybridization | Zhang et al. (2010b) |
| | | Cloning and deep sequencing of small RNA libraries | Tanurdzic et al. (2008) |

localization and functionality of the interacting partners. Cyclin-dependent protein kinases belong to the serine/threonine kinase family and are key players in the molecular regulation of cell cycle and cell division in eukaryotes. The *R2* gene of rice encodes a cyclin-dependent protein kinase. When over-expressed in tobacco, the gene was able to confer cell division and callus formation properties, instead of root organogenesis in an auxin-rich medium,

pointing to a role of this class of protein kinase in regulating the acquisition of cell fate (Yamaguchi et al. 2003). Members of the Sucrose non-fermenting 1 (SNF1)-related serine/threonine kinase (SnRK) family, for example, *Medicago truncatula* Stress kinase 1 (MtSK1), have been reported to be important for stress-induced somatic embryo formation (Nolan et al. 2006).

Receptor protein kinases are generally transmembrane protein kinases with an extracellular receptor-like ligand binding domain and an intracellular kinase domain. They play a prominent role in cellular signal transduction pathways in prokaryotes and eukaryotes. A signal transduction cascade is initiated by the binding of a specific ligand or signal followed by transduction of the signal inside the cell resulting in the altered transcription of an array of genes involved in development, stress or hormone response. Somatic embryogenesis receptor kinase (*SERK*), a leucine-rich repeat receptor-like kinase (LRR-RLK), identified in cultured carrot cells was the first receptor-like kinase suggested to play a major role in somatic embryo induction (Schmidt et al. 1997). Homologues of the *SERK1* gene also reportedly play equivalent roles in cultured cells of Arabidopsis (Hecht et al. 2001), sunflower (Thomas et al. 2004), rice (Hu et al. 2005), wheat (Singla et al. 2008), grapes (Schellenbaum et al. 2008a) and coconut (Pérez-Núñez et al. 2009). An exception to these reports is the identification of two maize homologues (*ZmSERK1 and 2*) that were found to be expressed in both embryogenic and non-embryogenic tissues (Baudino et al. 2001).

Another LRR-receptor-like kinase, Clavata 1 (CLV1), mediates signaling that restricts the size of the shoot meristem by negatively regulating the WUS transcription factor (Cary et al. 2002; Miwa et al. 2009). Ectopic overexpression of rapeseed *CLV1* gene in Arabidopsis led to a drastic reduction in somatic embryo production (Elhiti et al. 2010), thereby pointing to its negative regulatory effect in this process.

Proteins belonging to the histidine kinase family act as hormone receptors in plants. Cytokinin hormone signaling is mediated by a two component system that comprises histidine kinases involved in signal perception and response regulators that transduce the signal to downstream effectors. Arabidopsis histidine kinases 1, 2 and 3 (AHK1, AHK2 and AHK3), and cytokinin response 1 (CRE1/AHK4) form a family of transmembrane receptors that are involved in sensing and initiation of the cytokinin signaling cascade (Inoue et al. 2001; Higuchi et al. 2004; Romanov et al. 2006). Hormone habituation is a phenomenon by which plant cells and tissues lose the requirement of exogenous hormones to sustain cell division and development upon continuous culture (Meins 1989). The *CRE1* receptor expression was upregulated in Arabidopsis cultures habituated for cytokinin indicating an important role of hormone perception and signaling in this phenomenon (Pischke et al. 2006).

The receptor kinase activation is the starting point of the signaling cascade mediating developmental switches/hormone responses; it represents an important regulatory control point. However, the downstream signaling components and transcription factors modulate the ultimate response features of the species or explant of interest.

### *Transcription factors*

Transcription factors are regulatory proteins that are able to induce or repress the transcription of genes owing to their ability to bind to specific DNA sequences thereby regulating target gene expression. They are essential mediators of developmental transitions and cellular stress responses (Liu et al. 1999). Many genes that have established roles in regulating embryogenesis and meristem induction and development in vivo have also been found to regulate somatic embryo formation and organogenesis in vitro. Transcription factors of the Apetala2/Ethylene Response Factor (AP2/ERF) family like Bolita (BOL) (Marsch-Martinez et al. 2006), and wound-induced dedifferentiation 1 (WIND1) (Iwase et al. 2011) have been found to trigger cell dedifferentiation and proliferation leading to callus formation. Babyboom (BBM) (Boutilier et al. 2002) and Embryomaker (EMK) (Tsuwamoto et al. 2010), belonging to the same family, have established roles in somatic embryo induction and development. The BBM protein is reported to trigger cell proliferation by interacting with downstream target genes, including actin depolymerising factor (ADF9), which is important for actin reorganization affecting developmental switches (Passarinho et al. 2008). Enhancer of regeneration (ESR) 1 and 2 (Banno et al. 2001; Ikeda et al. 2006) are other members of this gene family that have been found to positively affect shoot organogenesis in Arabidopsis. The role of ethylene in somatic embryo development was demonstrated in *Medicago* protoplast cultures, where Somatic Embryo-Related Factor1 (MtSERF1), an ethylene responsive ERF subfamily transcription factor, was shown to be crucial for embryo induction and is therefore implicated in stress and developmental crosstalk (Mantiri et al. 2008).

Wuschel, a related protein Wuschel homeobox (WOX) (Palovaara and Hakman 2008; Park et al. 2010; Gambino et al. 2011), MADS domain containing protein Agamous-like15 (AGL15) (Harding et al. 2003), Leafy Cotyledon 1 (LEC1) (Zhang et al. 2002), Leafy Cotyledon 2 (LEC2) (Ledwoń and Gaj 2009), Fusca3 (FUS3), ABA Insensitive 3 (ABI3)/Viviparous1 (VP1) (Su et al. 2009), Leafy Cotyledon 1 like (LIL) (Alemanno et al. 2008; Schellenbaum et al. 2008a; Chiappetta et al. 2009) are some of the transcription factors believed to play a crucial role in somatic

embryogenesis. Mutants of the Leafy Cotyledon genes (*LEC1, LEC2* and *FUS3*) displayed drastically reduced somatic embryo production in vitro, thereby proving their key role in in vitro embryogenic response, despite a normal auxin gradient (Gaj et al. 2005). Another homeobox protein, Shootmeristemless (STM) (Elhiti et al. 2010), NAC family transcription factors like Cup shaped cotyledon 1 (CUC1) (Takada et al. 2001) and (CUC2) (Daimon et al. 2003; Motte et al. 2011) were found to be important for stem cell establishment and shoot formation in Arabidopsis.

The effect of auxin on meristem specification in somatic embryos was examined in Arabidopsis using molecular markers for auxin efflux protein Pinformed (PIN) and auxin responsive reporter, *DR5::GUS*. Expression of the auxin transporter *PIN4* gene was found to co-localize with the site for root initiation (root pole) in an auxin responsive manner, as evidenced by *DR5::GUS* expression (Bassuner et al. 2007). Localized expression of the homeobox transcription factor Wuschel (WUS) (Zuo et al. 2002) is considered a reliable marker for acquisition of competence for shoot regeneration in Arabidopsis (Che et al. 2007) and *Medicago* (Chen et al. 2009a). In these studies, the exact concentration of exogenous auxin supply was essential for inducing somatic embryogenesis. This is necessary for efficient polar localization of auxin mediated by PIN1 proteins, and establishment of an auxin gradient, which were prerequisites for the correct expression of *WUS* gene required for shoot apical meristem specification (Su et al. 2009).

Activation of transcription factors may imply the activation of their target genes. Hence, identifying master regulators of key processes and analyzing the underlying interactions and regulatory network (Zheng et al. 2009) may help us to understand and predict in vitro developmental phenomena.

### *Structural proteins and enzymes*

Biochemical, transcript and protein profiling studies have identified a large number of genes coding for proteins potentially involved in developmental events. These proteins belong to diverse metabolic pathways and play a pivotal role in cellular reprogramming in response to stress and hormonal cues in vitro.

Biochemical activity of cell wall enzymes such as $\beta$-1,3-glucanases in chicory (Helleboid et al. 1998; 2000), cell wall and membrane associated proteoglycan proteins such as glucosamine- and acetylated glucosamine-containing arabinogalactan proteins in carrot (van Hengel et al. 2001), abscisic acid (ABA) inducible expression of embryo-specific globulin protein in maize (Duncan et al. 2003), and germin-like proteins in conifer cultures (Mathieu et al. 2006) are found to be associated with somatic embryo induction. Recently, a mutant Arabidopsis was identified with an inability to regenerate shoots in in vitro culture, attributed to a defect in fasciclin-like arabinogalactan protein expression (Johnson et al. 2011).

The activity of mevalonate kinase, an enzyme involved in isoprenoid biosynthesis, has been reported to be a biochemical marker for root induction of white pine shoots (Tang and Newton 2005). Likewise, the expression of glutathione-*S*-transferase (GST), which is crucial for protecting cellular machinery from oxidative damage, was correlated with shoot regeneration in mustard (Gong et al. 2005). Interestingly, AUX 1, an auxin influx facilitating membrane protein, was also found to be involved in cytokinin-mediated stimulation of auxin accumulation in shoots (Kakani et al. 2009).

Early studies identified that the auxin-induced accumulation of ACT7, a protein involved in the formation of actin cytoskeleton, was essential for cell proliferation and callus formation in Arabidopsis cultures (Kandasamy et al. 2001). Actin deploymerising factor proteins (e.g. ADF9) have also been shown to express predominantly in calli, indicating the significance of actin cytoskeleton dynamics in determining meristematic activity (Ruzicka et al. 2007).

The differential expression and hormonal regulation of callus dedifferentiation and redifferentiation was probed by developing two-dimensional gel electrophoresis (2-DGE) protein reference maps in rice (Yin et al. 2007; 2008). A large number of cellular metabolism-related proteins such as those involved in carbohydrate metabolism (e.g. alpha amylase isoforms), stress/defense (e.g. mannose-binding lectins), cytoskeleton dynamics (e.g. actin, beta-tubulin), amino acid metabolism (e.g. *S*-adenosylmethionine synthetase), photosynthesis (e.g. photosystem I subunit), etc. were found to be differentially expressed in various callus stages. These approaches facilitate rapid identification of putative candidate genes and proteins involved in this developmental process and also aid in understanding the role of these proteins in mediating the in vitro responses.

## Genetic changes associated with tissue culture

Plant tissue culture-mediated micropropagation is also referred to as 'clonal propagation' implying that all the progenies generated as a result of this asexual method in vitro are 'clones' or 'true to types'. However, morphological off-types or variants observed among the progeny are found associated with permanent genetic changes or temporary, potentially reversible epigenetic changes to the DNA. These changes are believed to be a direct manifestation of cellular stress responses and genome evolution as proposed originally by McClintock (1984).

Genetic changes frequently associated with in vitro regenerated plants lead to stable, lasting modifications to

the genome that are inherited in subsequent generations. Some of these molecular changes are associated with phenotypic differences and hence referred to as somaclonal variations. This in vitro-induced variation has been exploited as a technology to develop new cultivars with improved and desirable agronomic traits such as yield, early maturity, and resistance to biotic and abiotic stresses (Jain 2001 and the references therein). The in vitro selection strategy has been employed successfully for desirable traits such as herbicide tolerance (Wrather and Freytag 1991), drought and abiotic stress tolerance (Lu et al. 2007) and disease resistance (Sotirova et al. 1999). This technology is particularly relevant in asexually propagated plants and self pollinated crops with a narrow genetic base.

Genetic variations observed in in vitro regenerated plants are largely stochastic, unpredictable, and non-reproducible, and have been classified as originating from the source/explant ('pre-existing') or induced during the culture process ('de novo') (Larkin and Scowcroft 1981). For example, in the flowering plant *Saintpaulia*, tissue culture regenerants show altered variegation and flower color phenotype because of the activation and mobility of *VGs1* transposon (Sato et al. 2011a). Percentages of 'pre-existing' mutated cells in the source tissue from different plant parts and the 'de novo' variations generated during culturing and regeneration process were estimated using quantitative real-time PCR technique and compared. It was demonstrated that heritable variations in regenerated plants are indeed predominantly associated with 'de novo' tissue culture-induced changes (Sato et al. 2011b). The extent of genomic instability depends on a range of factors, including genotype, explant type, in vitro system, genome size, age of the culture, presence of an intermediate callus phase, and nature or concentration of the exogenous growth hormone used in nutrient media (Bairu et al. 2011).

### *Chromosomal level changes*

Gross changes such as variation in ploidy level, number of chromosomes, and structural changes represent major alterations to the genome and they are often generated during in vitro proliferation and differentiation. Ploidy refers to the number of chromosomal sets in a given cell. Polyploid organisms have several sets of chromosomes in the genome, as opposed to that of normal diploid organisms (Leal et al. 2006). They arise as a consequence of endoreduplication, wherein the nuclear genome continues to replicate without the normally succeeding cell division (Weber et al. 2008). Aneuploidy denotes an extra or a missing chromosome state (Jin et al. 2008). Structural changes are associated with deletions, duplications, inversions or translocations of specific chromosomal segments (Larkin and Scowcroft 1981; Morgens et al. 1984; Fukuoka et al. 1994). In general, chromosomal structural alterations are observed more frequently than chromosome number changes in regenerated plants (Kaeppler and Phillips 1993a).

Chromosome breakage and rearrangements reportedly occur during the in vitro culture process (Gernand et al. 2007; Kaeppler et al. 2000 and the references therein). Chromosome breakpoints generally occur between distal heterochromatic knobs and the centromere in maize (Lee and Phillips 1987), or within the centromeric heterochromatin in oat (Johnson et al. 1987). This led to the hypothesis that delayed replication of heterochromatin in tissue culture due to altered cell cycle controls might be responsible for these aberrations (Pryor et al. 1980). The effect of DNA methylation on chromosomal aberrations by influencing heterochromatin formation has also been proposed (Kaeppler et al. 2000).

The type, concentration and combination of synthetic analogues of auxins [2,4-dichlorophenoxy acetic acid (2,4-D)] and cytokinins [6-benzylaminopurine (BA)] have been shown to affect chromosome number and ploidy levels in select species and genotypes (Bairu et al. 2011 and the references therein). The synthetic auxin 2,4-D, which is not transportable out of the cells, is believed to facilitate a meristematic state by altering the endogenous auxin gradient (Morris 2000). High concentrations of 2,4-D resulted in the generation of mixoploids and tetraploids in cucumber suspension culture (Ladyżyński et al. 2002). Although these chemicals may not be directly mutagenic, they may affect ploidy levels by triggering unorganized cell growth, disturbing cell cycle control leading to DNA synthesis and endoreduplication.

### *DNA sequence changes*

DNA sequence variations such as single base pair changes and small 'indels' are predominant in progenies generated by culturing tissues (Jiang et al. 2011). Single nucleotide substitution mutation could arise by deamination of methylated cytosine to thiamine resulting in a transition. Furthermore, the efficiency of DNA replication and repair machinery is altered due to reduced cellular controls, leading to a loss of sequence fidelity by generating transitions and transversion mutations (Phillips et al. 1994). In maize, tissue culture-regenerated independent mutants of the alcohol dehydrogenase gene have been reported with a single base change and amino acid substitution arising from an adenosine (A) to thymine (T) transversion (Brettell et al. 1986; Dennis et al. 1987). The variant enzyme generated as a result of glutamic acid to valine residue substitution in exon 7 had a slower electrophoretic mobility, whereas the second mutation resulted in a complete loss of enzyme production due to the incorporation of a nonsense codon in place of lysine in exon 4.

The molecular basis of somaclonal variations in a population of plants regenerated from a single root explant cultured in vitro was studied in detail in the model plant Arabidopsis (Jiang et al. 2011). Among the 28 selfed $R_1$ families derived from $R_0$ regenerants, 6 exhibited heritable phenotypic variants, such as late flowering and long hypocotyle. Whole genome sequencing analysis revealed single base substitutions, in protein coding genes, as the most predominant class of nucleotide changes likely to generate somaclones. The ratio of transitions to transversions and pattern of single base substitution were significantly different from spontaneous mutations found in sexually propagated plants. A characteristic series of nucleotide insertions, deletions and substitutions were observed that were postulated to be related to the reduced cellular competence for proof reading and maintenance of sequence integrity. Surprisingly, the contribution of transposable elements towards this variation was estimated to be minor (Jiang et al. 2011). This observation was also supported by studies in maize where the extent of transposon polymorphisms in in vitro generated plants was less prominent, in spite of their abundance in the genome (Yu et al. 2011).

*Gene amplification and transposition*

Gene amplification refers to selective multiplication of specific DNA sequences in the genome, leading to tandem repeats at the same locus or those dispersed throughout the genome. Ribosomal DNA repeats, DNA microsatellites and transposable elements are more sensitive to stress conditions and could be considered as hot spots for mutations (Linacero et al. 2000). Long-term callus culture (up to a year) of Welsh onion (*Allium fistulosum*) resulted in genomic instability as evident from large scale transposition and amplification of ribosomal DNA and telomeric repeat sequences (Gernand et al. 2007). An extensive increase in copy number of mainly 5S ribosomal DNA sequences and transposable elements in the medicinal plant *Plantago lagopus* resulted in the formation of an independent, supernumerary 'apparent B chromosome'. Genetic instability as visualized by recombination and transposition could be re-induced in these B chromosomes by means of in vitro stress (Kour et al. 2009).

Microsatellites or simple sequence repeat (SSR) are multiple repeats of simple, short stretches of a DNA sequence unit (1–6 bases). They can vary in size due to their unstable nature and they can also potentially affect the expression of adjacent genes. Investigations of the molecular basis for microsatellite instability in vitro in sorghum cultures revealed a correlation with reduced expression of the DNA mismatch repair gene, *MLH3* (Zhang et al. 2010a).

Transposable elements, as the name implies, are DNA elements that have the ability to transpose to non-native genomic locations. They constitute about 85% of the maize genome (Schnable et al. 2009). Most transposable elements are dormant during normal growth and development, but are mobilized under specific conditions such as cell culture (Grandbastien 1998; Hirochika 1993; Peschke et al. 1987), irradiation (Nakazaki et al. 2003), hydrostatic pressure (Lin et al. 2006), pathogen infection (Pouteau et al. 1994), wounding, freezing (Mhiri et al. 1997), spaceflight environment (Long et al. 2009) and alien DNA introgression (Liu and Wendel 2000). Transposon insertions can affect single genes, either by disrupting their function or influencing their regulation and can also be associated with chromosomal level deletions and rearrangements (Peschke et al. 1987). Long-term repeated sub-culture results in the activation of transposable elements and progressive inactivation of host genes at the newly inserted site (Ozeki et al. 1997). Potato somaclonal variants with purple tuber skin color generated from protoplast cultures of red-skinned potato (Momose et al. 2010) and flower color variants of the African violet plant, *Saintpaulia* cultivar Thamires (Sato et al. 2011a), were associated with excision and mobility of the transposons associated with the flavonoid 3′, 5′ hydroxylase (*F3′5′H*) gene and promoter sequence, respectively, thereby affecting its functionality and pigment biosynthesis.

Various classes of plant transposons and retroelements are active in the in vitro environment as summarized in Table 2. Retrotransposons (Class I elements) make up the most abundant class occupying almost 75% of the maize genome (Schnable et al. 2009). These transposable elements mobilize via an RNA intermediate. In contrast, transposable elements that move with a DNA intermediate belong to Class II and members of this group have been shown to be predominantly active at the transcriptional level in maize cell cultures (Vicient 2010).

The molecular basis of selective transcription and transposition of cryptic transposons has been studied in recent years. Early experiments elucidating the mechanism of stress-induced transposon mobilization were focused on rice (*Tos17*) and tobacco (*Tto1, Tnt1*, and *Tnp2*) elements. In rice, one of the *Tos17* copies in chromosome 7 transposes by a 'copy and paste' mechanism to generate multiple copies proportional to the duration of in vitro culture (Hirochika 2001). An array of rice mutant phenotypes including dwarf (Sato et al. 1999), viviparous (Agrawal et al. 2001), and virus resistance (Yoshii et al. 2009) were characterized and the corresponding genes were identified using *Tos17* transposon tagging strategy. In tobacco, analysis of the upstream sequence of the long terminal repeat (LTR) region of *Tnt1* and *Tto1* transposon promoters revealed regulatory motifs responsible for stress and

**Table 2** Tissue culture-induced transposable elements in plants

| No. | Name | Species | Type of transposon | Class | Activation | References |
|---|---|---|---|---|---|---|
| 1 | *Ac1* | Maize | DNA transposon | II | Low frequency transposition in in vitro culture-regenerated plants | Peschke et al. (1987) and Brettell and Dennis (1991) |
| 2 | *BARE-1* | Maize | DNA transposon | II | Proposed to be transpositionally active in in vitro culture | Planckaert and Walbot (1989) |
| 3 | *dTstu1* | Maize | DNA transposon | II | Low frequency transposition in in vitro culture-regenerated plants | Peschke and Phillips (1991) |
| 4 | *Karma* | Tobacco | Retrotransposon | I | Tissue culture | Pouteau et al. (1991) |
| 5 | *Llb* | Tobacco | Retrotransposon | I | Tissue culture | Hirochika (1993) |
| 6 | *LORE1* | Tobacco | Retrotransposon | I | Tissue culture, wounding, methyl jasmonate, and some fungal elicitors | Hirochika (1993) |
| 7 | *Lullaby* | Rice | Retrotransposon | I | Tissue culture, Introgression, one copy in chromosome 7 active in Nipponbare, reverts in the regenerated plant | Hirochika et al. (1996) |
| 8 | *MERE1* | Rice | Retrotransposon | I | Tissue culture | Hirochika et al. (1996) |
| 9 | *mPING* | Rice | Retrotransposon | I | Tissue culture | Hirochika et al. (1996) |
| 10 | *Mu* | Carrot | En/Spm like element | II | Long-term (>4 years) sub-culturing in suspension cultures | Ozeki et al. (1997) |
| 11 | *nDaiZ* | Rice | Tourist like MITE | II | Induced by anther culture, interspecific hybridisation | Kikuchi et al. (2003) |
| 12 | *Pong* | Rice | DNA transposon | II | Active in *indica* cell culture line, also in interspecific hybridization | Jiang et al. (2003) |
| 13 | *Popin* | Rice | Retrotransposon | I | Activated in $R_1$ plants, not in cultured cells or $R_0$ plants | Komatsu et al. (2003) |
| 14 | *RYS1* | Rye | Fold back transposon | | Activated by in vitro stress | Alves et al. (2005) |
| 15 | *Spm* | Maize | Rice Pong homologues | II | Transcriptionally active in in vitro culture, somaclonal variants in anther/callus cultures and lines | Barret et al. (2006) |
| 16 | *Tdc1* | Sweet potato | Retrotransposon | I | High transposition in callus and meristem culture | Yamashita and Tahara (2006) |
| 17 | *Tnt1* | Rice | MITES | II | One copy in Chromosome 5 activated by tissue culture, only in a few genotypes | Huang et al. (2009) |
| 18 | *Tos10* | Rice | Retrotransposon | I | Transposed in callus | Picault et al. (2009) |
| 19 | *Tos17* | Maize | Novel maize specific TE, small size (43 bp) | | Tissue culture | Rhee et al. (2009) |
| 20 | *Tos19* | Medicago | Retrotransposon | I | Low copy number, activated in tissue culture and shows preferential gene insertion | Rakocevic et al. (2009) |
| 21 | *Tto1* | Potato | Stowaway MITE | II | Tissue culture | Momose et al. (2010) |
| 22 | *Tto2* | Lotus | Retrotransposon | II | Activated in plants regenerated from dedifferentiated cells | Fukai et al. (2010) |
| 23 | *VGs1* | *Saintpaulia* sp. | DNA transposon | II | Tissue culture | Sato et al. (2011a) |
| 24 | *ZmTPAPong like -1* and *3* | Barley | Retrotransposon | I | Activated by tissue culture involving dedifferentiation | Evrensel et al. (2011) |

hormone inducibility (Grandbastien et al. 1997; Takeda et al. 1999), which may suggest a role for tissue culture activation of these elements.

Plant chromovirus is a retrotransposon with a chromodomain which is believed to regulate the choice of insertion site (Fukai et al. 2010). A recent study on characterizing the transposition pattern of the *LORE1* (Lotus retrotransposon 1), a chromovirus in *Lotus japonicas,* revealed that these elements can be activated in the male germ line by tissue culture de-differentiation stress. The *cis*-acting elements directing expression in pollen may also regulate male gametophyte-specific transposition in regenerated plants (Fukai et al. 2010).

## Epigenetic regulation in vitro

Epigenetic reprogramming of gene expression involves heritable, but potentially reversible, enzyme-mediated, chemical modifications to the DNA and associated proteins in the chromatin. These changes often lead to the generation of epigenetic marks or signatures. Epigenetic regulation in plant cell cultures orchestrated by methylation of DNA and histones, chromatin remodeling and small RNA-mediated regulation has been recently reviewed (Miguel and Marum 2011). The changes are considered 'epigenetic' because these variations are not 'coded' by the DNA, but still can be transmitted to the next generation.

### *DNA methylation*

DNA methylation is one of the primary, heritable epigenetic signatures that determine silencing of specific DNA sequences. This involves the addition, via covalent bonding, of a methyl group to the cytosine base in the DNA at the CpG, CpHpG and CpHpH sites, where H denotes any nucleotide other than guanine. DNA modification by methylation is mediated by DNA cytosine methyltransferase enzymes belonging to chromomethylase (CMT), domains rearranged methyltransferase (DRM), and methyltransferase (MET) families. In Arabidopsis, DRM2 acts as a de novo methylase and DRM2, MET1 and CMT3 act as maintenance methylases, capable of identifying and transferring methylation marks to the newly synthesized strand during DNA replication (Cao and Jacobsen, 2002).

The magnitude of DNA sequence and methylation variation present in in vitro regenerated plants has been analyzed in diverse crops such as barley (Bednarek et al. 2007; Li et al. 2007), cocoa (López et al. 2010), grapevine (Schellenbaum et al. 2008b), hop (Peredo et al. 2006), pea (Smýkal et al. 2007), *Doritaenopsis* (Park et al. 2009), plantago (Kour et al. 2009), Arabidopsis (Yu et al. 2010), maize (Yu et al. 2011) and medicinal plants such as *Codonopsis* (Guo et al. 2007). The frequency of these variations increased with culture age, although some studies also show a subsequent decline in very advanced culture regenerants, possibly due to the loss of totipotency and regeneration potential of extremely mutated cells as observed in cocoa cultures (López et al. 2010). In general, polymorphisms in these variants revealed through methylation assays are higher than that revealed through DNA sequence analyses (such as RAPD or RFLP), pointing to a higher level of epigenetic deregulation in the in vitro environment. In a recent study, the ability to maintain genomic homeostasis in vitro was compared between sorghum inbred lines and their hybrids (Zhang et al. 2009). The coordinated induction of DNA methyltransferases and glycosylases in the hybrid calli and regenerated plants was attributed to their greater ability to rebuff the mutational impact. However, parallel studies in maize hybrids revealed only minor superiority over inbreds in terms of genetic and epigenetic vulnerability in culture. Therefore, genotypic effect seems to play a major role in regulating the magnitude of such variations (Yu et al. 2011).

The effect of type and concentration of growth regulator present in the media, especially auxins, on genome-wide methylation levels has been studied in carrot embryogenic cultures (LoSchiavo et al. 1989). Increased 2,4-D concentration was found to promote cytosine methylation levels. Although similar observations were reported in pumpkin cultures, the correlation was not unique to auxins. Media components such as nitrogen source also affected methylation levels (Leljak-Levanić et al. 2004). Certain antibiotics such as cefotaxime, kanamycin and hygromycin also contributed to enhanced, irreversible methylation of repetitive sequences. In particular, the CpG sites were preferentially methylated (Schmitt et al. 1997).

Hormone habituation is a phenomenon by which plant cells and tissues lose the requirement of exogenous hormones to sustain cell division and development. This autonomous growth in tissues can be established by continuous in vitro culturing (Meins 1989), inclusion of anti-auxin chemical compounds in media (Christou 1988) or treatment of tissue cultures with 5-azacytidine, a chemical known to induce global hypomethylation (Durante et al. 1989). Genome-wide expression profiling of Arabidopsis cytokinin-habituated cultures revealed that enhanced expression of several putative DNA and chromatin modifiers may affect this phenomenon, along with cytokinin signaling components (Pischke et al. 2006).

Tissue culture-induced epigenetic somaclonal variation of an endogenous gene was demonstrated in maize by identifying novel epialleles of a myb transcription factor, *pericarp color 1*, which affects the flavonoid biosynthesis pathway in maize seeds (Rhee et al. 2010). The progenitor allele, *P1-wr*, consists of tandem copies of the gene and produces brick-red color in the cob glume. The tissue

culture-induced epiallelic variants with reduced (pink pigmentation) and even complete absence (colorless cob glumes) of pigmentation were associated with intron hypermethylation and almost complete loss of transcripts. Such variants generated due to epigenetic aberrations can be 'reproducible' under identical conditions (Smulders and de Klerk 2011).

Epigenetic mechanisms also play a role in orchestrating developmental events and response features in vitro. Promoter methylation of specific single-copy genes and consequent gene repression has been reported to contribute to the regulation of the undifferentiated state in Arabidopsis callus and suspension cultures (Berdasco et al. 2008). Transient expression of leafy cotyledon gene, *LEC1*, is important for somatic embryogenesis. The promoter of *LEC1* gene was found to undergo hypomethylation prior to somatic embryo formation, whereas these methylation levels increased during subsequent developmental events leading to vegetative growth. This indicates that *LEC1* promoter methylation effectively modulates *LEC1* gene expression changes in relation to embryogenesis in vitro (Shibukawa et al. 2009).

Recent studies involving characterization of Arabidopsis epigenetic mutants have revealed that the expression of *WUS* gene and auxin signaling components, which are crucial for de novo adventitious shoot initiation and regeneration, are regulated by DNA methylation and histone modification of regulatory sequences (Li et al. 2011). *MET1* mutants had reduced methylation levels, and consequently higher *WUS* gene expression, resulting in an earlier shoot primordial initiation as compared to wild type calli.

The pattern of methylation changes was examined in embryo-derived in vitro regenerated maize plants (Kaeppler and Phillips 1993b). It was speculated that a high tendency of decreased methylation in the regenerated plants provided an ideal environment for the activation of transposable elements. The role of DNA methylation in silencing the activity of endogenous transposable elements was first experimentally demonstrated in Arabidopsis (Miura et al. 2001), followed by rice where in vitro activation of elements such as *Tos17* and *mPing* was associated with hypomethylation of cytosine (Liu et al. 2004; Cheng et al. 2006; Ngezahayo et al. 2009). Furthermore, heritable methylation changes at the flanking sites also point to transposon-directed epigenetic regulation of host gene expression.

The precise, genome level epigenetic changes in transcriptional activation of transposable elements in Arabidopsis were investigated using chromosome immunoprecipitation (ChIP) analysis on tilling arrays (Tanurdzic et al. 2008). An apparent 'misregulation' of epigenetic changes was observed with an increase in methylation of genic, euchromatic regions and extensive hypomethylation of heterochromatin followed by reactivation of transposable elements. In general, transcriptional expression of transposable elements was highest in cell suspension cultures as compared to callus or seedling tissues, thereby suggesting a high level of genomic instability in immortalized suspension cultures (Tanurdzic et al. 2008).

Recent studies indicate a role of 5-methyl cytosine DNA glycosylase in transposon expression and transposition. In rice, this enzyme is believed to have a role in demethylation of *Tos17* thereby affecting its expression and transposition. Knock-out mutants of rice DNA glycosylase *DNG701* had lower *Tos17* expression and transposition due to elevated DNA methylation levels and over-expressers exhibited hyper transposition features (La et al. 2011).

### *Chromatin remodeling*

Chromatin refers to the complex of DNA and proteins, primarily histones, and is present in the nucleus of a eukaryotic cell. This combination enables DNA to be packaged inside the cell and it also aids in DNA replication and gene expression. The basic repeating unit of chromatin is the nucleosome, a 146-base pair unit of DNA wrapped around a histone octamer core, which consists of two copies each of histone proteins H2A, H2B, H3 and H4. Further compaction of the nucleosome to form a 30 nm fiber is facilitated by the histone protein H1. Transcriptionally active chromatin is called euchromatin and the more condensed, transcriptionally inactive form is termed heterochromatin. Efficient modification of chromatin structure is crucial in accomplishing epigenetic regulation of genes (Jarillo et al. 2009).

Post-translational modification of histone proteins, especially the N terminal tails that involve activities such as methylation of lysine and arginine, acetylation or ubiquitination of lysine, or phosphorylation of serine residues, is an essential regulatory mechanism affecting chromatin conformation. Enzymes such as DNA or histone methyltransferases, histone acetyl transferases (HATs), histone deacetylases (HDACs), and ATP-dependent chromatin remodeling enzymes, are essential for bringing about these specific changes. The non-histone proteins present in chromatin, such as the high mobility group (HMG) proteins can also be subject to enzymatic modification to facilitate chromatin modulation.

Dynamic histone modification, predominantly deacetylation of histone H3 and H4, reportedly occurs during mitosis in cultured tobacco cells as revealed by immunolabeling studies (Li et al. 2005). Chemicals such as trichostatin A have been found to inhibit histone deacetylases and its application led to cell cycle arrest, demonstrating

that these changes are vital for mitosis progression. Chromatin remodeling is believed to play a key role in cellular dedifferentiation and proliferation (Grafi et al. 2007), including embryonic development and organogenesis (Ogas et al. 1999; Dean Rider et al. 2003) and hormone response (Anzola et al. 2010; Furuta et al. 2011), as outlined in Table 3. The Arabidopsis *kyp*-2 mutant is defective in callus formation (Grafi et al. 2007). The kryptonite (*KYP*)/*SUVH4* gene codes for the enzyme histone H3 lysine 9 (H3K9) methyltransferase involved in the activation of cell cycle related genes, thereby initiating the meristematic state and cell division.

Chromatin remodeling proteins belonging to the SWI/SNF2 family like Pickle utilize the energy derived from ATP hydrolysis to alter chromatin structure. Some transcription factors such as the Viviparous 1/ABA insensitive-3 like (VAL) B3 domain proteins are also known to have additional domains such as plant homeodomain (PHD) zinc finger-like and CW zinc finger domains with conserved cysteine (C) and tryptophan (W), that can function in chromatin regulation. Due to the de-repression of leafy cotyledon genes occurring in the absence of the Pickle protein, Arabidopsis *pickle* mutant exhibits embryonic traits in seedlings (Ogas et al. 1997). Similarly, *val1 val2* double mutants exhibit ectopic embryo formation in roots and shoots. The *pickle* and *val1* mutant phenotypes are modulated by the application of gibberellin biosynthetic inhibitors, pointing to the role of chromatin structure in controlling gibberellin-mediated responses crucial for repression of embryonic traits in seedlings. An independent mutation in Pickle, *cytokinin-hypersensitive 2* (*ckh2*), conferred the ability to produce green callus at sub-optimal levels of cytokinins (Furuta et al. 2011). The addition of trichostatin A partly mimicked exogenous kinetin, thereby indicating that chromatin regulation by deacetylation is crucial for cytokinin-regulated callus regeneration.

Chemicals that can potentially modify the epigenetic state of plant cells have been utilized recently in conifer cultures to improve embryogenic efficiency (Uddenberg et al. 2011). Trichostatin treatment inhibited germination progression of embryos such that they could efficiently initiate proliferative embryogenic calli in a suitable medium. Maturation of somatic embryos was also blocked by this treatment and this was mediated by affecting the expression of embryogenesis-related leafy cotyledon genes, possibly via chromatin modification (Uddenberg et al. 2011).

Transposable element activation and mobility is also regulated at the chromatin level. Chromatin associated with transposable elements bears histone H3 lysine 9 methylated (H3K9 methylation) states, mediated by histone H3K9 methyltransferases, which is an epigenetic mark for transcriptional inactivation (Slotkin and Martienssen 2007). It has been found that chromatin remodeling protein decrease in DNA methylation 1 (DDM1) is required for silencing transposable elements and formation of transcriptionally inactive, condensed chromatin (Miura et al. 2001). More recent studies demonstrate the role of histone deacetylase 6 (HDA6) in co-operation with DNA methyltransferase (MET1) in mediating silencing of transposable elements (Liu et al. 2011).

### *Small RNA regulation*

Small, non-protein-coding, regulatory RNAs are emerging as key players governing epigenetic processes in plants. MicroRNAs and *trans*-acting small RNAs, are short, usually 21–22 nucleotides in length, and typically mediate posttranscriptional gene silencing by mRNA degradation owing to near-perfect complementarity and cleavage, or by repression of translation. An independent class of small interfering RNAs (siRNAs), 24–26 nucleotides long, usually originates from transposable elements and tandem repeats. They are capable of transcriptional gene silencing by targeting specific DNA and histone sequences for methylation and heterochromatinisation via a specialized RNA-dependent DNA methylation (RdDM) pathway. These RNA silencing pathways are vital to the negative regulation of several transcription factor genes, repetitive elements, mobile elements and viruses, which are essential for maintenance of genome stability and survival (Almeida and Allshire 2005).

Insertion of a transposable element in the reverse orientation in the genome is believed to result in the production of an antisense transcript, followed by double-stranded RNA formation, thereby triggering the RNA interference (RNAi) phenomenon. The double-stranded RNA is cleaved by dicer proteins into short, 21–30 nucleotide siRNAs, which are then loaded onto the RNA-induced silencing complex (RISC) constituted primarily of Argonaute proteins. These complexes are capable of targeting and cleaving the complementary transcript (as reviewed in Slotkin and Martienssen 2007). Transposon silencing mediated by RNA-dependent DNA methylation machinery in Arabidopsis involves proteins such as RNA polymerase IV for transcribing transposons and other genomic regions, RNA-dependent RNA polymerase enzyme 2 (RDR2) to generate double-stranded RNA, dicer-like 3 (DCL3) for the transposable element-derived siRNA formation by cleavage, and argonaute 4 (AGO4) to generate the RNA-induced transcriptional silencing (RITS) complex. This multimeric complex can subsequently target genomic regions and mediate repression of transcription (Slotkin and Martienssen 2007).

Deep sequencing of small RNAs in Arabidopsis suspension cultures revealed a specific enrichment of a

**Table 3** Epigenetic regulation of plant tissue culture response traits in Arabidopsis

| Gene | Function | Mechanism of regulation | References |
|---|---|---|---|
| DNA modification | | | |
| Methyl transferase (*MET1*) | DNA methyltransferase | Promoter hypermethylation of cell division and differentiation genes (e.g. *GSTU10, MAPK12* and *BXL1*) in callus tissue leading to reduced proliferation rate and diminished efficiency of callus formation in mutants | Berdasco et al. (2008) |
| | | Promoter demethylation of *WUS* gene and enhanced expression of *WUS* and auxin signaling components (*ARF3*) in the mutant callus. Higher frequency of shoot regeneration was observed in mutant calli derived from root explants | Li et al. (2011) |
| Chromatin modification | | | |
| Curly leaf (*CLF*) and Swinger (*SWN*) | Histone H3 lysine 27 methyltransferases (H3K27me3) | Polycomb repressive complex 2 (PRC2) family proteins involved in regulating the developmental transition from embryonic to seedling state. The *clf/swn* double mutants and *fie* homozygous mutants form callus-like structures on leaves and in culture respectively, which subsequently formed somatic embryos | Chanvivattana et al. (2004) |
| Fertilization independent endosperm (*FIE*) | | | Bouyer et al. (2011) |
| Histone deacetylases (*HDA6* and *HDA19*) | Histone deacetylase | Act as transcriptional repressors of embryo-specific transcription factor genes, *LEC1*, *FUS3*, and *Abscisic acid insensitive3* (*ABI3*). Mutants formed ectopic embryo-like structures from vegetative tissues | Tanaka et al. (2008) |
| Kryptonite (*KYP*)/*SUVH4* | Histone H3 lysine 9 (H3K9) methyltransferase | Activation of cell cycle-related genes, thereby initiating the meristematic state and cell division. Mutants exhibited defective proliferation and callus formation | Grafi et al. (2007) |
| Pickle (*PKL*) | ATP-dependent chromatin remodeling factor | De-repression of leafy cotyledon genes (*LEC1, LEC2 FUS3*) in *pkl* mutants leading to spontaneous somatic embryo formation from vegetative tissues | Ogas et al. (1997) |
| | | Enhanced responsiveness of cytokinin-inducible genes and regulated expression of photosynthesis related genes was observed. The *ckh2* mutants produced green calli at sub-optimal levels of cytokinin | Furuta et al. (2011) |
| Pickle related 2 (*PKR2*) | Chromatin remodeling factor (Pickle homologue) | Act in conjunction with PKL to repress Polycomb proteins for suppressing embryonic traits in seedlings. The *pkl pkr2* double mutants show enhanced dedifferentiation and somatic embryo production in seedlings | Aichinger et al. (2009) |
| Polycomb repressive complex 1 (PRC1)-like ring-finger proteins | Ubiquitination of lysine 119 of histone H2A (H2AK119) | De-repression of regulatory genes leading to the development of embryonic traits. Ring protein *Atring1a Atring1b* and ring-finger protein, *Atbmi1a Atbmi1b* double mutant plants produce ectopic embryo-like structures | Chen et al. (2010) |
| Proporz1 (*PRZ1*) | Chromatin remodeling component | Controls histone acetylation and expression of auxin responsive genes. Mutants showed enhanced sensitivity to auxin, forms callus on a low auxin callus induction medium | Anzola et al. (2010) |
| VP1/abscisic acid insensitive 3-like (VAL) | Transcription factors with B3, PHD-like and CW domain | De-repression of seed developmental genes including leafy cotyledon genes leading to the development of embryonic traits. *val1 val2* double mutant plants displayed ectopic induction of callus in seedlings and form embryo-like structures from apical meristems and cotyledon margins | Suzuki et al. (2007) |

particular class of small RNA (21 nucleotides) associated with reactivated transposable elements that had lost their heterochromatic signature. A similar association was not observed for the silent elements indicating that the siRNAs of the 24 nucleotide class play a role in targeting the silencing machinery to the transposable DNA sequences to prevent transcriptional and transpositional activation (Tanurdzic et al. 2008).

A large body of evidence suggests that microRNAs are the master regulators of gene expression required for normal growth, development and stress response (Willmann and Poethig 2007; Martin et al. 2010). MicroRNAs are small, endogenous, single-stranded transcripts capable of forming a stem-loop hairpin structure and can mediate target gene silencing by RNA cleavage or translational inhibition. They are believed to fine-tune gene expression programs and have been found to act cooperatively with transcription factors affecting cell differentiation, proliferation, and cell fate acquisition in human cell cultures (Tenedini et al. 2010). The potential role of microRNAs in contributing to somaclonal variation in vitro has been recently postulated (Rodriguez-Enriquez et al. 2011).

Studies on Arabidopsis have identified the role of miRNAs in regulating key transcription factors like Leafy cotyledon 2 (LEC2) and Fusca 3 (FUS3) in early embryogenesis (Willmann et al. 2011), Cup shaped cotyledon 1 and 2 (CUC1 and CUC2) in the initiation of organ primordia (Takada et al. 2001), and transcriptional regulators of auxin signaling networks (Marin et al. 2010). Such information could be exploited to address the possible parallel effects of miRNAs on mediating somatic embryogenesis, formation of meristem, hormonal signaling, and organogenesis in cell cultures.

Recent efforts are beginning to unravel the significance of microRNAs in epigenetic regulation of key transcription factors in plant cell cultures. MicroRNA profile of rice embryogenic calli revealed the presence of potential candidates (e.g. miR397 and miR398), responsible for maintaining the meristematic state, possibly by mediating gene silencing (Luo et al. 2006). Four miRNA families (miR171, miR159, miR169, and miR172) were differentially expressed between embryogenic and non-embryogenic calli in Japanese Larch (Zhang et al. 2010b). These miRNA candidates are known to target transcription factors involved in developmental events and these were also inducible by abiotic stress and the phytohormone abscisic acid (ABA). In yet another study, miRNA expression was analyzed in conjunction with target transcription factors in sweet orange tissues in different developmental phases of somatic embryo development. The microRNA candidates, miR156, 168 and 171 were associated with competence to form somatic embryos in the embryogenic callus. It was also suggested that the mRNA targets of miR164, 166 and 397 were involved in maintaining the meristematic property of the non-embryogenic callus (Wu et al. 2011). All these studies provide exciting new indications as to the role of microRNAs in developmental control in vitro, but their functional characterization is imperative.

Oil palm, which is an important source of vegetable oil, is not amenable to vegetative propagation and in vitro clonal propagation results in a high frequency of the abnormal flower phenotype termed 'mantled' which accumulates less oil in the fruit as compared to the wild type. The phenotype manifests only when the oil palm starts flowering and it is a serious economic handicap affecting oil yield. Efforts have been underway over the past two decades to develop early detection strategies and to characterize the genetic and epigenetic processes underlying this somaclonal variation. The current hypothesis is that the molecular basis of this abnormality might, in part, involve epigenetic regulation of MADS box floral identity genes at the level of chromatin condensation and small non-coding RNA-mediated effects (Jaligot et al. 2011).

## Candidate genes for potential applications in improving tissue culture response

Molecular regulation of the signaling pathways and developmental switches that govern the in vitro response of an explant remains largely unknown. However, functional affirmation of candidate genes and proteins involved in some of these processes has been elucidated based on the tissue culture phenotype of the transgenic plants. The transgenic upregulation of SERK1 kinase activity resulted in enhanced induction of somatic embryos in Arabidopsis (Hecht et al. 2001) and frequency of shoot regeneration in rice (Hu et al. 2005). This demonstrates that expression of this gene has important promoting roles in developmental transitions and thereby has the potential to desirably modify in vitro response features in monocots and dicots.

Transcription factors of the AP2/ERF family are reported to play key roles in mediating developmental transitions in vitro. Proteins like BOLITA (Marsch-Martinez et al. 2006) and wound-induced dedifferentiation 1 (WIND1) (Iwase et al. 2011) have been reported to stimulate cell dedifferentiation and proliferation essential for callus formation. *WIND1* was initially identified based on its preferential expression in Arabidopsis cell culture lines compared to seedlings and was found to be efficiently produced in response to wounding. The engineered overexpression of this gene was sufficient to initiate and maintain meristematic properties in differentiated tissues in a hormone-independent manner. Inducible expression of Babyboom1, another AP2 domain transcription factor, was found to enhance transformation and regeneration in

recalcitrant species (Heidmann et al. 2011). Constitutive expression of *bbm1* causes undesirable pleiotropic effects such as sterility. Hence, regulated expression strategies were employed to efficiently utilize this gene to improve somatic embryo competence and regeneration in sweet pepper, poplar and maize (Heidmann et al. 2011; Deng et al. 2009; Lowe et al. 2011).

Homeodomain-containing proteins are key players in developmental transitions. The gene, *Plant Growth Activator 6* (*PGA6*), was identified in a genetic screen intended for identifying gain-of-function mutants associated with vegetative to embryonic transitions. This activation-tagged line was able to generate somatic embryos from various vegetative tissues in a hormone-independent manner (Zuo et al. 2002). These somatic embryos could subsequently be germinated into mature plants. The mutated gene was identified as *Wuschel*, which encodes a homeobox protein. The homeobox protein knotted1 has demonstrated roles in shoot organogenesis in Arabidopsis (Chuck et al. 1996) and tobacco (Sinha et al. 1993). Another homeobox protein, *Shootmeristemless* (*STM*) was also reported to promote somatic embryo production in Arabidopsis callus and *Brassica* microspore cultures (Elhiti et al. 2010). Specific fluctuations in nucleotide metabolism, in terms of improved expression and enzyme activity involved in nucleotide salvage pathways that are important for somatic embryo development, were found associated with *STM* over-expression (Elhiti et al. 2011).

Agamous-like 15 (AGL15) is the sole member of the MADS box family that abundantly expresses in both sexual and asexual embryonic tissues (Harding et al. 2003). Ectopic over-expression of AGL15 promoted somatic embryo development in Arabidopsis and also helped in the maintenance of embryo cultures for extended periods. This transcription factor is believed to function in association with SERK1 kinase and embryo-related leafy cotyledon genes (Karlova et al. 2006; Zheng et al. 2009). The engineered soybean orthologue was able to improve somatic embryo formation in soybean cultures (Thakare et al. 2008).

Hemoglobins are iron-containing, oxygen-binding proteins. In plants, they are involved in binding and transporting oxygen in symbiotic plants and in scavenging nitric oxide in non-symbiotic species. Recent studies have documented the effect of plant hemoglobins of class I and II types, in affecting cell fate and shoot organogenesis in root explants of Arabidopsis (Wang et al. 2011). The altered hemoglobin levels are believed to affect genes involved in cytokinin sensing and signaling, resulting in enhanced shoot formation.

A list of candidate genes, predominantly transcription factors, with potential applications for modulating in vitro response features is presented in Table 4. The functions of many of these genes have been demonstrated in the model plant *Arabidopsis thaliana*. Some of the genes, like *HsfA2* and *SERK1*, have also been reported to have additional beneficial effects in adaptation to adverse environmental conditions in certain species. The constitutive expression of a vast majority of these genes is associated with undesirable pleiotropic effects on normal plant growth and development. Inducible or regulated strategies that confine expression to an appropriate stage of cell culture have immense potential in improving in vitro response features in diverse species. As discussed in "Small RNA regulation", small non-coding RNA is emerging as a category of potential regulators in many cellular pathways. Its involvement in development and hormone response is one area that needs to be explored and potentially exploited.

## Conclusions

Use of in vitro cell and tissue-based systems offer a tremendous tool for dissecting the physiological, biochemical and molecular regulation of plant development and stress response phenomena. Furthermore, they are extensively utilized for clonal propagation, as a gateway for genetic engineering of a vast majority of crops and as an economical and large scale production platform for native or engineered molecules. Although plants regenerated from these systems are expected to be homogenous, we now know that due to intrinsic and extrinsic factors affecting development under artificial conditions, there is a high probability of epigenomic and genomic changes, predominantly methylation changes, single base pair changes and small indels, which may or may not be associated with phenotypic changes should be carefully considered in all practical applications of these technologies.

The availability of complete genome sequences and the advent of state-of-the-art sequence detection techniques and imaging technology have provided researchers with excellent tools and resources to address fundamental biological questions related to differentiation and morphogenesis. It is now known that the type, amount and timing of exogenous growth regulators added in the media play a major role in determining endogenous hormonal gradients and subsequent gene expression, and development. The available knowledge base on gene expression regulation associated with developmental transitions could be efficiently deployed in recalcitrant genotypes and crop species to improve regeneration and morphogenesis, thereby enhancing transformation competence.

Although there is significant progress in understanding the genetic basis of plant in vitro culture and response, recent discoveries regarding epigenetic mechanisms and small RNA-mediated silencing mechanisms have shed new

**Table 4** Candidate genes for enhanced tissue culture response

| No. | Gene name | Properties | Species | Phenotype | Reference(s) |
|---|---|---|---|---|---|
| 1 | *Agamous-like 15* (*AGL15*) | MADS box transcription factor | *Arabidopsis thaliana* | Constitutive over-expression stimulated secondary somatic embryo formation in cultured zygotic embryos and also in shoot meristem | Harding et al. (2003) |
| | | | *Glycine max* | Constitutive over-expression elevated somatic embryo development | Thakare et al. (2008) |
| 2 | *Baby Boom* (*BBM1*) | AP2 domain transcription factor; AP2 sub-family | *Brassica napus* | Ectopic expression in *Arabidopsis* and *Brassica* resulted in the formation of embryo-like structures on seedlings | Boutilier et al. (2002) |
| | | | | Ectopic expression in tobacco resulted in sterility; inducible expression resulted in spontaneous organogenesis | Srinivasan et al. (2007) |
| | | | | Inducible expression in recalcitrant species *Capsicum annum* resulted in enhanced efficiency of regeneration and transformation | Heidmann et al. (2011) |
| | | | *Brassica campestris* | Constitutive over-expression regulated by heat inducible FRT/FLP system in Chinese white poplar resulted in somatic embryo formation and plant regeneration | Deng et al. (2009) |
| | | | *Glycine max* | Heterologous expression in *Arabidopsis* resulted in formation of embryo-like structures on vegetative organs | Ouakfaoui et al. (2010) |
| | | | *Zea mays* | Inducible over-expression in maize promotes callus and embryo formation from recalcitrant tissues like leaves, stem etc. | Lowe et al. (2011) |
| 3 | *Bolita* | AP-2/ERF like transcription factor | *Arabidopsis thaliana* | Constitutive over-expressers show development of callus with shoot identity at the root tip | Marsch-Martinez et al. (2006) |
| 4 | *Cup-Shaped Cotyledon1* (*CUC1*) | NAC family transcription factor | *Arabidopsis thaliana* | Ectopic over-expression triggered adventitious shoot formation on the adaxial surface of the cotyledons | Takada et al. (2001) |
| 5 | *Cyclin-dependent kinase* (*CDK*) | Cell cycle regulators | *Nicotiana tabacum* | Inducible expression stimulated callus formation in auxin-rich medium in the absence of cytokinin | Yamaguchi et al. (2003) |
| 6 | *Elaeis guineensis AP2-1* (*EgAP2-1*) | AP2 domain transcription factor; AP2 subfamily; BABYBOOM (BBM) and AINTEGUMENTA-like (AIL) protein | *Elaeis guineensis* | Constitutive ever-expression in Arabidopsis had a positive effect on regeneration competence | Morcillo et al. (2007) |
| 7 | *Embryomaker* (*EMK*) | AP2 domain transcription factor; AP2 sub-family | *Arabidopsis thaliana* | Constitutive over-expression led to the formation of embryo-like structures from cotyledons and elevated somatic embryo production in vitro | Tsuwamoto et al. (2010) |
| 8 | *Enhancer of shoot regeneration 1* (*ESR1*) | AP2 domain transcription factor, ERF sub family | *Arabidopsis thaliana* | Constitutive over-expression affected shoot organogenesis; inducible over-expression facilitated shoot regeneration independent of cytokinins and enhanced shoot regeneration efficiency was obtained with cytokinin | Banno et al. (2001) |
| 9 | *Enhancer of shoot regeneration 2* (*ESR2*) | AP2 domain transcription factor, ERF sub family | *Arabidopsis thaliana* | Constitutive over-expression affected shoot organogenesis; Inducible over-expression facilitated shoot regeneration independent of cytokinins and enhanced shoot regeneration efficiency was obtained with cytokinin, more efficient regeneration than ESR1 | Ikeda et al. (2006) |

**Table 4** continued

| No. | Gene name | Properties | Species | Phenotype | Reference(s) |
|---|---|---|---|---|---|
| 10 | *Gibberellin 2-oxidase 6* (*GA2ox6*) | Enzyme in Gibberellin metabolism | *Arabidopsis thaliana* | Constitutive over-expression positively affects somatic embryo production | Wang et al. (2004) |
| 11 | *Heat shock factor A2* (*HSFA2*) | Heat shock transcription factor | *Arabidopsis thaliana* | Constitutive over-expression caused enhanced callus formation in root explants in vitro and thermo-tolerance in vivo | Ogawa et al. (2007) |
| 12 | *KNAT1* | Knotted-like homeobox protein | *Arabidopsis thaliana* | Constitutive over-expression in *Arabidopsis* results in lobed leaves and formation of ectopic shoot meristems in the sinus region | Chuck et al. (1996) |
| 13 | *Knotted 1* (*KN1*) | Homeobox protein | *Zea mays* | Ectopic heterologous expression in tobacco resulted in reduced plant height and leaf size, with small shoots being produced from the leaf surface | Sinha et al. 1993 |
| 14 | *Leafy Cotyledon 1* (*LEC1*) | Homologous to HAP3 subunit of CBF transcription factors | *Arabidopsis thaliana* | Postembryonic over-expression stimulated formation of embryo-like structures | Lotan et al. (1998) |
| 15 | *Leafy Cotyledon 2* (*LEC2*) | B3 domain transcription factor | *Arabidopsis thaliana* | Postembryonic over-expression stimulated formation of embryo-like structures, auxin-free somatic embryo formation in vitro | Stone et al. (2001) and Ledwoń and Gaj (2009) |
| 16 | Plant hemoglobins (*GLB 1 and 2*) | Oxygen-binding proteins | *Arabidopsis thaliana* | Constitutive over-expression enhances the number of shoots formed on root explants in culture and promote shoot organogenesis at suboptimal levels of cytokinin | Wang et al. (2011) |
| 17 | *Shootmeristemless* (*STM*) | Knotted-like homeobox protein | *Brassica oleraceae* | Heterologous expression in *Arabidopsis*-enhanced somatic embryogenesis | Elhiti et al. (2010) |
| | | | *Brassica napus* | Ectopic over-expression resulted in profound increase in microspore-derived embryos | |
| 18 | *Somatic embryogenesis receptor kinase 1* (*SERK1*) | Leucine rich repeat transmembrane receptor-like kinase | *Arabidopsis thaliana* | Ectopic over-expressers displayed enhanced efficiency of somatic embryo induction in vitro | Hecht et al. (2001) |
| | | | *Oryza sativa* | Constitutive over-expression boosted the frequency of shoot regeneration in vitro and increased blast resistance in vivo | Hu et al. (2005) |
| 19 | *Wound-induced dedifferentiation 1* (*WIND1*) | AP2/ERF family transcription factor | *Arabidopsis thaliana* | Constitutive and inducible over-expression induced and maintained dedifferentiation of adult cells, in a hormone-independent manner | Iwase et al. (2011) |
| 20 | *Wuschel* (*WUS*) | Homeodomain transcription factor | *Arabidopsis thaliana* | Inducible over-expression strongly promoted somatic embryo formation in a hormone-free manner | Zuo et al. (2002) |

light on the intricacies of this important process. Promoter methylation of specific genes and, in some cases, whole genome methylation has been found to affect in vitro development and response. Chemicals that can modify chromatin remodeling and methylation can also affect acquisition of developmental fate and hormone response. The use of chemicals that can change the epigenetic state of tissues is emerging as a potential tool in favorably modulating the in vitro developmental features.

Small non-coding RNAs play a major role in transposon silencing/activation and also can potentially modulate the expression of candidate transcriptional regulators of developmental phenomena. Identification of cell culture-regulated epigenetic signatures and small RNA candidates will undoubtedly expand our current understanding of stress and hormone-dependent developmental mechanisms. Novel insights from genotype, stage, tissue, and stress-specific epigenome and transcriptome, and their possible hormonal regulation, will give us a more comprehensive understanding of regulatory networks and provide additional resources to improve the embryogenic capacity and regeneration, in recalcitrant species and cultivars. Furthermore, these investigations will help to devise strategies for minimizing the effects of phenomena such as

somaclonal variation in economically important species such as oil palm or better still help us tailor them to our advantage.

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## References

Agrawal GK, Yamazaki M, Kobayashi M, Hirochika R, Miyao A, Hirochika H (2001) Screening of the rice viviparous mutants generated by endogenous retrotransposon *Tos17* insertion. Tagging of a zeaxanthin epoxidase gene and a novel *OsTATC* gene. Plant Physiol 125:1248–1257

Aichinger E, Villar CBR, Farrona S, Reyes JC, Hennig L, Köhler C (2009) CHD3 proteins and polycomb group proteins antagonistically determine cell identity in Arabidopsis. PLoS Genet 5:e1000605

Alemanno L, Devic M, Niemenak N, Sanier C, Guilleminot J, Rio M, Verdeil J-L, Montoro P (2008) Characterization of *leafy cotyledon1-like* during embryogenesis in *Theobroma cacao* L. Planta 227:853–866

Almeida R, Allshire RC (2005) RNA silencing and genome regulation. Trends Cell Biol 15:251–258

Alves E, Ballesteros I, Linacero R, Vázquez AM (2005) *RYS1,* a foldback transposon, is activated by tissue culture and shows preferential insertion points into the rye genome. Theor Appl Genet 111:431–436

Andreev O, Spiridonova KV, Solovyan VT, Kunakh VA (2005) Variability of ribosomal RNA genes in *Rauwolfia* species: parallelism between tissue culture-induced rearrangements and interspecies polymorphism. Cell Biol Int 29:21–27

Anzola JM, Sieberer T, Ortbauer M, Butt H, Korbei B, Weinhofer I, Müllner AE, Luschnig C (2010) Putative Arabidopsis transcriptional adaptor protein (PROPORZ1) is required to modulate histone acetylation in response to auxin. Proc Natl Acad Sci USA 107:10308–10313

Atta R, Laurens L, Boucheron-Dubuisson E, Guivarćh A, Carnero E, Giraudat-Pautot V, Rech P, Chriqui D (2009) Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown in vitro. Plant J 57:626–644

Bairu MW, Aremu AO, Staden JV (2011) Somaclonal variation in plants: causes and detection methods. Plant Growth Regul 63:147–173

Banno H, Ikeda Y, Niu QW, Chua NH (2001) Overexpression of Arabidopsis *ESR1* induces initiation of shoot regeneration. Plant Cell 13:2609–2618

Bao Y, Dharmawardhana P, Mockler TC, Strauss SH (2009) Genome scale transcriptome analysis of shoot organogenesis in *Populus*. BMC Plant Biol 9:132–146

Barret P, Brinkman M, Beckert M (2006) A sequence related to rice *Pong* transposable element displays transcriptional activation by in vitro culture and reveals somaclonal variations in maize. Genome 49:1399–1407

Bassuner BM, Lam R, Lukowitz W, Yeung EC (2007) Auxin and root initiation in somatic embryos of *Arabidopsis*. Plant Cell Rep 26:1–11

Baudino S, Hansen S, Brettschneider R, Hecht VF, Dresselhaus T, Lörz H, Dumas C, Rogowsky PM (2001) Molecular characterisation of two novel maize LRR receptor-like kinases, which belong to the *SERK* gene family. Planta 213:1–10

Bednarek PT, Orłowska R, Koebner RMD, Zimny J (2007) Quantification of the tissue-culture induced variation in barley (*Hordeum vulgare* L.). BMC Plant Biol 7:10–18

Berdasco M, Alcázar R, García-Ortiz MV, Ballestar E, Fernández AF, Roldán-Arjona T, Tiburcio AF, Altabella T, Buisine N, Quesneville H, Baudry A, Lepiniec L, Alaminos M, Rodríguez R, Lloyd A, Colot V, Bender J, Canal MJ, Esteller M, Fraga MF (2008) Promoter DNA hypermethylation and gene repression in undifferentiated Arabidopsis cells. PLoS One 3:e3306

Bolibok H, Gruszczyńska A, Hromadajudycka A, Rakoczy-Trojanowska M (2007) The identification of QTLs associated with the in vitro response of rye (*Secale cereale* L.). Cell Mol Biol Lett 12:523–535

Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, Hattori J, Liu CM, van Lammeren AA, Miki BL, Custers JB, van Lookeren Campagne MM (2002) Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. Plant Cell 14:1737–1749

Bouyer D, Roudier F, Heese M, Andersen ED, Gey D, Nowack MK, Goodrich J, Renou JP, Grini PE, Colot V, Schnittger A (2011) Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PLoS Genet 7:e1002014

Brettell RIS, Dennis ES (1991) Reactivation of a silent *Ac* following tissue culture is associated with heritable alterations in its methylation pattern. Mol Gen Genet 3:365–372

Brettell RIS, Dennis ES, Scowcroft WR, Peacock WJ (1986) Molecular analysis of a somaclonal variant of alcohol dehydrogenase. Mol Gen Genet 202:235–239

Cao X, Jacobsen SE (2002) Locus-specific control of asymmetric and CpNpG methylation by the *DRM* and *CMT3* methyltransferase genes. Proc Natl Acad Sci USA 99:16491–16498

Cary AJ, Che P, Howell SH (2002) Developmental events and shoot apical meristem gene expression patterns during shoot development in *Arabidopsis thaliana*. Plant J 32:867–877

Chanvivattana Y, Bishopp A, Schubert D, Stock C, Moon YH, Sung ZR, Goodrich J (2004) Interaction of polycomb-group proteins controlling flowering in Arabidopsis. Development 131:5263–5276

Che P, Love TM, Frame BR, Wang K, Carriquiry AL, Howell SH (2006a) Gene expression patterns during somatic embryo development and germination in maize Hi II callus culture. Plant Mol Biol 62:1–14

Che P, Lall S, Nettleton D, Howell SH (2006b) Gene expression programs during shoot, root, and callus development in Arabidopsis tissue culture. Plant Physiol 141:620–637

Che P, Lall S, Howell SH (2007) Developmental steps in acquiring competence for shoot development in *Arabidopsis* tissue culture. Planta 226:1183–1194

Chen SK, Kurdyukov S, Kereszt A, Wang XD, Gresshoff PM, Rose RJ (2009a) The association of homeobox gene expression with stem cell formation and morphogenesis in cultured *Medicago truncatula*. Planta 230:827–840

Chen X-Y, Kim ST, Cho WK, Rim Y, Kim S, Kim S-W, Kang KY, Park ZY, Kim J-Y (2009b) Proteomics of weakly bound cell wall proteins in rice calli. J Plant Physiol 166:675–685

Chen D, Molitor A, Liu C, Shen WH (2010) The Arabidopsis PRC1-like ring-finger proteins are necessary for repression of embryonic traits during vegetative growth. Cell Res 20:1332–1344

Cheng C, Daigen M, Hirochika H (2006) Epigenetic regulation of the rice retrotransposon *Tos17*. Mol Genet Genomics 276:378–390

Chiappetta A, Fambrini M, Petrarulo M, Rapparini F, Michelotti V, Bruno L, Greco M, Baraldi R, Salvini M, Pugliesi C, Bitonti MB (2009) Ectopic expression of *LEAFY COTYLEDON1-LIKE* gene and localized auxin accumulation mark embryogenic

competence in epiphyllous plants of *Helianthus annuus* × *H. tuberosus*. Ann Bot 103:735–747
Cho WK, Chen XY, Chu H, Rim Y, Kim S, Kim ST, Kim S-W, Park Z-Y, Kim J-Y (2009) Proteomic analysis of the secretome of rice calli. Physiol Plant 135:331–341
Christou P (1988) Habituation in in vitro soybean cultures. Plant Physiol 87:809–812
Chuck G, Lincoln C, Hake S (1996) *KNAT1* induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. Plant Cell 8:1277–1289
Daimon Y, Takabe K, Tasaka M (2003) The *CUP-SHAPED COTYLEDON* genes promote adventitious shoot formation on calli. Plant Cell Physiol 44:113–121
Dean Rider S Jr, Henderson JT, Jerome RE, Edenberg HJ, Romero-Severson J, Ogas J (2003) Coordinate repression of regulators of embryonic identity by *PICKLE* during germination in Arabidopsis. Plant J 35:33–43
Deng W, Luo K, Li Z, Yang Y (2009) A novel method for induction of plant regeneration via somatic embryogenesis. Plant Sci 177:43–48
Dennis ES, Brettell RIS, Peacock WJ (1987) A tissue culture induced *Adh1* null mutant of maize results from a single base change. Mol Gen Genet 210:181–183
Duclercq J, Sangwan-Norreel B, Catterou M, Sangwan RS (2011) De novo shoot organogenesis: from art to science. Trends Plant Sci. doi:10.1016/j.tplants.2011.08.004
Duncan DR, Kriz AL, Paiva R, Widholm JM (2003) *Globulin-1* gene expression in regenerable *Zea mays* (maize) callus. Plant Cell Rep 21:684–689
Durante M, Cecchini E, Natali L, Citti L, Geri C, Parenti R, Nuti Ronchi V (1989) 5-Azacytidine-induced tumorous transformation and DNA hypomethylation in *Nicotiana* tissue cultures. Dev Genet 10:298–303
Elhiti M, Tahir M, Gulden RH, Khamiss K, Stasolla C (2010) Modulation of embryo-forming capacity in culture through the expression of Brassica genes involved in the regulation of the shoot apical meristem. J Exp Bot 61:4069–4085
Elhiti M, Ashihara H, Stasolla C (2011) Distinct fluctuations in nucleotide metabolism accompany the enhanced in vitro embryogenic capacity of Brassica cells over-expressing SHOOTMERISTEMLESS. Planta. doi:10.1007/s00425-011-1482-0
Evrensel C, Yilmaz S, Temel A, Gozukirmizi N (2011) Variations in BARE-1 insertion patterns in barley callus cultures. Genet Mol Res 10:980–987
Fukai E, Umehara Y, Sato S, Endo M, Kouchi H, Hayashi M, Stougaard J, Hirochika H (2010) Derepression of the plant Chromovirus LORE1 induces germline transposition in regenerated plants. PLoS Genet 6:e1000868
Fukuoka H, Kawata M, Tkaiwa M (1994) Molecular changes of organelle DNA sequences in rice through dedifferentiation, long-term culture or the morphogenesis process. Plant Mol Biol 26:899–907
Furuta K, Kubo M, Sano K, Demura T, Fukuda H, Liu Y-G, Shibata D, Kakimoto T (2011) The CKH2/PKL chromatin remodeling factor negatively regulates cytokinin responses in Arabidopsis calli. Plant Cell Physiol 52:618–628
Gaj MD, Zhang S, Harada JJ, Lemaux PG (2005) Leafy cotyledon genes are essential for induction of somatic embryogenesis of *Arabidopsis*. Planta 222:977–988
Gambino G, Minuto M, Boccacci P, Perrone I, Vallania R, Gribaudo I (2011) Characterization of expression dynamics of WOX homeodomain transcription factors during somatic embryogenesis in *Vitis vinifera*. J Exp Bot 62:1089–1101
Gernand D, Golczyk H, Rutten T, Ilnicki T, Houben A, Joachimiak AJ (2007) Tissue culture triggers chromosome alterations, amplification, and transposition of repeat sequences in *Allium fistulosum*. Genome 50:435–442
Gong H, Jiao Y, Hu W–W, Pua E-C (2005) Expression of glutathione-S-transferase and its role in plant growth and development *in vivo* and shoot morphogenesis in vitro. Plant Mol Biol 57:53–66
Grafi G, Ben-Meir H, Avivi Y, Moshe M, Dahan Y, Zemach A (2007) Histone methylation controls telomerase-independent telomere lengthening in cells undergoing dedifferentiation. Dev Biol 306:838–846
Grandbastien MA (1998) Activation of plant retrotransposons under stress conditions. Trends Plant Sci 3:181–187
Grandbastien M-A, Lucas H, Morel J-B, Mhiri C, Vernhettes S, Casacuberta JM (1997) The expression of the tobacco *Tnt1* retrotransposon is linked to plant defense responses. Genetica 100:241–252
Gruhler A, Schulze WX, Matthiesen R, Mann M, Jensen ON (2005) Stable isotope labeling of *Arabidopsis thaliana* cells and quantitative proteomics by mass spectrometry. Mol Cell Proteomics 4:1697–1709
Guo WL, Wu R, Zhang YF, Liu XM, Wang HY, Gong L, Zhang ZH, Liu B (2007) Tissue culture-induced locus-specific alteration in DNA methylation and its correlation with genetic variation in *Codonopsis lanceolata* Benth. et Hook. Plant Cell Rep 26:1297–1307
Harding EW, Tang W, Nichols KW, Fernandez DE, Perry SE (2003) Expression and maintenance of embryogenic potential is enhanced through constitutive expression of *AGAMOUS-Like 15*. Plant Physiol 133:653–663
Hecht V, Vielle-Calzada J-P, Hartog MV, Schmidt ED, Boutilier K, Grossniklaus U, de Vries SC (2001) The *Arabidopsis Somatic Embryogenesis Receptor Kinase 1* gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol 127:803–816
Heidmann I, de Lange B, Lambalk J, Angenent GC, Boutilier K (2011) Efficient sweet pepper transformation mediated by the BABY BOOM transcription factor. Plant Cell Rep 30:1107–1115
Helleboid S, Bauw G, Belingheri L, Vasseur J, Hilbert JL (1998) Extracellular $\beta$-1,3-glucanases are induced during early somatic embryogenesis in *Cichorium*. Planta 205:56–63
Helleboid S, Chapman A, Hendriks T, Inźe D, Vasseur J, Hilbert JL (2000) Cloning of $\beta$-1,3-glucanases expressed during *Cichorium* somatic embryogenesis. Plant Mol Biol 42:377–386
Higuchi M, Pischke MS, Mähönen AP, Miyawaki K, Hashimoto Y, Seki M, Kobayashi M, Shinozaki K, Kato T, Tabata S, Helariutta Y, Sussman MR, Kakimoto T (2004) *In planta* functions of the Arabidopsis cytokinin receptor family. Proc Natl Acad Sci USA 101:8821–8826
Hirochika H (1993) Activation of tobacco retrotransposons during tissue culture. EMBO J 12:2521–2528
Hirochika H (2001) Contribution of the Tos17 retrotransposon to rice functional genomics. Curr Opin Plant Biol 4:118–122
Hirochika H, Sugimoto K, Otsuki Y, Tsugawa H, Kanda M (1996) Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci USA 93:7783–7788
Hu H, Xiong L, Yang Y (2005) Rice *SERK1* gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. Planta 222:107–117
Huang J, Zhang K, Shen Y, Huang Z, Li M, Tang D, Gu M, Cheng Z (2009) Identification of a high frequency transposon induced by tissue culture, *nDaiZ*, a member of the hAT family in rice. Genomics 93:274–281
Ikeda Y, Banno H, Niu Q-W, Howell SH, Chua NH (2006) The *ENHANCER OF SHOOT REGENERATION 2* gene in Arabidopsis regulates *CUP-SHAPED COTYLEDON 1* at the

transcriptional level and controls cotyledon development. Plant Cell Physiol 47:1443–1456

Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. Nature 409:1060–1063

Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, Inoue Y, Seki M, Sakakibara H, Sugimoto K, Ohme-Takagi M (2011) The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. Curr Biol 21:508–514

Jain SM (2001) Tissue culture-derived variation in crop improvement. Euphytica 118:153–166

Jaligot E, Beulé T, Rival A (2002) Methylation-sensitive RFLPs: characterisation of two oil palm markers showing somaclonal variation-associated polymorphism. Theor Appl Genet 104:1263–1269

Jaligot E, Adler S, Debladis E, Beulé T, Richaud F, Ilbert P, Finnegan EJ, Rival A (2011) Epigenetic imbalance and the floral developmental abnormality of the in vitro-regenerated oil palm *Elaeis guineensis*. Ann Bot. doi:10.1093/aob/mcq266

Jarillo JA, Piñeiro M, Cubas P, Martínez-Zapater JM (2009) Chromatin remodeling in plant development. Int J Dev Biol 53:1581–1596

Jiang N, Bao Z, Zhang X, Hirochika H, Eddy SR, McCouch SR, Wessler SR (2003) An active DNA transposon family in rice. Nature 421:163–167

Jiang C, Mithani A, Gan X, Belfield EJ, Klingler JP, Zhu J-K, Ragoussis J, Mott R, Harberd NP (2011) Regenerant *Arabidopsis* lineages display a distinct genome-wide spectrum of mutations conferring variant phenotypes. Curr Biol 21:1385–1390

Jin S, Mushke R, Zhu H, Tu L, Lin Z, Zhang Y, Zhang X (2008) Detection of somaclonal variation of cotton (*Gossypium hirsutum*) using cytogenetics, flow cytometry and molecular markers. Plant Cell Rep 27:1303–1316

Johnson SS, Phillips RL, Rines HW (1987) Possible role of heterochromatin in chromosome breakage induced by tissue culture in oats (*Avena sativa* L.). Genome 29:439–446

Johnson KL, Kibble NA, Bacic A, Schultz CJ (2011) A fasciclin-like arabinogalactan-protein (FLA) mutant of *Arabidopsis thaliana*, *fla1*, shows defects in shoot regeneration. PLoS One 6:e25154

Jung Y-H, Jeong S-H, Kim SH, Singh R, Lee J, Cho Y-S, Agrawal GK, Rakwal R, Jwa N-S (2008) Systematic secretome analyses of rice leaf and seed callus suspension-cultured cells: workflow development and establishment of high-density two-dimensional gel reference maps. J Proteome Res 7:5187–5210

Kaeppler SM, Phillips RL (1993a) DNA methylation and tissue culture-induced variation in plants. In Vitro Cell Dev Biol 29P:125–130

Kaeppler SM, Phillips RL (1993b) Tissue culture-induced DNA methylation variation in maize. Proc Natl Acad Sci USA 90:8773–8776

Kaeppler SM, Kaeppler HF, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Mol Biol 43:179–188

Kakani A, Li G, Peng Z (2009) Role of AUX1 in the control of organ identity during in vitro organogenesis and in mediating tissue specific auxin and cytokinin interaction in *Arabidopsis*. Planta 229:645–657

Kandasamy MK, Gilliland LU, McKinney EC, Meagher RB (2001) One plant Actin isovariant, ACT7, is induced by auxin and required for normal callus formation. Plant Cell 13:1541–1554

Karlova R, Boeren S, Russinova E, Aker J, Vervoort J, de Vries S (2006) The *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 protein complex includes BRASSINOSTEROID-INSENSITIVE1. Plant Cell 18:626–638

Kikuchi K, Terauchi K, Wada M, Hirano HY (2003) The plant MITE *mPing* is mobilized in anther culture. Nature 421:167–170

Komatsu M, Shimamoto K, Kyozuka J (2003) Two-step regulation and continuous retrotransposition of the rice LINE-type retrotransposon *Karma*. Plant Cell 15:1934–1944

Koukalova B, Fojtova M, Lim KY, Fulnecek J, Leitch AR, Kovarik A (2005) Dedifferentiation of tobacco cells is associated with ribosomal RNA gene hypomethylation, increased transcription, and chromatin alterations. Plant Physiol 139:275–286

Kour GL, Kour B, Kaul S, Dhar MK (2009) Genetic and epigenetic instability of amplification-prone sequences of a novel B chromosome induced by tissue culture in *Plantago lagopus*. Plant Cell Rep 28:1857–1867

Krakowsky MD, Lee M, Garay L, Woodman-Clikeman W, Long MJ, Sharopova N, Frame B, Wang K (2006) Quantitative trait loci for callus initiation and totipotency in maize (*Zea mays* L.). Theor Appl Genet 113:821–830

Krsnik-Rasol M (1991) Peroxidase as a developmental marker in plant tissue culture. Int J Dev Biol 35:259–263

Kubis SE, Castilho AM, Vershinin AV, Heslop-Harrison JS (2003) Retroelements, transposons and methylation status in the genome of oil palm (*Elaeis guineensis*) and the relationship to somaclonal variation. Plant Mol Biol 52:69–79

La H, Ding B, Mishra GP, Zhou B, Yang H, Bellizzi Mdel R, Chen S, Meyers BC, Peng Z, Zhu JK, Wang GL (2011) A 5-methylcytosine DNA glycosylase/lyase demethylates the retrotransposon *Tos17* and promotes its transposition in rice. Proc Natl Acad Sci USA. doi:10.1073/pnas.1112704108

Ladyżyński M, Burza W, Malepszy S (2002) Relationship between somaclonal variation and type of culture in cucumber. Euphytica 125:349–356

Larkin PJ, Scowcroft WR (1981) Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Larkin PJ, Scowcroft WR (1983) Somaclonal variation and crop improvement. In: Kosuge T et al (eds) Genetic engineering of plants: an agricultural perspective. Plenum, New York, pp 289–314

Leal F, Loureiro J, Rodriguez E, Pais MS, Santos C, Pinto-Carnide O (2006) Nuclear DNA content of Vitis vinifera cultivars and ploidy level analyses of somatic embryo-derived plants obtained from anther culture. Plant Cell Rep 25:978–985

Ledwoń A, Gaj MD (2009) *LEAFY COTYLEDON2* gene expression and auxin treatment in relation to embryogenic capacity of Arabidopsis somatic cells. Plant Cell Rep 28:1677–1688

Lee ML, Phillips RL (1987) Genomic rearrangements in maize induced by tissue culture. Genome 29:122–128

Leljak-Levanić D, Bauer N, Mihaljević S, Jelaska S (2004) Changes in DNA methylation during somatic embryogenesis in *Cucurbita pepo* L. Plant Cell Rep 23:120–127

Li Y, Butenko Y, Grafi G (2005) Histone deacetylation is required for progression through mitosis in tobacco cells. Plant J 41:346–352

Li X, Yu X, Wang N, Feng Q, Dong Z, Liu L, Shen J, Liu B (2007) Genetic and epigenetic instabilities induced by tissue culture in wild barley (*Hordeum brevisubulatum* (Trin.) Link). Plant Cell Tiss Organ Cult 90:153–168

Li W, Liu H, Cheng ZJ, Su YH, Han HN, Zhang Y, Zhang XS (2011) DNA methylation and histone modifications regulate *de novo* shoot regeneration in Arabidopsis by modulating *WUSCHEL* expression and auxin signaling. PLoS Genet 7:e1002243

Lin X, Long L, Shan X, Zhang S, Shen S, Liu B (2006) In planta mobilization of *mPing* and its putative autonomous element *Pong* in rice by hydrostatic pressurization. J Exp Bot 57:2313–2323

Linacero RE, Alves F, Vazquez AM (2000) Hot spots of DNA instability revealed through the study of somaclonal variation. Theor Appl Genet 100:506–511

Linkiewicz A, Filipeck M, Tomczak A, Grabowska A, Malepszy S (2004) The cloning of sequences differentially transcribed during

the induction of somatic embryogenesis in cucumber (*Cucumis sativus* L.). Cell Mol Biol Lett 9:795–804

Liu B, Wendel JF (2000) Retrotransposon activation followed by rapid repression in introgressed rice plants. Genome 43:874–880

Liu L, White MJ, MacRae TH (1999) Transcription factors and their genes in higher plants. Functional domains, evolution and regulation. Eur J Biochem 262:247–257

Liu ZL, Han FP, Tan M, Shan XH, Dong YZ, Wang XZ, Fedak G, Hao S, Liu B (2004) Activation of a rice endogenous retrotransposon *Tos17* in tissue culture is accompanied by cytosine demethylation and causes heritable alteration in methylation pattern of flanking genomic regions. Theor Appl Genet 109:200–209

Liu X, Yu CW, Duan J, Luo M, Wang K, Tian G, Cui Y, Wu K (2011) HDA6 directly interacts with DNA methyltransferase MET1 and maintains transposable elements silencing in Arabidopsis. Plant Physiol. doi:10.1104/pp.111.184275

Long L, Ou X, Liu J, Lin X, Sheng L, Liu B (2009) The spaceflight environment can induce transpositional activation of multiple endogenous transposable elements in a genotype-dependent manner in rice. J Plant Physiol 166:2035–2045

López CMR, Wetten AC, Wilkinson MJ (2010) Progressive erosion of genetic and epigenetic variation in callus-derived cocoa (*Theobroma cacao*) plants. New Phytol 186:856–868

LoSchiavo F, Pitto L, Giuliano G, Torti G, Nuti-Ronchi V, Marazziti D, Vergara R, Orselli S, Terzi M (1989) DNA methylation of embryogenic carrot cell cultures and its variations as caused by mutation, differentiation, hormones and hypomethylating drugs. Theor Appl Genet 77:325–331

Lotan T, Ohto M, Yee KM, West MAL, Lo R, Kwong W, Yamagishi K, Fisher RL, Goldberg RB, Harada JJ (1998) *Arabidopsis* LEAF COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell 93:1195–1205

Lowe K, Hoerster G, Sun X, Scelonge C, Bao Z, Newman L, Zhang J, Shen B, Bruce W, Gordon-Kamm W (2011) Tetracycline-inducible expression of the ODP2 gene in maize produces callus from leaves. In vitro biology meeting proceedings, P-3018

Lu S, Peng X, Guo Z, Zhang G, Wang Z, Wang C, Pang C, Fan Z, Wang J (2007) In vitro selection of salinity tolerant variants from triploid bermudagrass (*Cynodon transvaalensis* × *C. dactylon*) and their physiological responses to salt and drought stress. Plant Cell Rep 26:1413–1420

Luo Y-C, Zhou H, Li Y, Chen J-Y, Yang J-H, Chen Y-Q, Qu L-H (2006) Rice embryogenic calli express a unique set of microRNAs, suggesting regulatory roles of microRNAs in plant post-embryogenic development. FEBS Lett 580:5111–5116

Madlung A, Comai L (2004) The effect of stress on genome regulation and structure. Ann Bot 94:481–495

Mangolin CA, Prioli AJ, Machado MF (1994) Isozyme patterns in callus cultures and in plants regenerated from calli of *Cereus peruvianus* (Cactaceae). Biochem Genet 32:237–247

Mantiri FR, Kurdyukov S, Lohar DP, Sharopova N, Saeed NA, Wang X-D, VandenBosch KA, Rose RJ (2008) The transcription factor MtSERF1 of the ERF subfamily identified by transcriptional profiling is required for somatic embryogenesis induced by auxin plus cytokinin in *Medicago truncatula*. Plant Physiol 146:1622–1636

Marin E, Jouannet V, Herz A, Lokerse AS, Weijers D, Vaucheret H, Nussaume L, Crespi MD, Maizel A (2010) miR390, *Arabidopsis TAS3* tasiRNAs, and their *AUXIN RESPONSE FACTOR* targets define an autoregulatory network quantitatively regulating lateral root growth. Plant Cell 22:1104–1117

Marsch-Martinez N, Greco R, Becker JD, Dixit S, Bergervoet JH, Karaba A, de Folter S, Pereira A (2006) *BOLITA*, an Arabidopsis AP2/ERF-like transcription factor that affects cell expansion and proliferation/differentiation pathways. Plant Mol Biol 62:825–843

Marsoni M, Bracale M, Espen L, Prinsi B, Negri AS, Vannini C (2008) Proteomic analysis of somatic embryogenesis in *Vitis vinifera*. Plant Cell Rep 27:347–356

Martin RC, Liu PP, Goloviznina NA, Nonogaki H (2010) MicroRNA, seeds, and Darwin? Diverse function of miRNA in seed biology and plant responses to stress. J Exp Bot 61:2229–2234

Mathieu M, Lelu-Walter MA, Blervacq AS, David H, Hawkins S, Neutelings G (2006) Germin-like genes are expressed during somatic embryogenesis and early development of conifers. Plant Mol Biol 61:615–627

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

Meins F (1989) Habituation: heritable variation in the requirement of cultured plant cells for hormones. Annu Rev Genet 23:395–408

Mhiri C, Morel J-B, Vernhettes S, Casacuberta JM, Lucas H, Grandbastien M-A (1997) The promoter of the tobacco Tnt1 retrotransposon is induced by wounding and by abiotic stress. Plant Mol Biol 33:257–266

Miguel C, Marum L (2011) An epigenetic view of plant cells cultured in vitro: somaclonal variation and beyond. J Exp Bot. doi:10.1093/jxb/err155

Miura A, Yonebayashi S, Watanabe K, Toyama T, Shimadak H, Kakutani T (2001) Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. Nature 411:212–214

Miwa H, Kinoshita A, Fukuda H, Sawa S (2009) Plant meristems: *CLAVATA3/ESR*-related signaling in the shoot apical meristem and the root apical meristem. J Plant Res 122:31–39

Momose M, Abe Y, Ozeki Y (2010) Miniature inverted-repeat transposable elements of *Stowaway* are active in potato. Genetics 186:59–66

Morcillo F, Gallard A, Pillot M, Jouannic S, Aberlenc-Bertossi F, Collin M, Verdeil JL, Tregear JW (2007) EgAP2-1, an AINTEGUMENTA-like (AIL) gene expressed in meristematic and proliferating tissues of embryos in oil palm. Planta 226:1353–1362

Morgens PH, Grabau EA, Gesteland RF (1984) A novel soybean mitochondrial transcript resulting from a DNA rearrangement involving the 5S rRNA gene. Nucleic Acids Res 12:5665–5684

Morris DA (2000) Transmembrane auxin carrier systems—dynamic regulators of polar auxin transport. Plant Growth Regul 32:161–172

Motte H, Verstraeten I, Werbrouck S, Geelen D (2011) CUC2 as an early marker for regeneration competence in Arabidopsis root explants. J Plant Physiol 168:1598–1601

Nakazaki T, Okumoto Y, Horibata A, Yamahira S, Teraishi M, Nishida H, Inoue H, Tanisaka T (2003) Mobilization of a transposon in the rice genome. Nature 421:170–172

Ngezahayo F, Xu C, Wang H, Jiang L, Pang J, Liu B (2009) Tissue culture-induced transpositional activity of mPing is correlated with cytosine methylation in rice. BMC Plant Biol 9:91–105

Nolan KE, Saeed NA, Rose RJ (2006) The stress kinase gene MtSK1 in *Medicago truncatula* with particular reference to somatic embryogenesis. Plant Cell Rep 25:711–722

Ogas J, Cheng J-C, Sung ZR, Somerville C (1997) Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant. Science 277:91–94

Ogas J, Kaufmann S, Henderson J, Somerville C (1999) PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc Natl Acad Sci USA 96:13839–13844

Ogawa D, Yamaguchi K, Nishiuchi T (2007) High-level overexpression of the *Arabidopsis HsfA2* gene confers not only increased

thermotolerance but also salt/osmotic stress tolerance and enhanced callus growth. J Exp Bot 58:3373–3383

Ouakfaoui SE, Schnell J, Abdeen A, Colville A, Labbé H, Han S, Baum B, Laberge S, Miki B (2010) Control of somatic embryogenesis and embryo development by AP2 transcription factors. Plant Mol Biol 74:313–326

Ozeki Y, Davies E, Takeda J (1997) Somatic variation during long term subculturing of plant cells caused by insertion of a transposable element in a *phenylalanine ammonia-lyase* (*PAL*) gene. Mol Gen Genet 254:407–416

Palama TL, Menard P, Fock I, Choi YH, Bourdon E, Govinden-Soulange J, Bahut M, Payet B, Verpoorte R, Kodja H (2010) Shoot differentiation from protocorm callus cultures of *Vanilla planifolia* (Orchidaceae): proteomic and metabolic responses at early stage. BMC Plant Biol 10:82

Palovaara J, Hakman I (2008) Conifer *WOX*-related homeodomain transcription factors, developmental consideration and expression dynamic of *WOX2* during *Picea abies* somatic embryogenesis. Plant Mol Biol 66:533–549

Park SY, Murthy HN, Chakrabarthy D, Paek KY (2009) Detection of epigenetic variation in tissue culture derived plants of *Doritaenopsis* by methylation sensitive amplification polymorphism (MSAP) analysis. In Vitro Cell Dev Biol Plant 45:104–108

Park S-Y, Klimaszewska K, Park J-Y, Mansfield SD (2010) Lodgepole pine: the first evidence of seed-based somatic embryogenesis and the expression of embryogenesis marker genes in shoot bud cultures of adult trees. Tree Physiol 30:1469–1478

Passarinho P, Ketelaar T, Xing M, van Arkel J, Maliepaard C, Hendriks MW, Joosen R, Lammers M, Herdies L, den Boer B, van der Geest L, Boutilier K (2008) BABY BOOM target genes provide diverse entry points into cell proliferation and cell growth pathways. Plant Mol Biol 68:225–237

Peredo EL, Revilla MA, Arroyo-García R (2006) Assessment of genetic and epigenetic variation in hop plants regenerated from sequential subcultures of organogenic calli. J Plant Physiol 163:1071–1079

Pérez-Núñez MT, Souza R, Sáenz L, Chan JL, Zúñiga-Aguilar JJ, Oropeza C (2009) Detection of a *SERK-like* gene in coconut and analysis of its expression during the formation of embryogenic callus and somatic embryos. Plant Cell Rep 28:11–19

Peschke VM, Phillips RL (1991) Activation of the maize transposable element *Suppressor-mutator* (*Spm*) in tissue culture. Theor Appl Genet 81:90–97

Peschke VM, Phillips RL, Gengenbach BG (1987) Discovery of transposable element activity among progeny of tissue culture-derived maize plants. Science 238:804–807

Phillips RL, Kaeppler SM, Olhoft P (1994) Genetic instability of plant tissue cultures: breakdown of normal controls. Proc Natl Acad Sci USA 91:5222–5226

Picault N, Chaparro C, Piegu B, Stenger W, Formey D, Llauro C, Descombin J, Sabot F, Lasserre E, Meynard D, Guiderdoni E, Panaud O (2009) Identification of an active LTR retrotransposon in rice. Plant J 58:754–765

Pischke MS, Huttlin EL, Hegeman AD, Sussman MR (2006) A transcriptome-based characterization of habituation in plant tissue culture. Plant Physiol 140:1255–1278

Planckaert F, Walbot V (1989) Molecular and genetic characterization of *Mu* transposable elements in *Zea mays*: behavior in callus culture and regenerated plants. Genetics 123:567–578

Pouteau S, Huttner E, Grandbastien MA, Caboche M (1991) Specific expression of the tobacco Tnt1 retrotransposon in protoplasts. EMBO J 10:1911–1918

Pouteau S, Grandbastien M-A, Boccara M (1994) Microbial elicitors of plant defence responses activate transcription of a retrotransposon. Plant J 5:535–542

Pryor A, Faulker K, Rhoades MM, Peacock WJ (1980) Asynchronous replication of heterochromatin in maize. Proc Natl Acad Sci USA 77:6705–6709

Rakocevic A, Mondy S, Tirichine L, Cosson V, Brocard L, Iantcheva A, Cayrel A, Devier B, Abu El-Heba GA, Ratet P (2009) *MERE1*, a low-copy-number *copia*-type retroelement in *Medicago truncatula* active during tissue culture. Plant Physiol 151:1250–1263

Rhee Y, Lin H, Buell R, Childs K, Kaeppler S (2009) *Ac2* allele of maize identified in regenerant-derived progeny from tissue culture results from insertion of a novel transposon. Maydica 54:429–437

Rhee Y, Sekhon RS, Chopra S, Kaeppler S (2010) Tissue culture-induced novel epialleles of a Myb transcription factor encoded by *pericarp color1* in maize. Genetics 186:843–855

Rodriguez-Enriquez J, Dickinson HG, Grant-Downton RT (2011) MicroRNA misregulation: an overlooked factor generating somaclonal variation? Trends Plant Sci 16:242–248

Romanov GA, Lomin SN, Schmülling T (2006) Biochemical characteristics and ligand-binding properties of Arabidopsis cytokinin receptor AHK3 compared to CRE1/AHK4 as revealed by a direct binding assay. J Exp Bot 57:4051–4058

Ruzicka DR, Kandasamy MK, McKinney EC, Burgos-Rivera B, Meagher RB (2007) The ancient subclasses of *Arabidopsis ACTIN DEPOLYMERIZING FACTOR* genes exhibit novel and differential expression. Plant J 52:460–472

Sato Y, Sentoku N, Miura Y, Hirochika H, Kitano H, Matsuoka M (1999) Loss-of-function mutations in the rice homeobox gene *OSH15* affect the architecture of internodes resulting in dwarf plants. EMBO J 18:992–1002

Sato M, Kawabe T, Hosokawa M, Tatsuzawa F, Doi M (2011a) Tissue culture-induced flower-color changes in *Saintpaulia* caused by excision of the transposon inserted in the *flavonoid 3′,5′ hydroxylase* (*F3′5′H*) promoter. Plant Cell Rep 30:929–939

Sato M, Hosokawa M, Doi M (2011b) Somaclonal variation is induced *de novo* via the tissue culture process: a study quantifying mutated cells in *Saintpaulia*. PLoS One 6:e23541

Schellenbaum P, Jacques A, Maillot P, Bertsch C, Mazet F, Farine S, Walter B (2008a) Characterization of *VvSERK1, VvSERK2, VvSERK3* and *VvL1L* genes and their expression during somatic embryogenesis of grapevine (*Vitis vinifera* L.). Plant Cell Rep 27:1799–1809

Schellenbaum P, Mohler V, Wenzel G, Walter B (2008b) Variation in DNA methylation patterns of grapevine somaclones (*Vitis vinifera L.*). BMC Plant Biol 8:78

Schmidt ED, Guzzo F, Toonen M, de Vries SC (1997) A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. Development 124:2049–2062

Schmitt F, Oakeley EJ, Jost JP (1997) Antibiotics induce genome-wide hypermethylation in cultured *Nicotiana tabacum* plants. J Biol Chem 272:1534–1540

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du F, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen W, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T,

Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn MW, Ying K, Yeh CT, Emrich SJ, Jia Y, Kalyanaraman A, Hsia AP, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia JM, Deragon JM, Estill JC, Fu Y, Jeddeloh JA, Han Y, Lee H, Li P, Lisch DR, Liu S, Liu Z, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Bennetzen JL, Dawe RK, Jiang J, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA, Wilson RK (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Schütz W, Hausmann N, Krug K, Hampp R, Macek B (2011) Extending SILAC to proteomics of plant cell lines. Plant Cell 23:1701–1705

Shibukawa T, Yazawa K, Kikuchi A, Kamada H (2009) Possible involvement of DNA methylation on expression regulation of carrot LEC1 gene in its 5′-upstream region. Gene 437:22–31

Singla B, Tyagi AK, Khurana JP, Khurana P (2007) Analysis of expression profile of selected genes expressed during auxin-induced somatic embryogenesis in leaf base system of wheat (*Triticum aestivum*) and their possible interactions. Plant Mol Biol 65:677–692

Singla B, Khurana JP, Khurana P (2008) Characterization of three somatic embryogenesis receptor kinase genes from wheat, *Triticum aestivum*. Plant Cell Rep 27:833–843

Sinha N, Williams R, Hake S (1993) Overexpression of the maize homeobox gene, *Knotted1*, causes a switch from determinate to indeterminate cell fates. Genes Dev 7:787–795

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 54:118–130

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet 8:272–285

Smulders MJM, de Klerk GJ (2011) Epigenetics in plant tissue culture. Plant Growth Regul 63:137–146

Smýkal P, Valledor L, Rodríguez R, Griga M (2007) Assessment of genetic and epigenetic stability in long-term in vitro shoot culture of pea (*Pisum sativum* L.). Plant Cell Rep 26:1985–1998

Song X, Han Y, Teng W, Sun G, Li W (2010) Identification of QTL underlying somatic embryogenesis capacity of immature embryos in soybean (*Glycine max* (L.) Merr.). Plant Cell Rep 29:125–131

Sotirova V, Shtereva L, Zagorska N, Dimitrov B, Bogatsevska N (1999) Resistance responses of plants regenerated from tomato anther and somatic tissue cultures to *Clavibacter michiganense*. Israel J Plant Sci 47:237–243

Sreedhar RV, Venkatachalam L, Bhagyalakshmi N (2007) Genetic fidelity of long-term micropropagated shoot cultures of vanilla (*Vanilla planifolia* Andrews) as assessed by molecular markers. Biotechnol J 2:1007–1013

Srinivasan C, Liu Z, Heidmann I, Supena ED, Fukuoka H, Joosen R, Lambalk J, Angenent G, Scorza R, Custers JB, Boutilier K (2007) Heterologous expression of the *BABY BOOM* AP2/ERF transcription factor enhances the regeneration capacity of tobacco (*Nicotiana tabacum* L.). Planta 225:341–351

Steward FC, Mapes MO, Kent AE, Holsten RD (1964) Growth and development of cultured plant cells. Science 143:20–27

Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ (2001) LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development. Proc Natl Acad Sci USA 98:11806–11811

Su YH, Zhao XY, Liu YB, Zhang CL, O'Neill SD, Zhang XS (2009) Auxin-induced WUS expression is essential for embryonic stem cell renewal during somatic embryogenesis in Arabidopsis. Plant J 59:448–460

Suzuki M, Wang HHY, McCarty DR (2007) Repression of the *LEAFY COTYLEDON 1/B3* regulatory network in plant embryo development by *VP1/ABSCISIC ACID INSENSITIVE 3-LIKE* B3 genes. Plant Physiol 143:902–911

Taguchi-Shiobara F, Yamamoto T, Yano M, Oka S (2006) Mapping QTLs that control the performance of rice tissue culture and evaluation of derived near-isogenic lines. Theor Appl Genet 112:968–976

Takada S, Hibara K, Ishida T, Tasaka M (2001) The CUP-SHAPED COTYLEDON1 gene of Arabidopsis regulates shoot apical meristem formation. Development 128:1127–1135

Takeda S, Sugimoto K, Otsuki H, Hirochika H (1999) A 13-bp cis-regulatory element in the LTR promoter of the tobacco retrotransposon Tto1 is involved in responsiveness to tissue culture, wounding, methyl jasmonate and fungal elicitors. Plant J 18:383–393

Tanaka M, Kikuchi A, Kamada H (2008) The *Arabidopsis* histone deacetylases HDA6 and HDA19 contribute to the repression of embryonic properties after germination. Plant Physiol 146:149–161

Tang W, Newton RJ (2005) Mevalonate kinase activity during different stages of plant regeneration from nodular callus cultures in white pine (*Pinus strobus*). Tree Physiol 26:195–200

Tanurdzic M, Vaughn MW, Jiang H, Lee TJ, Slotkin RK, Sosinski B, Thompson WF, Doerge RW, Martienssen RA (2008) Epigenomic consequences of immortalized plant cell suspension culture. PLoS Biol 6:2880–2895

Tenedini E, Roncaglia E, Ferrari F, Orlandi C, Bianchi E, Bicciato S, Tagliafico E, Ferrari S (2010) Integrated analysis of microRNA and mRNA expression profiles in physiological myelopoiesis: role of hsa-mir-299–5p in CD34+ progenitor cells commitment. Cell Death Dis 1:e28

Thakare D, Tang W, Hill K, Perry SE (2008) The MADS-domain transcriptional regulator AGAMOUS-LIKE15 promotes somatic embryo development in Arabidopsis and soybean. Plant Physiol 146:1663–1672

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

Tsuwamoto R, Yokoi S, Takahata Y (2010) *Arabidopsis EMBRYOMAKER* encoding an AP2 domain transcription factor plays a key role in developmental change from vegetative to embryonic phase. Plant Mol Biol 73:481–492

Uddenberg D, Valladares S, Abrahamsson M, Sundström JF, Sundås-Larsson A, von Arnold S (2011) Embryogenic potential and expression of embryogenesis-related genes in conifers are affected by treatment with a histone deacetylase inhibitor. Planta. doi:10.1007/s00425-011-1418-8

van Hengel AJ, Tadesse Z, Immerzeel P, Schols H, van Kammen A, de Vries SC (2001) *N*-Acetylglucosamine and glucosamine-containing arabinogalactan proteins control somatic embryogenesis. Plant Physiol 125:1880–1890

Verdeil J-L, Alemanno L, Niemenak N, Tranbarger TJ (2007) Pluripotent versus totipotent plant stem cells: dependence versus autonomy? Trends Plant Sci 12:245–252

Vicient CM (2010) Transcriptional activity of transposable elements in maize. BMC Genomics 11:601–610

Wang H, Caruso LV, Downie AB, Perry SE (2004) The embryo MADS domain protein AGAMOUS-Like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. Plant Cell 16:1206–1219

Wang Y, Elhiti M, Hebelstrup KH, Hill RD, Stasolla C (2011) Manipulation of hemoglobin expression affects Arabidopsis

shoot organogenesis. Plant Physiol Biochem. doi: 10.1016/j.plaphy.2011.06.005

Weber J, Georgiev V, Pavlov A, Bley T (2008) Flow cytometric investigations of diploid and tetraploid plants and in vitro cultures of *Datura stramonium* and *Hyoscyamus niger*. Cytometry A 73:931–939

Willmann MR, Poethig RS (2007) Conservation and evolution of miRNA regulatory programs in plant development. Curr Opin Plant Biol 10:503–511

Willmann MR, Mehalick AJ, Packer RL, Jenik PD (2011) MicroRNAs regulate the timing of embryo maturation in Arabidopsis. Plant Physiol 155:1871–1884

Wrather JA, Freytag AH (1991) Selection of atrazine tolerant soybean calli and expression of that tolerance in regenerated plants. Plant Cell Rep 10:44–47

Wu X-M, Liu M-Y, Ge X–X, Xu Q, Guo W–W (2011) Stage and tissue-specific modulation of ten conserved miRNAs and their targets during somatic embryogenesis of Valencia sweet orange. Planta 233:495–505

Yamaguchi M, Kato H, Yoshida S, Yamamura S, Uchimiya H, Umeda M (2003) Control of in vitro organogenesis by cyclin dependent kinase activities in plants. Proc Natl Acad Sci USA 100:8019–8023

Yamashita H, Tahara M (2006) A LINE-type retrotransposon active in meristem stem cells causes heritable transpositions in the sweet potato genome. Plant Mol Biol 61:79–94

Yin L, Tao Y, Zhao K, Shao J, Li X, Liu G, Liu S, Zhu L (2007) Proteomic and transcriptomic analysis of rice mature seed-derived callus differentiation. Proteomics 7:755–768

Yin L, Lan Y, Zhu L (2008) Analysis of the protein expression profiling during rice callus differentiation under different plant hormone conditions. Plant Mol Biol 68:597–617

Yoshii M, Shimizu T, Yamazaki M, Higashi T, Miyao A, Hirochika H, Omura T (2009) Disruption of a novel gene for a NAC-domain protein in rice confers resistance to rice dwarf virus. Plant J 57:615–625

Yu Y, Feng Z, Wang G, Li F, Du X, Zhu J (2010) Initiation of dedifferentiation and structural changes in in vitro cultured petiole of *Arabidopsis thaliana*. Protoplasma 241:75–81

Yu X, Li X, Zhao X, Jiang L, Miao G, Pang J, Qi X, Liu B (2011) Tissue culture-induced genomic alteration in maize (*Zea mays*) inbred lines and F1 hybrids. Ann Appl Biol 158:237–247

Zeng F, Zhang X, Zhu L, Tu L, Guo X, Nie Y (2006) Isolation and characterization of genes associated to cotton somatic embryogenesis by suppression subtractive hybridization and macroarray. Plant Mol Biol 60:167–183

Zhang S, Wong L, Meng L, Lemaux PG (2002) Similarity of expression patterns of *knotted1* and *ZmLEC1* during somatic and zygotic embryogenesis in maize (*Zea mays* L.). Planta 215:191–194

Zhang M, Xu C, Yan H, Zhao N, Wettstein DV, Liu B (2009) Limited tissue culture-induced mutations and linked epigenetic modifications in F1 hybrids of sorghum pure lines are accompanied by increased transcription of DNA methyltransferases and 5-methylcytosine glycosylases. Plant J 57:666–679

Zhang M, Wang H, Dong Z, Qi B, Xu K, Liu B (2010a) Tissue culture-induced variation at simple sequence repeats in sorghum (*Sorghum bicolor* L.) is genotype-dependent and associated with down-regulated expression of a mismatch repair gene, *MLH3*. Plant Cell Rep 29:51–59

Zhang S, Zhou J, Han S, Yang W, Li W, Wei H, Li X, Qi L (2010b) Four abiotic stress-induced miRNA families differentially regulated in the embryogenic and non-embryogenic callus tissues of *Larix leptolepis*. Biochem Biophys Res Commun 398:355–360

Zheng Y, Ren N, Wang H, Stromberg AJ, Perry SE (2009) Global identification of targets of the Arabidopsis MADS domain protein AGAMOUS-Like15. Plant Cell 21:2563–2577

Zimmerman L (1993) Somatic embryogenesis: a model for early development in higher plants. Plant Cell 5:1411–1423

Zuo J, Niu QW, Frugis G, Chua NH (2002) The WUSCHEL gene promotes vegetative-to-embryonic transition in Arabidopsis. Plant J 30:349–359