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Conservation and diversification of **SCARECROW** in maize

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Abstract

The **SCARECROW** (*SCR*) gene in *Arabidopsis* is required for asymmetric cell divisions responsible for ground tissue formation in the root and shoot. Previously, we reported that *Zea mays* **SCARECROW** (*ZmSCR*) is the likely maize ortholog of *SCR*. Here we describe conserved and divergent aspects of *ZmSCR*. Its ability to complement the *Arabidopsis scr* mutant phenotype suggests conservation of function, yet its expression pattern during embryogenesis and in the shoot system indicates divergence. *ZmSCR* expression was detected early during embryogenesis and localized to the endodermal lineage in the root, showing a gradual regionalization of expression. Expression of *ZmSCR* appeared to be analogous to that of *SCR* during leaf formation. However, its absence from the maize shoot meristem and its early expression pattern during embryogenesis suggest a diversification of *ZmSCR* in the patterning processes in maize. To further investigate the evolutionary relationship of *SCR* and *ZmSCR*, we performed a phylogenetic analysis using *Arabidopsis*, rice and maize **SCARECROW-LIKE** genes (*SCLs*). We found *SCL23* to be the most closely related to *SCR* in both eudicots and monocots, suggesting that a gene duplication resulting in *SCR* and *SCL23* predates the divergence of dicots and monocots.

Keywords

Arabidopsis; GRAS family; pattern formation; root development; **SCARECROW**

Introduction

In plants, highly regulated coordination of cell division, cell expansion and cell specification gives rise to the final shape of the organism. Asymmetric cell divisions play an important role in establishing and propagating the correct patterns of plant tissue. Radial patterning in the ground tissue (endodermis and cortex) of the *Arabidopsis* root is a well-characterized example of how regulatory genes control tissue patterning through a series of asymmetric cell divisions. In *Arabidopsis* roots, a set of initials undergo asymmetric cell divisions to regenerate the initials themselves and give rise to their daughter cells. The cortex/endodermis initial, for example, first divides transversely, and this asymmetric division produces another initial and a daughter cell (Dolan *et al.*, 1993; Scheres *et al.*, 1994). The daughter cell then divides longitudinally, and this second asymmetric division generates the endodermis and cortex cell lineages. **SCARECROW** (*SCR*) and **SHORT-ROOT** (*SHR*) are required for the second asymmetric cell

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division which gives rise to the cortex and endodermis (Di Laurenzio *et al.*, 1996; Helariutta *et al.*, 2000). Mutations in either *SCR* or *SHR* result in the absence of one of the ground tissue layers. In *scr* mutants, the remaining layer exhibits differentiated attributes of both cortex and endodermis, whereas in *shr* mutants it has characteristics only of cortex. Genetic and molecular analyses provided evidence that *SCR* acts downstream of *SHR*. In addition, it was demonstrated that ectopic expression of *SHR* under the *SCR* promoter could lead to supernumerary layers with endodermal characteristics (Nakajima *et al.*, 2001). These results indicate that *SCR* is essential for the asymmetric cell division but not specification of the endodermis, whereas *SHR* is required for both cell division and cell specification (Benfey *et al.*, 1993; Scheres *et al.*, 1995; Di Laurenzio *et al.*, 1996; Helariutta *et al.*, 2000; Nakajima *et al.*, 2001).

Both the *Arabidopsis SCR* and *SHR* genes encode putative transcription factors that belong to the GRAS family (Di Laurenzio *et al.*, 1996; Peng *et al.*, 1997; Silverstone *et al.*, 1998; Pysh *et al.*, 1999; Helariutta *et al.*, 2000). Thus far, it has been reported that at least 33 members of this plant-specific family are predicted in the *Arabidopsis* genome, including the founding members (GAI, RGA, and *SCR*) (Di Laurenzio *et al.*, 1996; Peng *et al.*, 1997; Silverstone *et al.*, 1998; Pysh *et al.*, 1999; Helariutta *et al.*, 2000; Bolle, 2004). Only a handful of the *GRAS* genes, however, have been studied in detail, revealing that they play diverse regulatory roles in plant growth and development (gibberellin signal transduction, axillary meristem initiation, shoot meristem maintenance, phytochrome A signal transduction, microsporogenesis, and root radial patterning) (Di Laurenzio *et al.*, 1996; Peng *et al.*, 1997; Silverstone *et al.*, 1998; Schumacher *et al.*, 1999; Bolle *et al.*, 2000; Helariutta *et al.*, 2000; Lee *et al.*, 2002; Stuurman *et al.*, 2002; Wen and Chang, 2002; Greb *et al.*, 2003; Kamiya *et al.*, 2003; Morohashi *et al.*, 2003). The functions of the majority of the *GRAS* genes remain to be studied.

In *Arabidopsis* roots, *SCR* expression was observed in the cortex/endodermis initials, the quiescent center (QC), and the endodermal cell lineage (Di Laurenzio *et al.*, 1996; Wysocka-Diller *et al.*, 2000). Recently, it was shown that the recovery of quiescent center (QC) identity and the stem cell niche in *scr* mutants depends on expression of *SCR* in the QC (Sabatini *et al.*, 2003). Further analysis of *SCR* expression in the *Arabidopsis* shoot system revealed that *SCR* was expressed in the seedling shoot apical meristem (SAM), young leaf primordia, bundle sheath cells of the leaf, and the endodermis/starch sheath of the inflorescence stem (Wysocka-Diller *et al.*, 2000). In addition, a detailed study of *SCR* expression during embryogenesis showed that it was consistently expressed in each ground tissue cell before longitudinal division and in the inner daughter cell after division (Wysocka-Diller *et al.*, 2000). Correlation of the *SCR* expression pattern with radial pattern defects in shoot and embryo provides further evidence that *SCR* is essential for key asymmetric cell division events, which give rise to different ground tissue layers.

Based on sequence similarity and its expression pattern in maize roots, we previously suggested that *ZmSCR* is the likely ortholog of *SCR* (Lim *et al.*, 2000). In addition, analysis of the dynamic expression pattern of *ZmSCR* during regeneration of the root tip after either whole or partial excision indicated the involvement of positional information as a primary determinant in regeneration of the root radial pattern in maize. Our results suggested that there is a common molecular basis for radial patterning of roots from the two distantly related species, despite the fact that the size and configuration of the QC is distinct in *Arabidopsis* and maize (Lim *et al.*, 2000).

In this study, we provide further evidence to support our hypothesis through molecular complementation of *Arabidopsis scr* mutants with *ZmSCR*, expression analyses during maize embryogenesis and in the shoot system, and a phylogenetic analysis of the *Arabidopsis*, rice and maize GRAS families. Interestingly, we found a novel gene (named *SCL23*) that is the

most closely related to *SCR* in *Arabidopsis*, rice and maize, suggesting that a gene duplication resulting in *SCR* and *SCL23* predates the divergence of dicots and monocots.

Taken together, our results suggest that *ZmSCR*, the maize *SCR* ortholog, employs similar as well as distinct molecular mechanisms to regulate radial patterning in maize, and that *SCR* function is conserved in eudicots and monocots.

Materials and methods

Molecular complementation of *scr* mutants with *ZmSCR*

The *pSCR::ZmSCR* construct was made by placing the full-length cDNA of *ZmSCR* immediately after the 2.5-kb region upstream of the *SCR* 620 translational start site in *pSCRHYG* (gift from Keiji Nakajima). The resulting plasmid (*pSCR::ZmSCR*) was used to transform *scr-1* mutants by the *Agrobacterium*-mediated, floral-dipping method (Clough and Bent, 1998). Seeds from the transformed plants were harvested and plated on medium containing hygromycin (15 µg/ml) to select transgenic plants harboring *pSCR::ZmSCR*. To confirm the genetic background of the transgenic plants, PCR products were amplified using the primers specific for *Arabidopsis SCR*. Subsequently, the amplified products were restriction-enzyme digested to observe polymorphisms between wild-type and *scr-1* plants. In addition, the primers specific for *ZmSCR* were used to verify the presence of the *pSCR::ZmSCR* construct by PCR analysis on the same DNA samples that were used for genotyping.

Histochemical techniques and in situ hybridization

Seeds of the maize inbred line B73 (*Zea mays* L.) were surface-sterilized, imbibed, and germinated in wet paper towels. When seedlings were approximately 3–4 cm long (6 days after planting) and all leaves were still enclosed in the coleoptile, the young shoots were excised and fixed in FAA (10% formaldehyde, 5% acetic acid and 50% ethanol [all v/v]) for plastic sectioning. For *in situ* hybridization, the samples were fixed in 4% paraformaldehyde in PBS overnight at 4 °C. Embryos of different stages were collected from inbred B73 plants grown and self-pollinated in the field, and the classification of stages was according to Randolph (1936). Subsequently, the collected samples were dehydrated, embedded and sectioned as previously described (Jackson, 1991; Di Laurenzio *et al.*, 1996; Fukaki *et al.*, 1998; Lim *et al.*, 2000). Sense and antisense riboprobes labeled with digoxigenin-11-UTP for *ZmSCR* were generated from the 3' UTR of *ZmSCR* and *in situ* hybridization was performed as described previously (Jackson, 1991; Lim *et al.*, 2000).

Plastic sections were used for both staining of Casparian strip and amyloplasts, using Technovit 7100 (Heraeus Kulzer). Samples for both Casparian strip and amyloplast staining were fixed as described previously (Di Laurenzio *et al.*, 1996; Fukaki *et al.*, 1998). In particular, to keep the growth orientation of stems with respect to the gravity vector, the upright primary inflorescence stems were cut from wild-type, *scr* and *scr* transformed with *pSCR::ZmSCR*, respectively. The segments of stems were fixed in FAA overnight at 4 °C in 1.5 ml tubes. After fixation, samples went through an ethanol series and were embedded in Technovit 7100 (Heraeus Kulzer) according to the manufacturer's instructions.

For Casparian strip detection, sections were counterstained in 0.1% aniline blue for 5–10 min as described by Di Laurenzio *et al.* 1996. The sections were visualized with a Leitz fluorescent microscope with FITC filter. For amyloplast detection, sections were stained in toluidine blue as described in Fukaki *et al.* 1998. Images were directly captured in a SONY CCD camera using scion NIH image software, and Adobe Photoshop 8.0 (Adobe Systems) was used to create composite figures.

Phylogenetic analysis

Sequence information was obtained from BLAST searches (Altschul *et al.*, 1990, 1997), and predicted proteins were aligned using ClustalX (Thompson *et al.*, 1997). In some cases, the alignments were manually adjusted using Se-Al program (Rambaut, 1996). We found that alignments using the full-length amino acid sequences with default parameters in ClustalX showed some regions that were difficult to align. Moreover, in the case where the sequence information of the full-length cDNAs was not available, the translational start sites for some GRAS members (especially if they were purely predicted by computational methods) were unclear, which led to ambiguous alignments. Thus, we used the signature motifs (VHIID, PFYRE, and SAW) of GRAS proteins for the best alignments as defined in Pysh *et al.* 1999. The culled alignment contained 548 characters in each of 135 taxa (33 *Arabidopsis*, 52 rice and 50 maize GRAS proteins), and data files used for our analysis are available in Supplement 1. Phylogenetic analysis using parsimony (PAUP* 4.0b10; Swofford, 2002) was used to infer the evolutionary relationships of the *Arabidopsis*, rice and maize GRAS members. The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using a human (*Homo sapiens*) STAT protein (HsSRC; NP004374) as an outgroup. The consensus tree with the culled alignment made no difference in our main conclusion, when compared to that with the 621 full-length alignment. Bootstrap analyses (Felsenstein, 1985) were conducted using 2000 replicates to measure node robustness.

Results

Molecular complementation of *scr* mutants

We previously reported the isolation and characterization of the putative maize *SCR* ortholog (*ZmSCR*) and its expression pattern in roots (Lim *et al.*, 2000). Our analysis revealed marked similarities in gene structure, deduced amino acid sequence and expression pattern between the two genes, raising the strong possibility of conserved function (Lim *et al.*, 2000).

To test the hypothesis of functional conservation, we transformed *Arabidopsis scr* mutants with *ZmSCR*. A binary plasmid *pSCR::ZmSCR*, which contains the open reading frame of *ZmSCR* under the control of the native 2.5-kb *Arabidopsis SCR* promoter, was generated and introduced into *scr-1* mutants. It was previously reported that the 2.5-kb *SCR* promoter with the full-length cDNA of *Arabidopsis SCR* was sufficient to rescue *scr* mutants and also mimic its native expression, when placed upstream of a reporter gene (Malamy and Benfey, 1997; Wysocka-Diller *et al.*, 2000). As shown in Figure 1, transverse sections of the primary root of transgenic plants showed a normal radial organization with endodermis and cortex layers. Histochemical staining for the Casparian strip, a marker for differentiated endodermis, confirmed the presence of endodermal characteristics in the inner layer of ground tissue (Figures 1D–F) indicating that *ZmSCR* was able to complement radial defects in *scr* roots (Figure 1F). In addition, the gravitropic response of *scr* mutant inflorescence stems was restored (Figure 1I). These results suggest that *ZmSCR* is functionally orthologous to *SCR* in regulation of the asymmetric cell divisions common to the shoot and root.

ZmSCR expression during embryogenesis

Although plant embryos lack most structures of the adult plant, radial patterning is established during embryogenesis and is propagated during postembryonic development (Steeves and Sussex, 1989). In *Arabidopsis*, it was shown that *SCR* expression appeared as early as the globular stage of embryogenesis (Wysocka-Diller *et al.*, 2000). Over the course of embryo development, *SCR* expression was consistently found in cells that were destined to undergo asymmetric cell divisions in the ground tissue of the embryonic root, hypocotyl, and presumptive cotyledon shoulder region (Wysocka-Diller *et al.*, 2000).

To investigate the temporal and spatial expression pattern of *ZmSCR* during embryogenesis, we performed an RNA *in situ* hybridization analysis in maize embryos. In the first phase in which the apical-basal asymmetry is established, the embryo is characterized by regionalization into the embryo proper and the suspensor (Sheridan and Clark, 1993, 1994). *ZmSCR* expression was found in the apical region of the embryo where the embryo proper would be formed (Figures 2A and B). When radial asymmetry was established, *ZmSCR* was expressed in a small group of cells on the anterior side of the embryo that would give rise to the prospective shoot and root meristems (Figure 2C). Once the shoot-root axis has formed, and as the rudiments of the coleoptile began to protrude from the anterior surface of the embryo just above the shoot meristem, expression of *ZmSCR* was localized to the root meristem at its basal pole similar to that seen in the primary root (Figure 2D). At the stage when a few leaf primordia were initiated by the shoot meristem, the root had been further elaborated, and vascular tissue was forming, localization of *ZmSCR* expression to the endodermal cell lineage became more evident (Figure 2E). Also, *ZmSCR* expression was detectable in leaf primordia and young leaves (Figure 2E). These results indicate that *ZmSCR* expression is a consistent feature of regionalization of the root meristem throughout embryogenesis.

***ZmSCR* expression in the maize shoot system**

We found that *ZmSCR* expression was localized to a small domain where both root and shoot meristems would develop and its expression remained in the leaves of mature embryos. To extend *ZmSCR* expression to the shoot system, we analyzed the *ZmSCR* expression pattern in the maize SAM and in young leaves. In *Arabidopsis* seedlings, *SCR* expression was primarily detected in the L1 layer of the SAM and in young leaf primordia (Wysocka-Diller *et al.*, 2000). Unlike *SCR* expression in the *Arabidopsis* shoot, no *ZmSCR* was observed in the SAM (Figures 3A and B). However, we did observe its expression in leaf primordia and in young leaves (Figures 3A and B). It was also reported that *SCR* expression in *Arabidopsis* leaves became progressively restricted to the bundle sheath cells that surround the vascular strands, as leaf primordia expand. The pattern of *SCR* expression and the morphological defects in these organs suggested a potential role of *SCR* in the formation of vascular bundles in leaves (Wysocka-Diller *et al.*, 2000). The patchy expression pattern of *ZmSCR* in young maize leaves led us to further investigate its expression in leaves. The maize leaf exhibits a typical Kranz anatomy of C4 plants in which mesophyll cells and bundle sheath cells are tightly arranged to facilitate the interchange of metabolites during photosynthesis (Esau, 1977). *ZmSCR* expression was observed in a series of parallel veins that extend the length of the leaves (Figures 3C–E). The *ZmSCR* expression domain appeared to be 2 to 3 cells wide (Figure 3E). In transverse sections, the spacing of *ZmSCR* expression appeared to be in places at which vascular bundles would be formed (Figure 3F). *ZmSCR* expression in young leaves suggests a potential role of *ZmSCR* in ground tissue formation during vascular development.

Phylogenetic analysis of SCARECROW in *Arabidopsis*, rice and maize

To understand the evolutionary relationship of *SCR* in *Arabidopsis* (eudicot) and maize (monocot), we sought to identify all members of the *Arabidopsis* and maize *GRAS* family and perform a comparative analysis. Currently, at least 33 genes are predicted to encode *GRAS* proteins in the *Arabidopsis* genome (AGI, 2000; Referred in Bolle, 2004). With *SCR* as a query, the TBLASTN program (Altschul *et al.*, 1990, 1997) was used to search the available maize genome databases, and a total of 50 *GRAS* genes were identified (Table 1). To verify expression, we also searched for expressed sequence tags (ESTs) and/or full-length cDNAs for the maize *GRAS* genes. The majority of predicted maize *GRAS* genes did not have counterparts among ESTs or full-length cDNAs (Table 1). To perform a comprehensive comparative analysis of the *GRAS* genes, we included the rice *GRAS* genes that were previously reported (Kamiya *et al.*, 2003; Tian *et al.*, 2004). In our phylogenetic analysis, we noticed that *GRAS* proteins could be divided into several major branches, which were named after a

common feature of the branch or one representative of their members as previously reported: PAT1, DELLA, SCL3, SCL4/7, LAS (SCL18), HAM, LISCL, SCR, and SHR branches (Bolle, 2004; Tian *et al.*, 2004). The inclusion of the rice GRAS members in our analysis lent support to these branches and allowed us to identify the genes most closely related to SCR (Figure 4). The most closely related gene in *Arabidopsis*, *SCL23*, was also conserved in rice and maize. When we compared the deduced amino acid sequence of *ZmSCL23* to those of *OsSCL23* and *SCL23*, the similarity among these predicted proteins extended throughout their entire length (data not shown). When compared at the amino acid level, *ZmSCL23* shows 85.7% identity to *OsSCL23*, and 64.8% identity to *SCL23*. Our analysis suggests that gene duplication resulting in *SCR* and *SCL23* occurred before the divergence of monocots and dicots.

Discussion

Evolutionary relationship between ZmSCR and SCR

We had previously suggested that *ZmSCR* is the likely ortholog of *SCR* based on two criteria: sequence similarity when compared with members of the GRAS family and similarity of expression patterns (Lim *et al.*, 2000). To test our hypothesis that *ZmSCR* is the functional ortholog of *SCR*, we investigated its ability to complement loss-of-function mutations of *SCR* and its evolutionary relationship to the *Arabidopsis SCR* gene. When *Arabidopsis scr* mutants were transformed with *ZmSCR* under the control of the native *SCR* promoter (Malamy and Benfey, 1997; Wysocka-Diller *et al.*, 2000), the radial defects in roots and shoots were restored indicating that *SCR* function appeared to be conserved in maize. Although *ZmSCR* under the native *SCR* promoter could complement the defects of *scr* mutants, it is important to note that this alone does not prove orthology.

In our comparative analysis, we noticed conservation and divergence of the GRAS family between monocots and eudicots, which have diverged 150–300 million years ago (Wolf *et al.*, 1989; Wikstrom *et al.*, 2001; Tian *et al.*, 2004). Interestingly, we found that the most closely related pair, *SCR* and *SCL23*, was conserved in *Arabidopsis*, rice and maize, strongly suggesting the orthologous relationship between *ZmSCR* and *SCR*. In *Arabidopsis*, *SCL23* expression in the published digital *in situ* datasets was below thresholds set for background levels and thus we could not reliably predict its expression pattern in the root (data not shown) (Birnbaum *et al.*, 2003). It is still possible that more closely related genes to *SCR* are present in maize, since the maize genome sequence is not yet completed. However, using the complete rice genome sequence information, which substantiates our analysis, it is suggestive that a gene duplication resulting in *SCR* and *SCL23* predates the divergence of dicots and monocots. With the efforts to elucidate the function of every gene in the *Arabidopsis* genome by the year of 2010 (Chory *et al.*, 2000), a large number of T-DNA insertion mutant lines and full-length cDNAs are now available. Thus, it will be of interest to elucidate the role of *SCL23* and the other *GRAS* genes in plant growth and development.

Our analysis now at four different levels: (i) sequence similarity, (ii) evolutionary relationship, (iii) expression pattern and (iv) functionality, provides strong evidence that *ZmSCR* is the functional maize ortholog of *SCR*.

ZmSCR expression during embryogenesis

The process of pattern formation during plant embryogenesis has been extensively studied resulting in a definition of the spatial relationships among the different parts of the embryo (Jürgens, 1995; Heckel *et al.*, 1999). However, comparative studies of patterning processes during embryogenesis between *Arabidopsis* and maize are problematic for several reasons. First, in contrast to *Arabidopsis*, cell divisions in maize embryos follow no obvious stereotypical pattern. Second, the apical-basal axis of the maize embryo, defined by the shoot

meristem and the root meristem, is oblique to the axis of the embryo proper and the suspensor while the *Arabidopsis* apical-basal axis is perpendicular. Third, unlike in *Arabidopsis* where the primordia for the shoot and root are set apart early in embryogenesis, in maize the primordia for both the shoot and root appear to arise from the anterior side of the embryo that would give rise to the prospective SAM and RAM. Finally, in maize, dormancy occurs later during embryogenesis, leading to the formation of 5–6 leaf primordia in the embryo in contrast to *Arabidopsis* where there are no leaf primordia and the embryo is much less mature (Sheridan, 1995; Heckel *et al.*, 1999). Without tissue-specific markers, it is difficult to compare fates of groups of cells in these two embryos. Function and expression of the patterning gene, *SCR*, appears to be conserved in both species, making it an informative marker for comparing the radial patterning process during *Arabidopsis* and maize embryogenesis. We analyzed *ZmSCR* expression during embryogenesis to determine its temporal and spatial expression. *ZmSCR* expression was found in the apical region as early as the appearance of apical-basal asymmetry. It is interesting to note that *ZmSCR* was expressed in a small group of cells on the anterior side of the embryo that would give rise to the prospective shoot and root meristems, when radial asymmetry was clearly established. *ZmSCR* expression in later stages was localized to the root meristem and leaf primordia, similar to what is seen in the maize seedling. As in *Arabidopsis*, *ZmSCR* expression was observed in the early stages of embryogenesis, suggesting that ground tissue formation is an early event in both monocot and dicot embryogenesis. However, because of the irregularity of the cell division pattern, it is difficult to follow the asymmetric cell division processes, which gives rise to cortex and endodermis as seen in the *Arabidopsis* embryo.

Isolation of mutant embryos that are morphologically abnormal and arrested at certain stages has implied that patterning processes during embryogenesis are under different genetic controls at specific times and places (Sheridan and Clark, 1993, 1994; Sheridan, 1995; Heckel *et al.*, 1999). It will be interesting to investigate *ZmSCR* expression as a marker in a collection of maize embryonic mutants to determine what developmental processes are impaired.

Tissue-specific expression of ZmSCR in the maize shoot system

It was demonstrated that in *Arabidopsis*, *scr* mutant hypocotyls and inflorescence stems were agravitropic and that these phenotypes correlated with the absence of one of the ground tissue cell layers (Fukaki *et al.*, 1998). Detailed analysis of *SCR* expression also revealed the possible origins of the endodermis/starch sheath in these organs (Wysocka-Diller *et al.*, 2000). In mature leaves, the strongest *SCR* expression was observed in bundle sheath cells associated with all veins (Wysocka-Diller *et al.*, 2000).

To understand how the *ZmSCR* gene is regulated in the maize shoot system, we analyzed the RNA expression pattern of *ZmSCR* in the maize shoot system. *ZmSCR* expression was found in the leaf primordia but not in the SAM in maize seedlings, which may reflect differences in ontogeny of lateral organs derived from the SAM between maize and *Arabidopsis*. In maize, a plumule with 5–6 leaf primordia enclosed within the coleoptile is formed during embryogenesis (Sheridan and Clark, 1993, 1994; Kiesselbach, 1999). In contrast to maize, all leaves are formed during postembryonic development in *Arabidopsis*. Expression of *ZmSCR* in young leaves was detected in the parallel veins that extended the length of the leaves. In transverse sections, *ZmSCR* expression was found with regular spacing in places at which vascular bundles would be formed, suggesting that *ZmSCR* may prepattern the vascular tissue. Comparison of the expression patterns of *ZmSCR* and *SCR* in the shoot system suggests that this regulatory gene plays a similar role in conserved developmental mechanisms for radial patterning in the two distantly related species.

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Figure 1.

Complementation of *scr* with *ZmSCR*. (A–C) Transverse sections of primary roots (wild type plants: A, D, G, J; *scr*: B, E, H, K; *scr* with *pSCR::ZmSCR*: C, F, I, L). (D–F) Casparian strip staining of transverse sections of primary roots. The white arrowheads point to Casparian strip staining in the endodermis, which indicates the restoration of endodermal characteristics in the inner ground tissue layer. (G–I) Gravitropic response of inflorescence stems. (J–L) Longitudinal sections through inflorescence stems. The black arrowheads indicate the amyloplasts that sediment in response to gravity. The direction of the gravity vector is shown by the arrows. Note the restoration of two ground tissue layers in *scr* with the introduction of

pSCR::ZmSCR. co, cortex; en, endodermis; ep, epidermis; m, mutant cell layer. Bars in (A) to (L) = 50 μ m.

Figure 2.

Analysis of *ZmSCR* expression during embryogenesis. Longitudinal sections through embryos at sequential stages of maize embryogenesis. (A) *ZmSCR* mRNA was localized in the apical region of the embryo (6 days after pollination [DAP]). (B) *ZmSCR* mRNA was detected in the apical region of the embryo proper, not in the suspensor (8 DAP). (C) Expression of *ZmSCR* was observed on the anterior side of the embryo that would give rise to the prospective SAM and RAM (10 DAP). (D) *ZmSCR* expression was restricted to the root meristem and leaf primordia (14 DAP). (E) *ZmSCR* expression was detected in the endodermal cell layer of the root, leaf primordia, and in young leaves, but not in the SAM (18 DAP). The stages are adapted from Randolph (1936). (F) Embryo probed with a sense probe (12 DAP). co, coleoptile; lp, leaf primordia; sc, scutellum; su, suspensor; yl, young leaf.

Figure 3.

Expression of *ZmSCR* in the maize shoot system. (A and B) *ZmSCR* mRNA was expressed in leaf primordia and young leaves, but not in the SAM. (C and D) *ZmSCR* expression was observed in the parallel veins of young leaves. (E) Close-up of the box in D is shown. *ZmSCR* expression was detected in 2–3 cells of the parallel veins. (F) *ZmSCR* mRNA was detected in places where vascular bundles would develop, showing characteristic morphology with regular spacing (transverse section of the young leaves). co, coleoptile; lp, leaf primordia; SAM, shoot apical meristem Bars in (A) and (B) = 50 μ m.

Figure 4.

Phylogenetic relationship of *Arabidopsis*, rice and maize *GRAS* genes. The Neighbor-joining tree was constructed using the culled alignment of 33 *Arabidopsis*, 52 rice and 50 maize *GRAS* proteins. The tree was rooted using a human STAT (HsSRC) as an outgroup as previously reported (Bolle, 2004). The pair of SCR and SCL23 is marked with a black box. Bootstrap values of 2000 replicates are shown for each branch.

Table 1

Identification of the maize GRAS genes. The TIGR entry and their expression as evidenced by ESTs/full-length cDNAs are listed (indicated by positive (+) or negative (–) signs, when there is at least one form of EST or full-length cDNA).

TIGR entry	EST/cDNA	Length (amino acids)
AZM4_101909	–	623
AZM4_102252	+	551
AZM4_102253	–	569
AZM4_112779	–	586
AZM4_112780	+	384
AZM4_114042	–	492
AZM4_124766	–	686
AZM4_125439	–	542
AZM4_133944	+	303
AZM4_134172	–	564
AZM4_135383	–	270
AZM4_137589	+	507
AZM4_15299	–	545
AZM4_23076	–	525
AZM4_23591	+	506
AZM4_34779	–	449
AZM4_38107	+	558
AZM4_39321 (ZmSCL23)	+	452
AJ242530 (ZmD8)	+	625
AZM4_69218	–	645
AZM4_60857	–	599
AZM4_69126	–	440
AZM4_69128	+	354
AZM4_69803	–	379
AZM4_80696	+	640
AZM4_80707	+	459
AZM4_81199	+	467
AZM4_81973	–	481
AF263457 (ZmSCR)	+	668
AZM4_89455	–	459
AZM4_90639	+	475
AZM4_91631 (ZmSCL1)	+	508
AZM4_101910	–	623
AZM4_94295	–	329
AZM4_22747	–	366
AZM4_101950	+	809
AZM4_33193	–	416
AZM4_40669	–	454
AZM4_91142	–	486
AZM4_22014	+	721
AZM4_60927	–	379
AZM4_112683	+	648
AZM4_33931	+	488
AZM4_69865	–	497
AZM4_34292	–	372
AZM4_91364	–	732
AZM4_51309	–	689
AZM4_13034	–	338
AZM4_112778	+	669
AZM4_2733	–	494