Mutants for Biochemical and Molecular Insights into Embryogenesis in Plants**

Amita Bhattacharya, Preeti Sharma and Paramvir Singh Ahuja*
Institute of Himalayan Bioresource Technology, Palampur 176 061, India

Despite the advancements that have been made in understanding the intricate mechanisms of embryogenesis, a lot remains elusive. However, the understanding of the mutants of different stages of embryo development viz. *tsII*, *gnom*, *mickey*, *lee* etc. have opened up new vistas towards gaining insights into their biochemical and molecular basis of embryogenesis. Today, studies on mutants have not only played a major role in the easier manipulation of PGRs *in vitro* but have also led to the important discoveries of genes that are involved in the process of embryo pattern formation and in elucidating the underlying genetic, molecular and physiological mechanisms of inducing polarity, cotyledon initiation and pro-vascular tissue differentiation. The present review deals with the different mutants and the role they have played in the understanding of molecular and biochemical insights of somatic embryogenesis.

Keywords: mutants, somatic embryos, PGRs, biochemical, molecular, morphogenesis, maturation and germination

Introduction
The unique phenomenon of producing embryos from any vegetative cell and its ability to eventually develop into a complete plant has traversed a long path since its discovery by Reinert (1958) and Steward *et al* (1958). As a result of this, efficient somatic embryogenesis systems have been developed in a wide variety of plants (Zimmerman, 1993). Somatic embryogenesis has also contributed significantly towards the understanding of zygotic embryogenesis.

Embryogenesis represents the critical stage in the development of a plant at which its basic organization and body plan have their inception and extensive work has been done on the morphological, biochemical and molecular aspects (Kaplan & Cooke, 1997). However, one of the most basic question in developmental biology with respect to how does an undifferentiated mass of cells become programmed to behave as an embryo and more importantly how the undifferentiated mass of cells that comprises the early embryo takes on a pattern in which different cell layers and/or regions adopt particular developmental fates, still remains elusive (Chasan, 1993). Despite the fact that a lot needs to be understood regarding the above questions, mutants have played a significant role (Kaplan & Cooke, 1997; Gallois, 2001).

Identification of mutants at different stages of development of somatic and zygotic embryos has opened new vistas towards gaining insights into their biochemical and molecular basis of embryogenesis (Krautwig & Lorz, 1996; Mordhorst *et al*, 1998). The *in vitro* technique of ‘embryo culture’ has shown that isolated zygotic embryos have the ability to complete their development *in vitro*. This enabled the identification of several forms of mutants of plant growth hormones that are important for the normal development of embryos (Reinbothe *et al*, 1992; Liu *et al*, 1993). These studies not only helped in easier manipulation of plant growth regulators, *in vitro*, but also led to important discoveries of genes that are involved in the process of embryogenesis and also their expression (Sheridan, 1995; Heberle *et al*, 1997; Shen *et al*, 2001; Magioli *et al*, 2001). The ‘embryo culture technique’ when utilized for studies on mutants of the model system ‘carrot’ revealed the involvement of several secreted proteins during embryo development (Cordewener *et al*, 1991; De Jong *et al*, 1992). Mutants have also helped in dispelling the earlier belief of maternal or post-fertilization events being the absolute requirement for specifying pattern and for inducing polarity in the plant embryos.

A conceptual model of basic pattern formation in plants was presented for the first time by Jurgens *et al* (1991). However, this was largely derived from the studies on *Drosophila* developmental genetics. The very same year, the mutants of embryo development were discovered in *Arabidopsis thaliana*. Mayer *et al* (1991) and Meinke (1991a; 1991b) demonstrated embryo pattern formation in plants to be genetically controlled. Mutants with disturbed pattern formation...
contributed much to the knowledge of ‘control of regional specificity in plant embryos’. The understanding of the underlying molecular mechanisms of the polarity inducing processes gained considerably from these phenotypic mutants.

The present article reviews the role of mutants in the understanding of the process of embryo pattern formation and also in elucidating the underlying genetic, molecular and physiological mechanisms of inducing polarity, cotyledon initiation and pro-vascular tissue differentiation.

The Process of Embryogenesis

In plants, the process of zygotic embryogenesis commences after fertilization wherein, the single celled zygote undergoes gradual morphological differentiation in order to give rise to successive developmental stages. The striking similarity of these stages in most angiosperm and gymnosperm plant species suggests the existence of a common gene expression programme for pattern formation and cell specialization (Jurgens et al, 1991). The process of development in somatic embryos closely resembles that of zygotic embryos, both morphologically as well as temporally. In dicots, the stages of development include, the first recognizable globular stage and the oblong stage followed by the heart stage and the torpedo stage (Zimmerman, 1993). While the shift from the globular stage to the oblong stage signals the shift from the isodiametric to bilaterally symmetrical growth and beginning of the heart stage, the globular to heart transition is clearly marked by the outgrowth of the two cotyledons, the elongation of the hypocotyl and the beginning of radicle development. This transition continues through the torpedo and the plantlet stages (Schiavone & Cooke, 1987). Despite being similar, an important difference between the somatic and zygotic embryogenesis includes the ability of the somatic embryos to develop outside the physical constraints and the informational context of maternal tissue (Zimmerman, 1993). The other important difference includes the absence of endosperm and/or suspensor differentiation in somatic embryogenesis.

The question of whether it is the differential ability of somatic cells or the presence of specific responsive cell type that is responsible for somatic embryogenesis is still not defined (De Jong et al, 1993). The identification of the original cells actually involved in the developmental processes of somatic embryogenesis is difficult and can proceed either from an asymmetrical cell cluster, a symmetrical cell or an aberrantly shaped cell cluster. The first embryonic developmental stage or initiation of morphogenesis is intimately linked to the development of polarity and subsequent asymmetric division leading to cell differentiation. Although, the first asymmetric cell division in case of zygotic embryogenesis is initiated by a gradient of light wherein, the plane of division is generally perpendicular to the light axis (Dodeman et al, 1997), yet, other stresses are also known to be important.

Conceptually, the entire process of embryogenesis in higher plants can be summarized into three overlapping phases during morphogenesis (i) a polar axis of the plant body is defined with specification of the shoot and root apices wherein the embryonic tissue and organ systems are formed, (ii) the second phase of embry maturation is characterized by accumulation of storage reserves and acquisition of desiccation tolerance and (iii) the final phase of desiccation and developmental arrest (West & Harada, 1993).

Role of Phenotypic Mutants in Understanding the Morphological and Physiological basis of Morphogenesis

In Arabidopsis thaliana studies on six mutants of apical-basal pattern for mutation viz. gnom, gurke, fackel monopteros, rootless and shoot meristemless, the three mutants of radial defects i.e. keule, knolle and raspberry and the three mutants with altered shape i.e. fass, knopf, and mickey) have confirmed the fact that polarity of the female gamete or zygote is genetically pre-determined (Weigel, 1993; Meinke et al, 1994; Yadegiri et al, 1994). The gnom mutants not only fail to elongate but also produce an enlarged apical cell. This is due to the inclination of the plane of division at an abnormal degree as a result of which phenotypic mutants like the ball-shaped seedlings without root and cotyledons are produced. Gnom mutants led to the discovery of the fact that the Gnom gene is responsible for the early events in embryo morphogenesis (Mayer et al, 1991) and also to the conclusion that the alignment of the microtubules is in a fashion perpendicular to the axis prior to asymmetric division.

Cell polarity followed by asymmetric divisions are important for the initiation of somatic embryogenesis. In general, auxins promote asymmetric divisions in the explant tissue to give rise to small daughter cells from which the somatic embryos arise. These cells either single (Backs-Husemann & Reinert, 1970; No-
mura & Komamine, 1985) or in tight clusters are
designated as the pro-embryonic masses or the PEMs (Halperin, 1966). PEMs generally develop on nutrient
media containing high concentrations of auxins (Schiavone & Cooke, 1987; Michalczuk et al, 1992a, b).
These PEMs are cells or cell clusters with dense
cytoplasm and are enriched with proteins, mRNAs,
etc. They are replete with gene products necessary to
complete the globular stage of embryogenesis (Zimmerman, 1993). The PEMs also contain many other
mRNAs and proteins whose continued presence generally inhibits the elaboration of the embryogenic
process. The removal of auxins results in inactivation of these genes so as to allow the embryogenic pro-
gramme to proceed smoothly. However, different factors like the presence of other PGRs, pH shift,
electrical fields (Smith & Krikorian, 1990), temperature, other environmental or cellular signals (Hause et
al, 1993) or nutrients in the medium may also play an
important role. Till date it has been possible only to
compare the somatic and the zygotic embryos from
the globular stage onwards. Heterogeneity or vari-
ability have been reported in the initial patterns of
both somatic and zygotic embryogenesis (Toonen et
al, 1994). However, it is still not clear whether it is
the specific responsive cell type or the differential
ability of the somatic cells that become embryogenic
are actually responsible for the initial steps of somatic
embryogenesis (De Jong et al, 1993).

Role of Mutants in Understanding Pattern Forma-
tion during Embryogenesis

The importance of heterogenous partitioning for
controlled cell expansion and asymmetric division in
embryogenesis has been indicated (De Jong et al, 1993). In other words, co-ordinately regulated cell
divisions are important for generating cells that will expand, take on a pattern and finally form the plant
body. This was confirmed by the studies involving the
dominant negative cdc2aAt mutant of Arabidopsis thaliana, which showed that these co-ordinated cell
divisions are brought about by plant cyclin dependent
kinases (CDKs) and cyclins are modulated by a vari-
ety of internal and external signals (Hemerly et al,
2000). Since cell division events are essential for em-
byro patterning and morphogenesis (Mansfield & Bri-
arty, 1991), it is not surprising that the cdc2aAt mu-
tant of Arabidopsis shows a high range of distortions
on the apical-basal pattern, defective leaves and incor-
rect phyllotactic pattern.

However, polarized cell expansion and division
plane alignment are not required for spatial develop-
ment (Tras et al, 1995). This was proven by the gurke
and fackel mutants with disturbed apical and basal
patterns. Generally, the first asymmetric division in
zygotic embryos is followed by three crucial stages
viz. (i) the octant stage composed of two to four cells,
(ii) the protoderm formation and (iii) initiation of
primordia. The small apical cell produced after the
first asymmetric division neither enlarges during the
above three steps nor during the subsequent periclinal
divisions when the protoderm is formed. This was
proved by the emb101-1 mutant of Arabidopsis thal-
liana. While the protoderm formation prevents cell
eexpansion and is essential for the remaining develop-
mental phases, the emb101-1 mutants of Arabidopsis
show uncontrolled cell expansion leading to the pro-
duction of enlarged cells which fill the whole seed.
Embryo specific mutants of Zea mays i. e. emb*-8518
and emb*-8521 fail to form protoderm and hence
cannot acquire bilateral symmetry as compared to the
emb*-853 and emb*-8542 embryos that exhibit the
wild type characteristics. Similarly the knolle mutants
proved that specification of radial axis is required for
a normal apical and basal pattern and the Knolle gene
is involved in vesicular trafficking or in membrane
fusion and hence in cytokinesis (Lukowitz et al,
1996). About 40 genes are reported to control the
formation of embryo axis pattern elements (Mayer et

Role of Mutants in Understanding the Formation
of Meristem or Initiation of Primordia

Orientation of dividing cells and their subsequent
cell morphogenesis and differentiation are tightly
controlled (Berleth, 1998). While patterning in the
embryos is not important for tissue differentiation,
positional control is of considerable importance. This
was proven true by the raspberry mutants. Rather
development of root meristem or somatic tissues or in
other words, determination of cell fate requires
positional control. In this regard, the monopteros or
the gnom mutants have indicated that while the
monopteros gene is responsible for the organization of
the basal region, the gnom is epistatic to the monop-
teros (Berleth & Jurgens, 1993). These studies have
now made it possible to conclude that the root devel-
opment is totally dependant on organized and
positional segmentation and cell-cell interaction.

Shoot apical meristems are a group of indetermi-
nate stem cells that are organized during embryogene-
sis. Each of the mutants pt and clv (clavata) of Arabidopsis thaliana embryos indicated that their defects are first detected at early heart stage and broad shoot apical meristems (SAM) are produced. Double mutants i.e. pt clv showed additive effects with enlarged SAMs which later produced short fasciated inflorescence stems and also had an additive effect on the number of rosette leaves. These mutants indicated that the CLV and PT genes act in an independent manner on pathways that control SAMs (Mordhorst et al., 1998). A recessive mutant of maize, abphyll, indicated that defects in them first appeared during embryogenesis and played a vital role in regulating morphogenesis and in determining the future phyllotaxy of shoots. The embryo mutants of maize, emb*-853 and emb*-8542 are important in overcoming the mutants defective in development of functional shoot apical meristems and leaf primordia.

Role of Mutants in Understanding the Hormonal Regulation of Embryogenesis

Mutants have shown that development entails a series of interacting processes where the alteration of one factor triggers successive abnormal events (Goebel-Tourand et al., 1993). Generally, these interacting processes are governed by plant growth regulators which act as signaling molecules during embryogenesis. Mutants with a disturbed balance of auxins and cytokinins have perturbed spatial patterns like multiple or fused cotyledons, shoot-meristemless embryos, etc. While the shoot-meristemless mutants either completely lack or have only partially organized apical meristems that do not interfere with the development of other parts of the embryo (Barton & Poethig, 1993; Long et al., 1996), the pin1 mutants of Arabidopsis thaliana have fused cotyledons due to the inhibition of polar transport of auxins (Okada et al., 1991; Liu et al., 1993; Cooke et al., 1993). Liu and co-workers used excised zygotic embryos of Brassica juncea to show that polar transport of auxin is essential for the establishment of bilateral symmetry during embryogenesis. Using a wide array of inhibitors of polar transport of auxins on globular embryos they showed that cotyledon formation became decoupled from the development of bilateral symmetry wherein instead of two cotyledons forming on opposite sides of the embryo, a ring of cotyledon tissue emerged to encircle the embryo. In Arabidopsis, embryos homozygous for the mutant pin1 also develop a collar like or fused cotyledon. This was further elucidated by the use of an auxin antagonist like 2-p-chlorophenoxy isobutyric acid or CIPB that does not have any effect on polar auxin transport. Based on the fact that the use of CIPB did not produce any aberrant structures, Liu et al. (1993) and Cooke et al. (1993) postulated that the auxin polar transport was an absolute requirement for the establishment of bilateral symmetry in globular embryos. The activity of polar transport of auxin determines where the two cotyledons will be formed at the side towards which the embryo accumulates maximum auxin becomes the future site of cotyledon formation. The cells at these sites not only amplified their own levels of polar auxin transport but also inhibited the polar transport of auxin to all the neighbouring cells except the most distant one (Chasan, 1993).

The mickey mutants of Arabidopsis (Mayer et al., 1991) indicated that an optimal balance of hormones was required for normal vasculature and subsequent meristem development but any disturbance in this balance resulted in abnormalities as is evident in their phenotypic mutants. The shoot-meristemless mutants displayed reduced meristems, abnormal fuzzy vascular strands and/or hypertrophic cell distortion (Goebel-Tourand et al., 1993). A similar response was observed in species like soybean (Barwale et al., 1986), alfalfa (Dos Santos et al., 1983) and Vitis longii (Gray & Mortensen, 1987).

Role of Mutants in understanding the Underlying Biochemical Basis of Embryogenesis

Mutants have shown that the process of embryogenesis is characterized by well marked stages, of which, the transition from the globular to heart shape is the most crucial. Recent studies with mutants have indicated that certain cell wall proteins either promote or inhibit the process of somatic embryogenesis (Cordewener et al., 1991; De Jong et al., 1992; Kruenger & Van Holst, 1993; De Jong et al., 1993). Giuliano et al. (1984) produced a chemical mutant that was sensitive to temperature. This temperature sensitive ts11 mutant showed a transitional arrest from the globular to the heart stage. Aberrant protoderm formation in these mutants was actually responsible for this developmental arrest at non-permissible temperatures. However, the addition of a mixture of proteins secreted by the embryogenic wild type cell lines of carrot was capable of overcoming this developmental arrest (Lo Schiavo et al., 1990). These proteins were later found to be glycosylated extra-cellular proteins that were
involved in different stages of development. While the extra-cellular protein 3 (EP3), an acidic endo-
chitinase of 32 kDa was responsible for the rescue of ts11 embryos, (De Jong et al, 1992), the EP1 protein
was found to be secreted only by the non-
embryogenic cells (Van Engelen et al, 1991). In the
same year, another extra-cellular protein (EP2) was
identified to be involved in lipid transfer and was
synthesized only by embryogenic cells and somatic
showed that the EP3 proteins could be substituted by
the N-acetylglucosamine containing lipooligosaccha-ides or nodulation factors or NodRlv-V(Ac C18:4) of
Rhizobium leguminosarum bacterial variety viciae.

Maturation and Germination

The stages of somatic and zygotic embryo devel-
opment are similar in all aspects, except for the fact
that the somatic embryos do not become dormant.
Genetic studies based on mutants have revealed the
influence of late embryogenesis specific genes in-
volved in maturation. The leafy cotyledon or lec mu-
tants of Arabidopsis cause defects in the differentia-
tion process of cotyledons and in the maturation spe-
cific events such as storage reserve accumulation,
desiccation tolerance and maintenance of quiescence
(Meinke et al, 1994). While the wild type LEC1 gene
functions in both regions of the embryo axis and lack
storage organelles, the Lee mutations result in the
transition of cotyledons of embryos and seedlings into
leaf like structures, characterized by trichomes, stom-
ata and mesophyll cell differentiation and absence of
protein and lipid storage bodies. Therefore, the lec
mutant embryos germinate precociously, implying
that embryonic and post-germinative programmes
occur simultaneously.

The fusca mutants of Arabidopsis, which accumu-
late anthocyanins in their cotyledons during late em-
byogenesis and fail to germinate, showed the genes
required for post-embryonic development are active
in late embryogenesis (Goldberg et al, 1994). Gener-
ally, the fusca mutants show normal embryogenesis
with exception to fus3 mutants which exhibit leafy
like phenotype. The lethal nature also indicated that
FUSCA genes are essential for critical developmental
processes wherein, the accumulation of anthocyanin
was a secondary effect (Castle & Meinke, 1994).

Some regulatory genes that prepare the embryos for
subsequent germination.

Molecular Insights

Sung and his group (1984) and Goldberg et al
(1989) considered somatic embryogenesis to be an
excellent model system for studying gene regulation
during embryogenesis. Fujimura & Komamine,
(1980) indicated that active RNA synthesis and sub-
stantial changes in gene expression at transcriptional
levels dictate the embryo development process. Since
then several laboratories have been searching for
genes that were regulated during somatic embry-
ogenesis (Zimmerman et al, 1992). Based on their
work on protein gel analysis Sung and Okimoto
(1983) showed that the embryogenic process may not
involve the mRNAs. Therefore, the search for 'em-
broyo enhanced genes' began. Approaches like cDNA
libraries and screening of the libraries by raising anti-
sera against embryo proteins or radiolabelling led to
significant successes ( Choi et al, 1987; Sterk et al,
Thomas, 1993).

A number of 'embryo enhanced genes' have been
isolated whose functions are still unknown. However,
some genes like DC8, DC3, EMB-1 are now known
to belong to the family of proteins called the ''Late
Embryogenesis Abundant'' proteins (Galau et al,
1986; Dure et al, 1989). These genes are generally
ABA inducible and are thought to contribute to desic-
cation tolerance. A second group of embryo abundant
genes have been isolated for the EP2 proteins (Sterk
et al, 1991) The EP2 gene is expressed in the proto-
derm cells of both somatic and zygotic embryos and is
transiently expressed in epidermal cells of leaf pri-
mordia and is considered to be a marker for proto-
derm development in early embryos.

Apuyala & Zimmerman, (1992) isolated two other
genes, the ATP-2 gene and the translation elongation
factor EF-1α and found that they were specifically
expressed in globular embryos of carrot. Also the
studies on mutants of Arabidopsis like extracotyledon
2 or xtc2 accumulation of MADS protein domain
AGAMOUS 15 in the embryonic tissues of dandelion,
Arabidopsis, oil seed rape and alfalfa indicate that
these genes were active during early embryogenesis
(Perry et al, 1999).

Genes responsible for embryo development like the
embryo specific PEII and those responsible for the
transition from globular to heart stage (Li & Thomas,
1998), the meristem formation i. e. the kn1 for shoot
meristem and ZnHox for shoot and root meristems
have been isolated from mutants like the *tl* of *Arabidopsis* (Sheridan, 1995).

**The Indian Scenario**

In India, considerable work has been done in the recent past on different aspects of somatic embryogenesis in the plant species is listed in Table 1. Although, the major focus has been on the production of large number of plants for either biotechnological improvement or for mass propagation of important plants using somatic embryogenesis, no work has yet been done on the mutants of somatic embryogenesis.

Table 1—Work done by Indian workers on somatic embryogenesis in the different plant species in the last ten years

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Explants used</th>
<th>Mutant if any</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trees</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santalum indicum</td>
<td>Hypocotyls</td>
<td>—</td>
<td>Bapat &amp; Rao, 1984</td>
</tr>
<tr>
<td>Acacia nitotica</td>
<td>Endosperm</td>
<td>—</td>
<td>Garg <em>et al.</em>, 1996</td>
</tr>
<tr>
<td>Terminalia arjuna</td>
<td>Leaves</td>
<td>—</td>
<td>Kumari <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>Pinus roxburghii</td>
<td>Immature megagametophytes</td>
<td>—</td>
<td>Mathur <em>et al.</em>, 2000</td>
</tr>
<tr>
<td>Gloriosa superba</td>
<td>Shoot apices</td>
<td>—</td>
<td>Jadhav &amp; Hegde, 2001</td>
</tr>
<tr>
<td>Hardwickia banata</td>
<td>Semi mature zygotic embryos</td>
<td>—</td>
<td>Chand &amp; Singh, 2001</td>
</tr>
<tr>
<td><strong>Oilseeds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brassica juncea</td>
<td>Mesophyll Protoplasts</td>
<td>—</td>
<td>Kirti &amp; Chopra, 1987</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Immature zygotic embryos</td>
<td>—</td>
<td>Sujatha &amp; Prabakaran, 2001</td>
</tr>
<tr>
<td>Brassica juncea</td>
<td>Protoplast derived hypocotyls</td>
<td>—</td>
<td>Kirti &amp; Chopra, 1990</td>
</tr>
<tr>
<td>Brassica nigra</td>
<td>Hypocotyls</td>
<td>—</td>
<td>Narasimhula <em>et al.</em>, 1992</td>
</tr>
<tr>
<td>Archis hypogea</td>
<td>Immature leaflets</td>
<td>—</td>
<td>Venkatachalam, <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>safflower</td>
<td>Cotyledons</td>
<td>—</td>
<td>Mandal <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Medicinal plants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asparagus cooperi</td>
<td>Spear callus</td>
<td>—</td>
<td>Ghosh &amp; Sen, 1991</td>
</tr>
<tr>
<td>Podophyllum hexandrum</td>
<td>Zygotic embryos</td>
<td>—</td>
<td>Arumugam &amp; Bhojvani, 1990</td>
</tr>
<tr>
<td>Hyoscyamus muticus</td>
<td>protoplasts</td>
<td>—</td>
<td>Giri &amp; Ahuja, 1990</td>
</tr>
<tr>
<td>Aconitum heterophyllum</td>
<td>Leaves and petioles</td>
<td>—</td>
<td>Giri <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Solanum sarrachoides</td>
<td>Leaves</td>
<td>—</td>
<td>Banerjee <em>et al.</em>, 1994</td>
</tr>
<tr>
<td>Psoralea corylifolia</td>
<td>Leaves and stem</td>
<td>—</td>
<td>Rout &amp; Das, 2001</td>
</tr>
<tr>
<td><strong>Cereals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td>Protoplasts</td>
<td>—</td>
<td>Nayak &amp; Sen, 1991</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>Scutellar callus of immature zygotic embryos</td>
<td>—</td>
<td>Nambisan &amp; Chopra, 1992</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>callus</td>
<td>—</td>
<td>Basu <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>Mature embryos</td>
<td>—</td>
<td>Shankdher <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Pulses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick pea</td>
<td>Cotyledons and immature embryo axes</td>
<td>—</td>
<td>Sagare <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Chick pea</td>
<td>Embryo axes</td>
<td>—</td>
<td>Suhasini <em>et al.</em>, 1994</td>
</tr>
<tr>
<td>Pigeon pea</td>
<td>Cotyledon and leaf explants</td>
<td>—</td>
<td>Sreenivasu <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>Chick pea</td>
<td>Leaves</td>
<td>—</td>
<td>Kumar <em>et al.</em>, 1994</td>
</tr>
<tr>
<td>Vigna radiata</td>
<td>Immature cotyledons</td>
<td>—</td>
<td>Giri <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><strong>Commercial crops</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bamboo</td>
<td>Seeds</td>
<td>—</td>
<td>Nadgauda <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Bamboo</td>
<td>Nodal segments</td>
<td>—</td>
<td>Godbole, 2002</td>
</tr>
<tr>
<td>Hevia brasiliensis</td>
<td>Immature inflorescence</td>
<td>—</td>
<td>Kumari <em>et al.</em>, 2000</td>
</tr>
<tr>
<td>Anacardium occidentale</td>
<td>Radicle end of immature zygotic embryos</td>
<td>—</td>
<td>Cardoza <em>et al.</em>, 2000</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Cotyledons</td>
<td>—</td>
<td>Jha <em>et al.</em>, 1992</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>Cotyledons</td>
<td>—</td>
<td>Mondal <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>Cotyledons</td>
<td>—</td>
<td>Mondal <em>et al.</em>, 2001</td>
</tr>
</tbody>
</table>

(Contd)
Conclusion

While most of the genes that control polarity establishment, tissue differentiation and elaboration of patterns during embryo development have been identified and characterized, the regulatory mechanisms which co-ordinate the asymmetrical division and subsequent determination is still unknown. The only exception is the regulatory gene, shoot-meristemless.

However, investigations on the developmental basis of a number of mutant phenotypes have enabled the identification of gene activities promoting asymmetric cell division and polarization leading to heterogeneous partitioning of the cytoplasmic determinants necessary for the initiation of embryogenesis e.g. gnom.

It can thus be concluded that, mutants have now enabled one to predict not only the direct or indirect induction potential of somatic embryogenesis in different plants and explants (Ogas et al., 1997; Dupire et al., 1999; Limanton et al., 2000) but also on whether there would be a potential for recurrent embryogenesis (Recklinghausen et al., 2000). These studies are indeed valuable in manipulating cells and tissues of non-embryogenic nature to embryogenic types. Identification of a super-embryogenic mutant, Jemalong 2HA is specially valuable for providing insights into the nature of totipotency in plants (Rose et al., 1999).

References


Table I—Work done by Indian workers on somatic embryogenesis in the different plant species in the last ten years—Contd

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Explants used</th>
<th>Mutant if any</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Camellia sinensis</em></td>
<td>Cotyledons</td>
<td>—</td>
<td>Mondal et al, 2002</td>
</tr>
<tr>
<td><em>Coffea arabica</em></td>
<td>Cultured roots and hypocotyl segments</td>
<td>—</td>
<td>Santa et al, 1999</td>
</tr>
<tr>
<td>Cotton</td>
<td>Hypocotyl</td>
<td>—</td>
<td>Kumar &amp; Pental, 1998</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>Mangifera indica</em></td>
<td>Protoplasts</td>
<td>—</td>
<td>Ara et al, 2000</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>Nucellus</td>
<td>—</td>
<td>Ara et al, 2000</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Eggplant</td>
<td>Cotyledons, leaves and hypocotyls</td>
<td>—</td>
<td>Sharma et al, 1995</td>
</tr>
<tr>
<td>Tomato</td>
<td>Leaves and shoots</td>
<td>—</td>
<td>Chandel &amp; Katiyar, 2000</td>
</tr>
</tbody>
</table>


