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Genes Involved in *Phaseolus* Embryogenesis

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ABSTRACT

Within the genus *Phaseolus*, the common bean, *Phaseolus vulgaris* L., is the most important species widely distributed in the world and occupies more than 90% of production areas sown to *Phaseolus* species. Interspecific hybridization in the genus *Phaseolus*, with the aim to introgress desired traits to the common bean, leads to the abortion of immature embryos usually at the globular or early heart-shaped developmental stages. Plant zygotic embryogenesis is controlled by many genes and malfunction of these genes can disrupt embryo formation. In this paper, we reviewed some of these genes i.e. *KNOX*, *BELL1*, *LEUCINE ZIPPER*, *PHD-FINGER*, *GLABRA2*, *WUSHEL*, *HEAT SHOCK PROTEIN*, *LIPID TRANSFER PROTEIN*, *PASTICCINO*, *LEAFY COTYLEDON* and *TITAN*, from model plants such as *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa*, *Medicago truncatula*, *Solanum lycopersicum*. This study helps us to identify the genes involved in *Phaseolus* embryogenesis and to verify their expression in ovules at different steps of embryos development.

Keywords: common bean, embryo abortion, embryo development, genes, model plants

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INTRODUCTION

The common bean, *Phaseolus vulgaris* L., is the most important species widely distributed in the world and occupies more than 90% of production areas sown to *Phaseolus* species (Baudoin *et al*. 2001). Its dry seeds are a major component in the diet of human populations in Latin America, as well in Central and East Africa. Legumes usefully complement cereal seeds in the nutritional value of daily diet, mainly because of their protein and amino acid contents. *P. vulgaris* is adapted to several cropping systems, particularly in association with other food crops like maize, sorghum or pearl millet. In tropical regions, the common bean is characterized by low and unstable seed yield. This is mainly due to the susceptibility of this crop to numerous pests and diseases: more than 200 pathogens have been reported attacking beans, some of them causing considerable economic losses (Graham and Ranalli 1997; Singh 1999). Another constraint limiting yield is the lack of improved varieties tolerant to abiotic stresses (poor soil, high temperature, drought, etc.). Agronomists and breeders have not found sufficient genetic variability within the primary gene pool of *P. vulgaris*. However, the alien gene pools offer very good breeding potentialities (Debouck and Smartt 1995; Debouck 1999; Baudoin 2001; Broughton *et al*. 2003). This is particularly the case of the two species *P. coccineus* L. and *P. polyanthus* Greenm., which show interesting traits not, or only poorly, expressed in the primary gene pool of *P. vulgaris*, such as resistance to two diseases Ascochyta blight and *Bean Golden Yellow Mosaic Virus* (BGYMV). Ascochyta blight is caused by *Phoma exigua* Desmaz. var. *diversispora* (Bubak) Boerema and prevails in highland areas. The symptoms include black or brown concentric lesions on leaves and pods, as well as collapsed and black nodes, petioles, and stems (Schwartz *et al*. 1981; Hanson *et al*. 1993). BGYMV is economically important in Latin America, and is caused by a geminivirus transmitted by whiteflies of the genus *Bemisia*. Disease symptoms include intense yellowing, mosaic, stunting, and distortion of leaves and pods (Markham *et al*. 1994; Bracero *et al*. 2003). The extensive genetic variability of the pathogens requires the constant development of new resistant cultivars in common

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bean. Breeders take benefit of the interspecific hybridizations between the recurrent species, *P. vulgaris*, and the two donor species: *P. coccineus* and *P. polyanthus*, belonging to the secondary gene pool of the common bean. Indeed, different sources of resistance to these two diseases have been identified among these two donor species (Hanson *et al.* 1993; Bianchini 1999; Beaver *et al.* 2005). In addition, other desired traits can be found from these two taxa, such as cold tolerance, long multi-nodded inflorescences, and thick stems (Camarena 1988; Schmit and Baudoin 1992; Baudoin *et al.* 2001).

In the interspecific hybridizations between *P. vulgaris* and the two donor species, the use of *P. vulgaris* as a female parent increases the percentage of successful hybrids but, unfortunately, the presence of *P. vulgaris* cytoplasm provokes a quick reversal to the recurrent species, at the expense of the donor species (Baudoin 2001). Reciprocal crosses, a condition favouring the introgression of desired genes to the common bean, avoid the reversal process showed above, but are more difficult to succeed. Indeed, theses crosses lead to abortion of immature embryos, usually at the globular or early heart-shaped developmental stages, with most embryos aborting 3-8 days after pollination, 79% for the crosses *P. polyanthus* × *P. vulgaris* and 84% for the crosses *P. coccineus* × *P. vulgaris* during trials conducted under greenhouse in Gembloux Agricultural University (Baudoin *et al.* 2004). Early interspecific embryo abortion is attributed to several factors such as nutritional barriers related to a deficient endosperm or suspensor development, endothelium proliferation and, in some extent, hypertrophy of the vascular elements (Lecomte 1997; Geerts 2001; Toussaint *et al.* 2004). Plant zygotic embryogenesis is controlled by many genes and their malfunction can disrupt interspecific embryos formation. Transcripts of these genes can be localized in the embryo proper, in endosperm or in maternal tissues around the embryo (Raghavan 1997).

In this paper, we review some of these genes involved in model plants embryogenesis, such as *KNOX*, *BELL1* (*BEL*), *LEUCINE ZIPPER* (*HD-ZIP*), *PHD-FINGER*, *GLABRA2* (*HD-GL2*), *WUSHEL* (*WUS*), *HEAT SHOCK PROTEIN* (*HSP*), *LIPID TRANSFER PROTEIN* (*LTP*), PASTICCINO (PAS), *LEAFY COTYLEDON* (*LEC*) and *TITAN* (*TTN*) genes. Alignment and comparison of the sequences from different species help us to design DNA primers targeting conserved domains of these genes, to isolate related genomic sequences from *Phaseolus* by PCR, and to verify the expression of these genes at different steps of *Phaseolus* embryos development.

PLANT EMBRYOGENESIS AND GENE EXPRESSION

Fertilization and embryogenesis are the first stages in the development of new life in both animals and plants. In early dicot embryogenesis, cell division occurs regularly. The first transverse division is asymmetric, which gives rise to one smaller apical cell and one larger basal cell. The smaller apical cell will form most of the embryo proper, and the larger basal cell will develop into the suspensor. The suspensor anchors the embryo to the endosperm and serves as a conduit for nutrients and growth factors for the developing embryo. The smaller apical cell (the embryo proper) divides longitudinally twice and transversely once, and results in an eight-cell embryo (Goldberg *et al.* 1994; Laux and Jürgens 1997; Rademacher and Weijers 2007). Early embryogenesis in monocots differs from that of dicots mainly in the two following ways: first, cell division occurs randomly; and second, the protodermal cell layer is not morphologically formed until the embryo size reaches more than 100 mm in length (more than 200 cells). This means that the period leading up to organ differentiation, including protoderm differentiation, is much longer than that in dicots. Further cell divisions lead to the globular stage. The three basic tissue systems (dermal, ground, and vascular) can be recognized at this point based on characteristic cell division patterns. The globular shape of the embryo is then lost as the cotyledons (embryonic leaves) begin to form. The formation of two cotyledons in dicots gives the embryo a heart-shaped appearance, while in monocots only a single cotyledon forms. Upright cotyledons can give the embryo a torpedo shape, and at this time the suspensor is degenerating while the shoot apical meristem and root apical meristem (SAM and RAM) are established. At this stage, embryogenesis is arrested, the mature seed dessicates and remains dormant until germination. The meristems will give rise to the adult structures of the plant upon germination (Steeves and Sussex 1989; Harada 1997; Laux and Jürgens 1997; Weijers and Jürgens 2005).

In higher plants the embryo passes through four developmental stages after fertilization (Umehara *et al.* 2007). The globular stage is the pattern formation, in which the axis of the plant body is defined, tissue layers organized, and earliest organs established. The heart stage is the cell diversification and specification phase, in which cell types such as the suspensor, provascular tissue, shoot and root meristems are defined. The torpedo stage is growth and morphogenesis, in which cells become expanded. During the last process, the embryo enters into maturation, in which cell division is completed, embryo storage reserves as proteins, starch, and lipids accumulate and the embryo acquires dormancy and desiccation. The first three stages occur concurrently in the developing embryo and are also known as early embryogenesis, while maturation is a distinct process that begins later in embryogenesis (West and Harada 1993; Harada 1997; Berleth and Chatfield 2002). The plant body is the basis of the future plant, controls the three next phases and consists of two superimposed patterns: apical-basal pattern along the main axis of the plant, and radial pattern of concentrically arranged tissue systems.

Seed development in higher plants such as *Arabidopsis* requires coordinated differentiation of the embryo proper, suspensor, endosperm tissue, and seed coat. Plant embryos are morphologically simple, but molecularly complex. Many genes must be expressed as the zygote divides in a regulated manner, completes morphogenesis, and differentiates into a mature embryo capable of surviving desiccation and producing a viable plant (McElver *et al.* 2001). Interactions between these components have been explored in part through the analysis of embryo-defective mutants in *Arabidopsis* (Meinke 1995). These recessive mutants have been recovered either by screening immature siliques for abnormal seeds (Meinke 1994) or by screening at the seedling stage for defects indicative of a disruption of normal embryogenesis (Jürgens *et al.* 1994) or through forward genetic screens of T-DNA insertion lines (McElver *et al.* 2001). These genes show temporal as well as spatial patterns of gene expression during seed development. The stage-specific cDNAs have been used as markers of cell differentiation and to follow the normal development of the embryos (Jofuku and Goldberg 1989; Elster *et al.* 2000; Bommert and Werr 2001). Gene products identified to date include a wide array of metabolic enzymes (Lukowitz *et al.* 2001), transcription factors (Lotan *et al.* 1998), chloroplast and mitochondrial proteins (Apuya *et al.* 2001), and proteins required for vesicle trafficking (Rojo *et al.* 2001). From the frequency at which embryo-lethal mutants appear in *Arabidopsis*, many genes are required for normal embryogenesis; however, only ten percent of them affect pattern formation in embryogenesis. A vast number of genes represented by embryo-lethal mutants may be required for normal embryogenesis, but they may not be master regulators of the process (Howell 1998). However Franzmann *et al.* (1995) and McElver *et al.* (2001) identified and analyzed a large number of embryo-lethal mutants in *Arabidopsis*. They estimated that 750 genes can be easily mutated to give an embryo lethal phenotype. On the other side, Tzafrir *et al.* (2004) showed that 250 genes control distinct phenotypes and are essential to have a normal phenotype during *Arabidopsis* seed development.

In the next sections, some of these genes involved in

plant embryogenesis are discussed. Focus is made on their characteristics, roles and expression patterns during plant embryogenesis, as well on their relationships.

HOMEOBOX GENES

Homeotic genes are important regulatory genes in the speciation of cell fate and body plan at the early stage of embryogenesis in higher organisms (Gehring and Hiromi 1986; Yang *et al.* 2002). Initially identified in *Drosophilia* (McGinnis *et al.* 1984) as a single gene mutation capable of altering the identity of complex morphological structures, homeotic genes have been isolated and characterized from a wide range of animal and plant species (Chasan 1992; Gehring 1992). A common feature of many homeotic genes of both plants and animals is a conserved nucleotide sequence known as the homeobox which encodes the homeodomain (HD). The HD consists of an approximately 60 amino acids with a helix-turn-helix structure conferring a specific DNA binding function (Kissinger *et al.* 1990).

Plant homeoboxes were discovered at the beginning of the nineties, and the first family of homeobox genes reported in plant species was *KNOX* (*KNOTTED1*-like homeobox), isolated from maize, which is involved in leaf development (Vollbercht *et al.* 1991). Subsequent to the cloning of the *KN1* gene from maize, using library screening with previously identified gene or degenerate olinucleotides deduced from HDs as probes or primers, differential screening, mutant based cloning, etc., many plant homeobox genes have been isolated from various plant species: *Arabidopsis* (Carabelli *et al.* 1993; Söderman *et al.* 1999; Western and Haughn 1999; Hanson *et al.* 2001; Johannesson *et al.* 2001), parsley (Korfhage *et al.* 1994), rice (Sato *et al.* 1996; Sato *et al.* 1998; Postma-Haarsma *et al.* 1999; Sentoku *et al.* 1999; Ito *et al.* 2002; Yang *et al.* 2002), apple (Watillon *et al.* 1997; Dong *et al.* 2000), wheat (Takumi *et al.* 2000), soybean (Ma *et al.* 1994; Moon *et al.* 1996), tomato (Tornero *et al.* 1996), tobacco (Nishimura *et al.* 1999, 2000), Norway spruce (Sundas-Larsson *et al.* 1998; Hjortswang *et al.* 2002; Ingouff *et al.* 2003), *Phaleonopsis* (Nadeau *et al.* 1996).

Based on amino acid sequence similarities within the homeodomain and the presence of additional distinctive domains outside the HD, plant homeobox genes can be grouped into several families (Chan *et al.* 1998; Ito *et al.* 2002a): the homeodomain *LEUCINE ZIPPER* (*HD-ZIP*) family characterized by a leucine zipper dimerization motif adjacent to the homeodomain (Söderman *et al.* 1999; Tang *et al.* 2001), the plant homeodomain *FINGER* (*PHD-FINGER*) family distinguished by a conserved cysteine-rich motif (Überlacker *et al.* 1996; Yang *et al.* 2003), the family of *Arabidopsis GLABRA2*-like (*HD-GL2*, known as *HD-ZIP IV*) proteins (Lu *et al.* 1996; Ito *et al.* 2002b), the family of maize *KNOX* proteins (Vollbercht *et al.* 1991; Bowman and Eshed 2000), the family of *BELL1* (*BEL*) HD protein (Dong *et al.* 2000; Becker *et al.* 2002), and the family of *WUSHEL* (*WUS*) HD protein (Brand *et al.* 2002; Haecker *et al.* 2004). Within each family, several defined groups can be distinguished, each comprising proteins from different species (including monocot and dicot).

The involvement of homeobox genes in plant embryogenesis has been reported in several studies (Schena and Davis 1992; Ma *et al.* 1994; Sato *et al.* 1996; Ito *et al.* 2002a, 2002b; Haecker *et al.* 2004). In order to follow the normal development of the embryo proper, such genes are often used as molecular markers to study their cellular expression pattern during embryogenesis in wild-type and mutant embryos, using different techniques such as *in situ* hybridization, cDNA libraries screening, southern and northern blots analysis, RT-PCR reactions. Plant homeobox genes, by analogy of the functional roles of animal homeobox genes, have been expected to encode transcriptional regulators that mediate important developmental processes during embryogenesis (Sato *et al.* 1998; Dong *et al.* 2000; Elster *et al.* 2000; Bommert and Werr 2001; Yang *et al.* 2002).

KNOX genes

As specified above, *KNOX* genes have been the first homeobox gene family identified in plant species and is the most extensively characterized gene family. These genes have been shown to be involved in meristem formation and maintenance in maize, rice and barley among the monocots (Kerstetter *et al.* 1997; Bowman and Eshed 2000). Studies revealed that the shoot apical meristem (SAM) is formed during embryogenesis, and after seed germination it continuously generates various organs and tissues, such as leaves, stems and flowers, throughout plant life (Steeves and Sussex 1989; Vollbrecht *et al.* 2000; Hake *et al.* 2004; Belles-Boix *et al.* 2006). Based on comparative analysis of the *KNOX* HD, this family has been subdivided into class 1 and class 2 which are 73-89% and 55-58% identical to maize *KN1* respectively. **Fig. 1** shows the alignment of deduced amino acid sequences of *KNOX* genes from maize (Vollbrecht *et al.* 1991), pea (Hofer *et al.* 2001), rice (Matsuoka *et al.* 1993), *Medicago truncatula* (Koltai *et al.* 2001), soybean (Ma *et al.* 1994) and *Arabidopsis thaliana* (Lincoln *et al.* 1994). The class 1 genes studied are mainly expressed in the SAM but not in lateral primordia, and some loss-of-function mutations affect meristem formation and/or maintenance. Two locations of expression patterns of the class 1 genes in the SAM have been identified: at the center of the meristem dome and at the base of leaf primordia (Reiser *et al.* 2000). Furthermore, all class 1 genes analyzed caused dramatic alteration of leaf morphology when ectopically expressed (Hake *et al.* 1995; Long *et al.* 1996). These results indicate that the class 1 genes play important roles in SAM. In contrast, the class 2 genes studied show more diverse expressions and are found not only in the SAM but also in differentiated organs such as roots, leaves and flowers. Ectopic expression of class 2 genes does not cause altered morphology (Serikawa *et al.* 1996, 1997; Sentoku *et al.* 1998).

The first plant homeobox gene shown to be involved in embryogenesis was *SHOOT MERISTEMLESS* (*STM*) isolated from *Arabidopsis thaliana* (Long *et al.* 1996; Groß-Hardt and Laux 2003). Embryos of *stm* mutants lack the capacity to form a shoot meristem, whereas other embryonic organs, for example cotyledons, hypocotyl and radicle, develop normally. *STM* belongs to class 1 of the *KNOX* gene familly and is expressed in the SAM during embryogenesis (Tsiantis and Hay 2003; Hay *et al.* 2004; Belles-Boix *et al.* 2006). The *KNOX* gene *SOYBEAN HOMEOBOX1* (*SBH1*) is expressed during soybean somatic embryogenesis (Ma *et al.* 1994). The deduced *SBH1* protein shares a high amino acid identity with maize *KN1* protein (47.00% overall and 87.50% for the homeodomain). The expression of *SBH1* is development- and tissue-specific. The transcript of *SBH1* was present in early-stage somatic embryos, increased prior to cotyledon formation and decreased thereafter. *SBH1* was weakly expressed in soybean stems and hypocotyls but was not detected in other plant tissues and nonembryogenic materials. In rice, members of the *KNOX* gene family are differentially expressed during embryogenesis. Sato *et al.* (1996) examined the spatial and temporal expression patterns of rice homeobox gene, *OSH1*, during rice embryogenesis. They showed that *OSH1* expression was first detected in a specific ventral region of globular embryo. This indicates that the cellular differentiation at the gene expression level has already occurred at this stage and it is plausible that *OSH1* may play an important role in the cellular differentiation preceeding organ formation. Considering the possibility that the putative *in vivo* function of *OSH1* is a *trans*-acting factor, *OSH1* may function as a regulator switching on and off the developmental program of embryonic cells located in a specific region. With the advancement of embryonic maturation, the expression level of *OSH1* is reduced. The down-regulation of *OSH1* expression at later stages suggests that its primary function resides in the early embryogenesis. This result also suggests a possibility that the main function of *OSH1* in embryo is to establish cellular identity in the ventral region at the globular stage. Once the

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M_trunc   : NSTNNNNNHYTNC--NNNTSSIMLQNNH---QNTPGLGYYFMD---------NINNHGSSSSSSSSSVKSKIMAHPHYHRL : 137
P_sat     : NSTNNNN-HYTN---NNNSNNTMLQNNHHTTQNTPGLGYYFMD---------NHNNNGASSSSSSSAVKAKIMAHPHYHRL : 127
G_max     : NSTGTPSIMLHN---NHNNNKTDDDDNN----NNTGLGYYFMESDHHHHHGNNNNNGSSSSSSSSAVKAKIMAHPHYHRL : 137
O_sat     : LTLNTAAAA-------VGNPVLQLANG--------SLLDACGKAK-------EASASAS-YAPDVEAIKAKIISHPHYSSL : 117
Z_mays    : LTLNTVAATGNS--GGSGNPVLQLANGG-------GLLDACVKAK-------EPSSSSP-YAGDVEAIKAKIISHPHYYSL : 117
A_thal    : QTTENCFRSDHDQPNNNNNPSVKSEASSSRINHYSMLMRAIHNTQ-------EANNNNNDNVSDVEAMKAKIIAHPHYSTL : 147

M_trunc   : LEAYINCQKVGAPSEVVARLEEACATAVRMGG--DAVGSGCLGEDPALDQFMEAYCEMLIKYEQELSKPLKEAMLFLQRIE : 216
P_sat     : LEAYINCQKVGAPSEVVTRLEEACASAVRMGG--DAVGSGCIGEDPALDQFMEAYCEMLIKYEQELSKPLKEAMLFLQRIE : 206
G_max     : LAAYVNCQKVGAPPEVVARLEEACASAATMAGGDAAAGSSCIGEDPALDQFMEAYCEMLTKYEQELSKPLKEAMLFLQRIE : 218
O_sat     : LAAYLDCQKVGAPPEVAARLT-AVAQDLELR-QRTALGVLGAATEPELDQFMEAYHEMLVKYREELTRPLQEAMEFLRRVE : 196
Z_mays    : LTAYLECNKVGAPPEVSARLT-EIAQEVEAR-QRTALGGLAAATEPELDQFMEAYHEMLVKFREELTRPLQEAMEFMRRVE : 196
A_thal    : LQAYLDCQKIGAPPDVVDRIT-AARQDFEARQQRSTPSVSASSRDPELDQFMEAYCDMLVKYREELTRPIQEAMEFIRRIE : 227
                                             KNOX Domain

M_trunc   : VQFKNLTVSSSSDNIACSEGGDRNGSSEEDHVDLYNN-----MIDPQAEDRELKGQLLRKYSGYLGSLKQEFMKKRKKGKL : 292
P_sat     : VQFKNLTVSSSSDNIACNEGGDRNGSSEEDQVDLYNN-----MIDPQAEDRELKGQLLRKYSGYLGSLKQEFMKKRKKGKL : 282
G_max     : CQFKNLTISSS--DFASNEGGDRNGSSEED-VDLHN------MIDPQAEDRDLKGQLLRKYSGYLGSLKQEFMKKRKKGKL : 290
O_sat     : TQLNTLSISGRSLRNILSS-----GSSEEDQEG-SGGETELPEIDAHGVDQELKHHLLKKYSGYLSSLKQELSKKKKKGKL : 271
Z_mays    : SQLNSLSISGRSLRNILSS-----GSSEEDQEG-SGGETELPEVDAHGVDQELKHHLLKKYSGYLSSLKQELSKKKKKGKL : 271
A_thal    : SQLSMLCQSPIHILNNPDGKSDNMGSSDEEQENNSGGETELPEIDPRAEDRELKNHLLKKYSGYLSSLKQELSKKKKKGKL : 308
                                                                 ELK Domain

M_trunc   : PKEARQQLLEWWSRHYKWPYPSESQKLALAESTGLDQKQINNWFINQRKRHWKPSEDMQFVVMDPSHPHYYMDNVLTNSYP : 373
P_sat     : PKEARQQLLEWWSRHYKWPYPSESQKLALAESTGLDQKQINNWFINQRKRHWKPSEDMQFVVMDPTHPHYYMDNVLTNSFP : 363
G_max     : PKEARQQLLEWWNRHYKWPYPSESQKLALAESTGLDQKQINNWFINQRKRHWKPSEDMQFVVMDPSHPHYYMDNVLGNPFP : 371
O_sat     : PKDARQQLLNWWELHYKWPYPSESQKVALAESTGLDLKQINNWFINQRKRHWKPSDEMQFVMMDGYHPTNAAAFYMDGHFI : 352
Z_mays    : PKEARQQLLSWWDQHYKWPYPSETQKVALAESTGLDLKQINNWFINQRKRHWKPSEEMHHLMMDGYHTTN--AFYMDGHFI : 350
A_thal    : PKEARQKLLTWWELHYKWPYPSESEKVALAESTGLDQKQINNWFINQRKRHWKPSEDMQFMVMDGLQHPHHAALYMDGHYM : 389
                          HomeoDomain

M_trunc   : MDLSNTML~~ : 381
P_sat     : MDLSNTML~~ : 371
G_max     : MDLSHPML~~ : 379
O_sat     : NDGGLYRLG~ : 361
Z_mays    : NDGGLYRLG~ : 359
A_thal    : GDG-PYRLGP : 398
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Fig. 1 Alignment of deduced amino acid sequences of KNOX genes of *Zea mays* (Z_mays, KN1 X61308), *Pisum sativum* (P_sat, AF080104), *Oryza sativa* (O_sat, D16507), *Medicago truncatula* (M_trunc, AF308454), *Glycine max* (G_max, L13663) and *Arabidopsis thaliana* (A_thal, ATU14174).

ventral identity is established then, the organogenesis is started; *OSH1* may finish its function early during embryogenesis with a decrease in its expression level. Sentoku *et al.* (1999) showed that all or some of the rice *OSH* homeobox genes may be involved in regionalization of the shoot area and/or the establishment of the SAM itself before shoot formation occurring early in embryogenesis. After shoot formation, however, the functions of the homeobox genes appear to differ. Some of these genes may maintain SAM activity in an indeterminate condition through continuous expression in the SAM, whereas others may be involved in pattern formation of the segmental units of the plant body and/or internodes development. Pre-patterning of specific cells by the expression of homeobox genes before morphological organ formation is the same as in organ formation during animal embryogenesis. Even though the body structures of plants and animals are quite different, there may be some common mechanisms in organ establishment and development in terms of homeobox function. Comparing the pattern expression of *KNOX* (*KN1*) gene in maize wild-type and mutant embryos, Bommert & Werr (2001) detected no *KN1* transcripts in *emb**-8518 and *emb**-8521 embryos between 15 and 30 DAP. *KN1* amplicons are detected in wild-type embryos, but are missing in the two mutants. These results revealed that both mutants are arrested before establishment of a functional SAM. Recently, Belmonte *et al.* (2007) pointed out that *HBK3*, a *KNOX* class I gene, improves the development of Norway spruce (*Picea abies*) somatic embryos. Indeed, in lines overexpressing *HBK3* (*HBK3-S*), somatic immature embryos showed enlarged embryogenic heads and were able to produce fully developed cotyledonary embryos at higher frequency. Furthermore, *HBK3-S* embryos had enlarged shoot apical meristems (SAMs). Lines in which *HBK3* was down-regulated (*HBK3-A*) had reduced ability to produce immature somatic embryos and were not able to complete the whole maturation process. Overall, these data confirm the importance of *KNOX* genes during development.

BELL1 (*BEL*) genes

The BEL family is another plant homeobox gene family that is closely related to the *KNOX* genes (Bürglin 1997; Chan *et al.* 1998; Becker *et al.* 2002). *BEL* transcription factors are essential for inflorescence and fruit development (Dong *et al.* 2000; Byrne *et al.* 2003; Roeder *et al.* 2003; Smith and Hake 2003; Bao *et al.* 2004; Bhatt *et al.* 2004; Smith *et al.* 2004). Based on genetic studies, an *Arabidopsis BEL* gene *PENNYWISE* (*PNY*) is involved in regulating early internode patterning events and is also necessary for replum development during fruit maturation (Byrne *et al.* 2003; Roeder *et al.* 2003; Smith and Hake 2003; Bao *et al.* 2004; Bhatt *et al.* 2004; Kanrar *et al.* 2006). Genetic analysis of *BEL* in *Arabidopsis* showed that expression of this transcription factor regulated the development of ovule integument. The loss-of-function phenotype of *bel* mutant ovules indicates that determination of integument initiation and organ identity is controlled by expression of the *BEL* homeobox gene. *BEL* appears to interpret positional information and control morphogenesis of the integuments through the regulation of genes within the chalazal domain (Robinson-Beers *et al.* 1992). In *bel* mutant ovules, the inner integument fails to form, the outer integument develops abnormally, and the embryo sac arrests at a late stage of

megagametogenesis. During later stages of ovule development, cells of the outer integument of a *bel* ovule sometimes develop into a carpel-like structure with stigmatic papillae and second-order ovules. The frequency of carpel-like structures was highest when plants were grown under conditions that normally induced flowering and was correlated with ectopic expression in the ovule of *AGAMOUS* (*AG*), an organ-identity gene required for carpel formation (Modrusan *et al*. 1994). The *BEL* gene family was identified in several plants such as tomato (Ron *et al*. 2001, Direct Submission AF375964), potato (Chen *et al*. 2003), *Arabidopsis* (Reiser *et al*. 1995), apple (Dong *et al*. 2000), barley (Muller *et al*. 2001) and *Gnetum gnemon* (Becker *et al*. 2002).

LEUCINE ZIPPER (*HD-ZIP*) genes

HD-ZIP protein can be divided into three classes by their sequence similarity of the HD and the presence or absence of some motifs outside of the HD. The functions of the *HD-ZIP* proteins have been investigated and studies have revealed that these proteins do not have a common function, even in the same subclass. The class I and class II proteins are primarily involved in signal transduction pathways in response to various environmental stimuli (Hanson *et al*. 2001; Ito *et al*. 2002). The class III proteins play a role in initiation and function of the shoot apical meristem (SAM) as well as in initiation of axillary SAMs and their functions appear to be critical for the formation of an embryonic SAM (Emery *et al*. 2003; Prigge *et al*. 2005). In relation with their role in SAM establishment, *HD-ZIP* class III proteins are initially expressed throughout the proembryo but their expression later becomes restricted to a central apical position (Emery *et al*. 2003; Prigge *et al*. 2005; Floyd *et al*. 2006).

PHD-FINGER genes

The *PHD-FINGER* homeobox genes encode proteins with the zinc-finger domain, named the PHD-FINGER domain, a cysteine-rich region in the N-terminal region of the homeodomain. The HD is at C-terminal region, as in the case of *KNOX* genes (Ito *et al*. 2002; Ito *et al*. 2004). Some proteins of this family have two homeodomains together with the PHD-FINGER domain. In maize, *PHD-FINGER* genes are mainly expressed in meristematic cells and are restricted to the early developmental stage of embryogenesis (Klinge *et al*. 1996; Ito *et al*. 2002). Deletion of the PHD-FINGER domain from the *PHD-FINGER* protein of *Arabidopsis* causes severe disturbances in DNA binding (Schindler *et al*. 1993). Overexpression of *ZMHOX1*, a maize *PHD-FINGER* gene, in tobacco lead to several developmental defects such as dwarfism, adventitious shoot formation (due to the loss of apical dominance), and homeotic transformations of floral organs (Uberlacker *et al*. 1996). *PHD-FINGER* proteins are thought to function in certain developmental process in maize by controlling expression of target genes (Comelli *et al*. 1999; Ito *et al*. 2004). Ito *et al*. (2004) showed that *HAZ1*, a rice *PHD-FINGER* protein isolate from three days old embryos, was similar in its entire amino acid sequence to *ZMHOX1*a (52% identity) and *ZMHOX1*b (50% identity), *PHD-FINGER* proteins of maize. But overexpression of *HAZ1* does not affect the phenotype either in tobacco or in rice (Ito *et al*. 2004). These authors also demonstrated that *HAZ1* was expressed at a higher level in the outer layers of a developing globular embryo (3 DAP) than in the inner parts. At 4 and 5 DAPs, the expression of *HAZ1* was concentrated at the ventral part of an embryo. Recently, it was proved that *Arabidopsis* contain 14 members of this family gene. All members of this family are expressed predominantly or exclusively in floral tissue, indicating a likely regulatory role during floral development (Tan and Irish 2006).

GLABRA2 (*HD-GL2*) genes

The analysis of the *HD-GL2* protein region adjacent to the homeodomain reveals the presence of a truncated leucine zipper-like segment at the same position as the *HD-ZIP* proteins. Due to these similarities, *GLABRA2*-like proteins are sometimes included in class IV of the HD-ZIP family (Di Cristina *et al*. 1996), while others have considered it as a separate family, since its components have characteristic features that differentiate them from the HD-ZIP family (Lu *et al*. 1996; Palena *et al*. 1997; Chan *et al*. 1998). This homeobox gene family encodes a protein required for normal trichome and root hair development. From the fertilization to the eight cell stage of embryogenesis, the *Arabidopsis ATML1* gene, which is a member of *HD-GL2* homeobox gene family, is expressed uniformly in the embryo proper. The expression of *ATML1* is then restricted to the outer cell layer belonging to the protoderm (Lu *et al*. 1996). Based on this expression pattern, *ATML1* is considered to be a good molecular marker for the protoderm and also for radial pattern formations. Since protoderm differentiation takes place at a very early stage (16- to 32-cell stage), it appears that the radial pattern formation, the protoderm differentiation and the protoderm-specific *ATML1* mRNA accumulation occur simultaneously in *Arabidopsis* embryogenesis. The same observations were shown in rice with *ROC1*, a GL2-type homeobox gene (Rice outermost cell-specific gene1). *ROC1* is specifically expressed in the protoderm (epidermis) and this expression is established shortly after fertilization, much earlier than protoderm differentiation (Yang *et al*. 2002). The maize *HD-GL2* genes *ZMOCL1*, *ZMOCL3*, *ZMOCL4* and *ZMOCL5* essentially exhibit the L1/protoderm/epidermis-specific expression patterns during early embryogenesis and in meristematic regions or young organ primordia at later stages (Ingram *et al*. 1999; Ingram *et al*. 2000). These observations show that the transcripts of *HD-GL2* genes are restricted to epidermal tissues or their precursor cells (Yang *et al*. 2002). The *GLABRA2* may represent a unique family of homeobox genes that specialize in control of lateral patterning in embryos and maintaining cell-layer identity in meristematic regions (Chan *et al*. 1998). *GLABRA2* gene was also involved in the control of seed oil accumulation in *Arabidopsis* (Shen *et al*. 2006).

WUSHEL (*WUS*) protein

A *WUS* gene was first characterized in *Arabidopsis* by Laux *et al*. (1996). The homeodomain of *WUS* gene has two and four additional amino acids in the loop between helix I and helix II and the turn between helix II and helix III, respectively. *WUS* proteins were initially considered to play important roles in the speciation of the stem cells in the shoot apical meristem of developing embryos (Laux *et al*. 1996; Hamada *et al*. 2000; Kieffer *et al*. 2006). In the mature embryo of *Arabidopsis wus* mutant, shoot apical meristem organization is aberrant with only a few vacuolated cells. After germination, the shoot apical meristem is terminated prematurely as a flat enlarged apex. In the shoot apical meristem of the *wus* mutant, the stem cells appear to undergo differentiation, instead of maintaining a pluripotant state in the functional shoot apical meristem (Laux *et al*. 1996). Further studies showed also that these genes were involved in the specification and the maintenance of the stem cells in the root apical meristem, by a mechanism similar to that in the shoot apical meristem (Kamiya *et al*. 2003; Haecker *et al*. 2004). The rice *WUS* gene was found to be specifically expressed in the central cells of the root. During embryogenesis and crown root formation, rice *WUS* gene expression is observed prior to the morphological differentiation of the root. This expression pattern was different between radicle and crown root formation during the process of the root apical meristem development (Kamiya *et al*. 2003).

HEAT SHOCK PROTEIN GENES

The *HEAT SHOCK RESPONSE* (*HSR*) is an evolutionarily conserved reaction to elevated temperatures (heat shock or heat stress) that is essential to the survival of eukaryotic cells and organisms. Indeed severe heat stress induces alterations in the conformation of cellular proteins, and leads to protein denaturation or aggregation and cell death. In response to increased temperature, the transcription and translation of many cellular proteins are repressed or arrested, whereas the expression of a small subset of specialized *HEAT SHOCK PROTEINS* (*HSPs*) is increased preferentially. The *HSPs* are molecular chaperonins that regulate protein homeostasis and membrane fluidity and ultimately prevent or delay cell death during heat stress. Most plant tissues and cells are competent to induce the HSR during thermal stress. However, two stages in the plant life cycle, pollen germination and early embryogenesis (i.e. before cotyledon formation), are notable for their inability to invoke the full *HSR*. As a result, these tissues are especially sensitive to thermal stress (Schöffl *et al*. 1998; Fu *et al*. 2002). The *HSPs* are usually divided into high-molecular-mass (HMM) proteins of more than 30 kDa and low-molecular-mass (LMM) protein of about 17 to 30 kDa (Lindquist and Craig 1988; Vierling 1991). In contrast to animal systems, plants synthesize more LMM *HSPs* than HMM *HSPs*. The plant LMM *HSPs* superfamily is unusually complex, consisting of at least six gene families based on DNA sequence analysis, immunological cross-reactivity, and intracellular localization; proteins encoded by the different LMM *HSPs* gene families are targeted to different cellular compartments, including the cytosol, chloroplasts, mitochondria, and endoplasmic reticulum (ER) (Waters *et al*. 1996). Plants LMM *HSPs* are represented by over 45 complete protein coding sequences including sequences from many different angiosperms, and a gymnosperm, *Pseudotsuga menziesii* (Tranbarger and Misra 1995). These sequences comprise four gene families encoding proteins localized in the cytosol (classes I and II), the chloroplast and the ER. A fifth class of *smHSPs* gene encoding a protein localized in mitochondria has been identified from *Pisum sativum* (Lenne and Douce 1994). A potential sixth class of LMM *HSPs* is represented by a single cDNA from *Glycine max* (LaFayette *et al*. 1996; Waters *et al*. 1996).

In addition to being part of the heat shock response, some plant LMM *HSPs* genes have been shown to be expressed at normal growth temperatures during zygotic embryogenesis. Expression of heat-shock genes occurs during embryogenesis from somatic cells, microspores, and developing pollen in alfalfa and tobacco (Gyôrgyey *et al*. 1991; Zarsky *et al*. 1995). Changes in concentrations of artificial phytohormones, heat shock, and starvation are known to induce somatic or microspore embryogenesis. Despite these largely different conditions, microspore-derived embryos from tobacco and somatic embryos from alfalfa express LMM *HSPs* during the globular and heart stages but not during the following torpedo stage. These data raise the question of whether heat-shock gene expression during early somatic embryogenesis is a general phenomenon that is also relevant to zygotic embryogenesis. In zygotic embryos, expression of *HEAT-SHOCK* genes occurs during the maturation stage of the seed, when cell division has ceased and seeds become tolerant to dessication. In sunflower, expression of class II LMM *HSPS* seems to parallel roughly storage protein and lipid accumulation, whereas expression of class I coincides with seed desiccation (Coca *et al*. 1994). It has been proposed that *HSPS* are important for desiccation tolerance of the embryo or are required for germination upon rehydration. Similar to other plants, *Arabidopsis thaliana* accumulates a specific set of *HSPS* (*ATHSP17.4* and *ATHSP17.6*) during seed maturation, whereas *ATHSP18.2* is not expressed (Wehmeyer *et al*. 1996). The expression of subsets of *HEAT-SHOCK* genes during gametogenesis and embryogenesis suggests that the developmentally expressed *HSPs* play certain functions that may differ to some extent from those required for coping with environmentally stressed vegetative tissue. Furthermore, these findings may indicate differences in the signal transduction pathway (Schöffl *et al*. 1998). Helm and Abernethy (1990) showed the presence of LMM *HSPs* mRNAs during wheat embryos development in normal condition. They concluded that the expression of these LMM *HSPs* is a normal part of wheat embryo development, and is not a consequence of heat stress experienced by the parental plants during embryogenesis. Fu *et al*. (2002) studied the expression of *HSPs* during a maize mutant embryo development. A recessive mutation at the locus *EMPTY PERICARP2* (*EMP2*) led to dramatically increased expression of heat shock genes, retarded embryo development, and early-stage abortion of embryogenesis. The developmental timing of *emp2* mutant embryo lethality was correlated with the initial ability of maize kernels to invoke the *HSR*. The developmental retardation of *emp2* mutant kernels before the *HSR* suggests an additional role for *EMP2* during embryo development distinct from the *HSR*.

LIPID TRANSFER PROTEIN GENES

LIPID-TRANSFER PROTEINS (*LTPS*) facilitate the transfer of lipids between membranes. The binding of lipids to *LTPS* can be both specific and nonspecific. The nonspecific lipid transfer proteins (*nsLTPs*; Rueckert and Schmidt 1990) have an affinity for a variety of hydrophobic molecules, such as monoacylated and diacylated lipid molecules including fatty acids (Han *et al*. 2001), fatty acyl CoA (Lerche *et al*. 1997), lyso-phosphatidylcholine (Charvolin *et al*. 1999), and phosphatidylglycerol (Sodano *et al*. 1997). They have been isolated from a diverse range of organisms, from bacteria and yeast to higher plants and animals (Rueckert and Schmidt 1990; Smolenaars *et al*. 2007). Plant nonspecific *LIPID TRANSFER PROTEINS* (*nsLTPs*) were first isolated from spinach leaves (Kader *et al*. 1984) and then have been isolated from rice, wheat, barley, maize, peaches, and apricots (Hollenbach *et al*. 1997; Poznanski *et al*. 1999; Han *et al*. 2001; Pons *et al*. 2003). *nsLTPs* are widely distributed and form a superfamily of related proteins subdivided into two families: *nsLTP1* (~9 kDa) and *nsLTP2* (~7 kDa; Kader 1996). Both families are multigenic, and more than 150 sequences of plant *nsLTPs* are listed in data bases. The presence of multiple members of the *nsLTP* family may be a consequence of gene duplications and subsequent sequence variation, while those residues crucial to function are conserved (Vignols *et al*. 1997; Clark and Bohnert 1999). Although the nucleotide and amino acid sequences have considerable divergences, several features are highly conserved in the encoded *nsLTPs*, including eight cysteine residues to form four disulphide bonds (Kader 1996).

nsLTPs were originally assumed to participate in phospholipid transfer between membranes, membrane biogenesis, modification of the lipid composition of a membrane, and in the function of membrane-bound enzymes using lipids as substrates (Kader *et al*. 1982; Arondel and Kader 1990; Wirtz 1991; Kader 1996). However, observations demonstrated that *LTPs* are extracellularly located and secreted (Sterk *et al*. 1991; Tsuboi *et al*. 1992; Coutos *et al*. 1993; Thoma *et al*. 1993). These findings suggest *LTPs* involvement in other roles such as cutin formation (Meijer *et al*. 1993; Hendriks *et al*. 1994; Pyee and Kolattukudy 1995), embryogenesis (Sterk *et al*. 1991; Thoma *et al*. 1994; Kader 1997), defense reactions against pathogens (Molina *et al*. 1993; Garcia-Olmedo *et al*. 1995), and adaptation of plants to environmental and abiotic changes (Jung *et al*. 2003; Wu *et al*. 2004). In addition, several members of the *nsLTP* family have been identified as relevant allergens in plant foods and pollens. Their high resistance to heat treatment, chemical denaturation and enzymatic digestion has been related with the induction by these allergens of severe symptoms in many patients (De Oliveira and Gomes 2007; Salcedo *et al*. 2007).

The expression pattern of *nsLTPs* is complex, character-

ized by strong developmental and tissue specificity with distinct patterns of expression for the different genes. For example, strong cell specificity, mainly in epidermal cells, has been observed in both maize seedlings (Sossountzov *et al*. 1991) and carrot embryos (Sterk *et al*. 1991). However, in rape seedlings, a *nsLTP* gene was found to be expressed in the entire cotyledon (Soufleri *et al*. 1996). In contrast, another *nsLTP* gene is specifically expressed in the tapetal cell layer in rape seed (Foster *et al*. 1992) and *Lilium henryi* (Crossley *et al*. 1995). Recent studies on embryogenesis were based on the position-specific expression of an *nsLTP* gene from *Arabidopsis* named *ATLTP1*. This gene is highly expressed in embryo protoderm, which is the precursor of the plant epidermis. The expression of the gene was studied in several *Arabidopsis* mutant embryos (*knolle*, *keule* and *gnom*) in which the mutation affects the formation of the apical-basal and radial axes. Using *in situ* hybridization, as well as by following *ATLTP1* promoter-β-glucuronidase transgene expression, it was observed that pattern formation in the embryo is reflected in the position-specific expression of this *LTP* gene (Vroemen *et al*. 1996). It was also found that *ATLTP1* is expressed in the outer cell layer of *Arabidopsis raspberry* embryos, which are morphologically arrested at the first stage (globular) of their formation (Yadegari *et al*. 1994). Together, these observations suggest that cell differentiation is uncoupled from morphogenesis during embryo development. The studies also show the utility of *nsLTP* genes as tissue-specific markers in mutant embryos.

In *P. vulgaris*, *nsLTP* (*PVLTP*) gene was induced by water deficit and ABA treatment. The deduced protein presents similarity with *LTPs* from different plants, showing the highest identity (57%) with maize *nsLTP*, *MZEPLTP* (Colmenero-Flores *et al*. 1997). Under water deficit conditions, the experiments showed that the *PVLTP* transcript accumulates mainly in the aerial regions of the plant (stems and leaves) while its accumulation is very low in stressed roots. This organ-specific expression can also be observed in well irrigated plants where mRNA can be detected in stems and leaves but not in roots. Such specific expression can be that of a gene whose product responds to the need for a higher impermeabilization of the plant surface (epidermal regions) in order to decrease water loss, particularly under water-limiting conditions.

PASTICCINO (*PAS*) GENES

The growth and differentiation of higher plants is also greatly dependent on environmental stimuli, such as light and temperature, and on endogenous factors, such as phytohormones. For instance, cell division and proliferation, controls of which are essential for proper plant development, are tightly regulated by cytokinin and auxin. Cytokinins (CKs) were originally identified by their ability to stimulate division and sustained tobacco pith cell growth when added in combination with auxin (Davies 1995). Cytokinins have an important role in various physiological processes and were one of the first plant hormones to be isolated. Since their discovery, numerous reports have demonstrated that cytokinins are implicated in a wide variety of plant growth and development processes including cell division, organ formation and regeneration, apical dominance, vascular development, nutrient mobility, and senescence (Noodén 1988; Estruch *et al*. 1991; Mok 1994; Schmulling 2002).

The *PASTICCINO* (*PAS*) genes, which are involved in the control of cell division, proliferation and differentiation, are required for normal organization of the apical region in the embryo. *Pasticcino* mutants, which belong to three complementation groups (*pas1*, *pas2*, *pas3*), were isolated in the progeny of independent ethyl methane sulfonate and T-DNA mutagenized *A. thaliana* plants. These mutants show a range of severe developmental defects throughout the growth stages: embryo formation is altered at the heart stage when cotyledon primordia are initiated; cotyledons do not form correctly, leading to a flat apex; seedlings possess short, thick hypocotyls and misshaped cotyledons; and mature plants are characterized by abnormal compact rosettes with multiple shoots. Irregular root development is also observed and consists of short primary root and no or very rare secondary root formation. *pas* mutants show altered response to exogenous cytokinin but are not affected in cytokinin biosynthesis (Faure *et al*. 1998; Bellec *et al*. 2002).

The *A. thaliana pas1* mutants were identified due to their abnormal seedling phenotypes. The mutants have more cell layers in the cotyledons and hypocotyls than wild type, a characteristic which is exaggerated by hyper proliferation when the seedlings are grown in the presence of exogenous cytokinins. The *pas1* phenotype is complex; these mutants have altered embryo development, shorter primary roots and reduced elongation of lateral roots compared to wild type, short bushy compact rosettes, and infertile flowers. The predicted protein sequence encoded by the PAS1 gene is similar to those of the FK506-binding protein (FKBP) class of immunophilins (Faure *et al*. 1998; Vittorioso *et al*. 1998). The cytokinin induction of primary cytokinin response markers *ARR1* and *ARR6* is enhanced and prolonged in *pas* mutants, suggesting that *PAS* functions to repress the cytokinin response. Finally, down-regulation of the primary auxin response genes *IAA4* and *IAA1* in *pas* mutants suggests an alteration in auxin response (Harrar *et al*. 2003). Mutants of the *Arabidopsis* gene *PAS2* develop abnormal shoot phenotypes from slow tumor-like cell proliferation. In absence of cytokinins, *pas2* mutant development is severely altered with an abnormal embryo development leading to embryos with short and large hypocotyl and very reduced round cotyledons (Faure *et al*. 1998). After germination, both hypocotyl and cotyledons failed to develop normally, leading to seedlings with short and wide hypocotyl and reduced finger-shaped cotyledons. *pas2* cotyledons showed a complete loss of bilateral symmetry. Drastic *pas2* defects are detectable in embryogenesis at the heart stage for the first time and are later manifested mainly in shoot while root is only retarded in its growth (Haberer *et al*. 2002). A striking feature of *pas2* adult plants was the occurrence of spontaneous fusion between organs such as leaves, stems, flowers, or siliques (Bellec *et al*. 2002). *pas3* like *pas1* mutant displayed variable phenotypes such as one or no cotyledons. Seven days after germination, this mutant has shorter primary root than in the wild type and the other *pas* mutants, and also show rare secondary roots (Faure *et al*. 1998).

LEAFY COTYLEDON (*LEC*) GENES

Arabidopsis Leafy Cotyledon (*LEC*) genes are central regulators of embryogenesis that play key roles in processes that occur during both the morphogenesis and maturation phases. *LEC* genes are defined by mutations at three loci: *LEC1*, *LEC2*, and *FUSCA3* (*FUS3*) that have major effects on embryo development. All three mRNA have been found to be expressed only in embryos (Lotan *et al*. 1998; Nambara *et al*. 2000; Baumbusch *et al*. 2004; Baumbusch 2006). *LEC1* and *LEC2* were shown to regulate the expression level of the third one, *FUS3* during seed filling (Wang *et al*. 2007). On the other hand, *LEC* genes are regulator factors involved in oil accumulation during seed maturation (Baud *et al*. 2007; Wang *et al*. 2007). During the morphogenesis phase, the *LEC* genes are required to maintain suspensor cell identity. In *lec* mutants, the suspensor which in *Arabidopsis* normally consists of a single file of cells, undergoes abnormal cell divisions, resulting in a structure that is several cell layers wide. In some genotypes, the abnormal suspensor continues to proliferate and gives rise to a secondary embryo. Another early function of the *LEC* genes is the specification of cotyledon identity. The adaxial surfaces of *lec* mutant cotyledons have trichomes, a leaf trait in *Arabidopsis*, and the anatomy of some *lec* mutant cotyledons is intermediate between cotyledons and leaves. These observations suggest that in the absence of LEC gene activity cotyledons are incompletely specified and revert partially to leaf-like organ (Lotan *et al*. 1998; West *et al*. 1994; Harada 2001;

Kwong *et al*. 2003; Lee *et al*. 2003; Kagaya *et al*. 2005). The *LEC1* gene encodes a transcription factor. mRNA accumulation resulting from this gene starts from preglobular to cotyledon stage, is limited to seed development, and is high during seed maturation. *FUS3* transcripts accumulation was observed during mid-embryogenesis (West *et al*. 1994; Lotan *et al*. 1998; Baumbusch *et al*. 2004; Baumbusch 2006). *LEC2* quickly and directly activates the expression of seed specific genes such as *S3 OLEOSIN* (*S3*) and *AT2S3 ALBUMIN*. In a second phase, it triggers the accumulation of the three regulatory genes leading to another increase of the level of *S3* and *AT2S3* mRNAs. Interestingly, this induction led to the accumulation of seed specific triacylglycerols in leaves (Mendoza *et al*. 2005).

lec mutants of Arabidopsis are unable to distinguish between embryonic and vegetative patterns of plant development. *lec* mutations exhibit morphological characteristics such as altered cotyledon morphology, desiccation intolerance and occasional vivipary. The *fus3* mutation in *Arabidopsis* affects several aspects of embryogenesis, provoking a *lec*-like phenotype with ectopic trichomes, dessication intolerance and precocious germination (Meinke 1992; West *et al*. 1994; Nambara *et al*. 2000).

TITAN (*TTN*) GENES

The *TITAN* (*TTN*) genes encode chromosome scaffold proteins of the condensing and cohesion classes named *STRUCTURAL MAINTENANCE OF CHROMOSOME* (*SMC*) proteins (condensins and cohesins) required for chromosome function at mitosis. They are conserved in most prokaryotes and all eukaryotes examined and play essential roles in networks that are responsible for sister chromatid cohesion, chromosome condensation, dosage compensation and recombination repair (Michaelis *et al*. 1997; Lieb *et al*. 1998; Liu *et al*. 2002; Tzafrir *et al*. 2002; Liu and Makaroff 2006). *SMC* proteins perform essential functions during embryo and endosperm development. At least nine different genes controlling titan phenotype have been identified; *TTN* genes played a direct role in either cell cycle control or the structural mechanics of mitosis (Liu and Meinke 1998; Liu *et al*. 2002; Tzafrir *et al*. 2002).

The *titan* mutants of Arabidopsis exhibit striking defects in seed development. These mutants are defective in karyokinesis and the defining feature is the presence of abnormal endosperm with giant polyploid nuclei which is up to 100 times larger than the wild-type, during early stages of seed development. Embryo development is arrested shortly after fertilization in most *ttn* mutants and in some cases is accompanied by dramatic cell enlargement. Mutant embryos differ in cell size, morphology and viability, depending on the locus involved (Liu and Meinke 1998; McElver *et al*. 2000; Liu *et al*. 2002; Tzafrir *et al*. 2002).

Liu and Meinke (1998) discussed three *titan* mutants with related but distinct phenotypes in *Arabidopsis*. Chromosome condensation has been observed in all *titan* mutants, while mitotic figures have only been seen in *titan3*. Development of *titan1* and *titan2* embryos is arrested at the 1-2 cell stage, whereas *titan3* can produce fertile homozygous mutant plants. The *ttn1* phenotype includes extraordinary enlargement of nuclei in the embryo and endosperm, similar enlargement of cells in the arrested embryo, and a disruption of endosperm nuclear migration to the chalazal end of the seed. Defects in *ttn2* are limited to early embryonic lethality and enlargement of endosperm nuclei. Embryo development in *ttn3* is surprisingly normal but is accompanied by the formation of giant endosperm nuclei early in development and the appearance of aberrant mitotic figures with numerous condensed chromosomes. Another unique feature of the *ttn3* phenotype is cellularization of the mutant endosperm late in development.

The *ttn4* mutant identified by Wu (1999) resembles *ttn2* in phenotype and appears to encode plant-specific proteins of unknown function. The *ttn5* mutant is most similar to *ttn1* except that migration of endosperm nuclei is not disrupted (McElver *et al*. 2000). The *ttn6* (Tzafrir *et al*. 2002) embryo cells often appeared rounded and disorganized. Endosperm cellularization was also disrupted. Defects visible at the heart stage of normal development included: increased size and reduced number of endosperm nuclei and nucleoli; and developmental arrest of the embryo proper. Endosperm nuclear enlargement was similar to that observed with other *titans* (Liu and Meinke 1998; McElver *et al*. 2000). Embryo proper and endosperm nucleolus size increases after the heart stage. A number of small nucleoli with a diameter of 5 to 6 µm were also found in the mutant endosperm, and their size remained constant between the heart and cotyledon stages. This variability in nuclear size within a single seed is a common feature of *titan* mutants. Most *ttn6* seeds at the heart stage contained between 20 and 50 endosperm nuclei. This number did not increase later in development and remained far below the number found in wild-type seeds. Therefore, endosperm nuclear division is completed at about the same time in mutant and wild-type seeds (Tzafrir *et al*. 2002). *TTN9* with a *titan* endosperm phenotype encodes a novel plant-specific protein of unknown function. The *ttn9* embryo, which contained at most four small cells, was typical of this class and resembled the cohesin (*ttn7* and *ttn8*) knockouts. *TTN9* appears to be a single copy gene that is expressed in siliques based on EST data (Liu *et al*. 2002; Tzafrir *et al*. 2002). The **Table 1** summarizes some distinguishing features observed in *titan* mutant seeds.

Table 1 Phenotypic variation observed in *Arabidopsis titan* mutant seeds.

	Endosperm phenotype		Embryo phenotype			References
Mutant	**Nuclear size**	**Nuclear migration**	**Nuclear size**	**Cell morphology**	**Embryo viability**	
titan1	Giant (~ 60µm, observed in 72.5% of seeds)	Absent	Giant	Giant	Inviable small embryos with only one or few large cells	Liu and Meinke1998; Tzafrir *et al*. 2002
titan2	Giant	Present	Normal	Aborted	Inviable embryos composed of several small cells	
titan3	Giant	Present	Normal	Normal	Viable	
titan4	Giant, observed in 75% of seeds of seeds observed					Tzafrir *et al*. 2002
titan5	Giant (~ 23.5µm)	Present		Giant, can exceed 150 µm in diameter		McElver *et al*. 2000
titan6	Giant, (~ 21µm, observed in 77.20% of seeds)			(~ 56.5µm)	Arrested at the preglobular/globular stage	Tzafrir *et al*. 2002
titan7	Giant, observed in 77% of seeds					Liu *et al*. 2002
titan8	Giant, observed in 88% of seeds					
titan9	Giant, observed in 73% of seeds					Tzafrir *et al*. 2002

Wild-type seeds have 150 to 300 endosperm nuclei at the developmental heart stage. Normal nucleolus size is 4 µm in diameter. Normal embryo cell diameter is 8 µm.

CONCLUSION AND PROSPECTS

Some major genes playing an important role in plant embryogenesis were described. Mutations in these genes affect normal development of plant embryos. After alignment of mRNA coding sequences from each gene family in the model plant, DNA primers were designed to target conserved domains of these genes and to identify related genomic sequences in *Phaseolus* (results not shown). Expression of the genes involved in embryogenesis was studied from mRNA extracted from *Phaseolus* ovules. RT-PCR reactions revealed several bands from all the described genes, with the exception of *KNOX* genes; and also showed different accumulation levels of *LTP* gene during *Phaseolus* embryo development.

In the following steps of our investigation, *Phaseolus* amplified fragments from RT-PCR will be extracted from agarose and inserted into the pCR2.1 vector plasmid. Ligated fragments will be sequenced with LICOR System (IR2, DNA Analyzer). BLASTN sequence homology analyses will be performed by using the BLAST network. Gene sequences with high homologies and unknown in *Phaseolus* will be submitted to NCBI for registration.

On the other side, mutants deficient in seed development were isolated from an ethyl methane sulphonate (EMS)-induced mutant collection of common bean (*Phaseolus vulgaris* cv. 'BAT93') from the University of Geneva (Switzerland) (Pankhurst *et al.* 2004). Seeds of these mutants aborted within 15 to 25 days after anthesis. These mutants will be used to study the expression of embryogenesis genes described above by RT-PCR and to isolate major genes involved in seed development by Suppression Subtractive Hybridization (SSH) technique (Diatchenko *et al.* 1996; Marenda *et al.* 2004). This method is based on the construction of subtracted cDNA libraries that allow the identification and isolation of differentially expressed transcript. SSH will allow isolating different cDNAs between degenerated seeds of mutants and normal seeds of wild type. Isolated cDNAs will be sequenced and analysed with BLAST network service.

We mentioned that immature embryos from the crosses *P. polyanthus* (as female) x *P. vulgaris* and *P. coccineus* (as female) x *P. vulgaris* aborted usually at the globular or early heart-shaped developmental stages. SSH technique will be used to isolate specific genes involved in embryo abortion, and to analyze allele sequences of these genes in the different collections of *P. vulgaris*, *P. polyanthus* and *P. coccineus* genotypes. This type of analysis will allow to understand better some gene disruption occurring during *Phaseolus* hybrid embryo development and device the most efficient ways to overcome post-zygotic barriers in this genus.

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