Journal of Experimental Botany, Vol. 58, No. 11, pp. 2887–2896, 2007
doi:10.1093/jxb/erm103 Advance Access publication 13 July, 2007
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RESEARCH PAPER

The *SERK1* gene is expressed in procambium and immature vascular cells

Mark A. C. J. Kwaaitaal* and Sacco C. de Vries

Laboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, the Netherlands

Received 5 December 2006; Revised 10 March 2007; Accepted 17 April 2007

Abstract

The *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1* (*SERK1*) gene is expressed in the procambium of the vascular bundles in roots, hypocotyls, and inflorescence stems. In younger parts of roots and hypocotyls, *SERK1* expression was less restricted and was also observed in protoxylem cells, immature metaxylem cells and phloem companion cells. In roots, *SERK1* expression was first detected in root vascular stem cells and was notably absent from the QC. In general, the SERK1 protein level as visualized by expression of a SERK1-YFP fusion protein closely followed the pattern of gene expression. In hypocotyls, prolonged application of 2,4-D resulted in extensive unorganized proliferation of *SERK1* expressing cells originating from the procambium and pericycle. In roots, 2,4-D treatment results in an increase in *SERK1* transcription that results in a moderate increase in the amount of SERK1-YFP fusion protein. The restricted vascular pattern of *SERK1* expression in roots remains unaffected after 2,4-D treatment.

Key words: 2,4-D, procambium, SERK1, somatic embryogenesis, stem cells, transit amplifying (TA) cells, vascular tissue.

Introduction

In plants, somatic embryos can develop from a single competent cell (Nomura and Komamine, 1985; Toonen *et al.*, 1994), but the origin of these competent cells remains uncertain. In general, competent cells arise from explants cultured in media supplemented with strong synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) (Mordhorst *et al.*, 1997, 1998). 2,4-D may have several roles in this process, acting as an auxin directly or modifying intracellular IAA metabolism and/or as a 'stressor' (Feher *et al.*, 2002, 2003). Other plant hormones, such as cytokinin and abscisic acid and several non-hormonal, stress-inducing factors can also induce the formation of cells finally capable of somatic embryogenesis (reviewed by Feher *et al.*, 2003). Once somatic embryos have been formed, they employ the same developmental mechanisms as zygotic embryos (Mordhorst *et al.*, 1997, 2002).

Two groups of genes involved in the acquisition of embryogenic competence in *Arabidopsis* have been identified. Negative regulators show an increased embryogenic competence upon mutation. Examples are *primordia timing* (*pt*), *clavata* (*clv*) *1* and *3* (Mordhorst *et al.*, 1998), and *pickle* (*pkl*) (Ogas *et al.*, 1997, 1999; Dean Rider *et al.*, 2003). Positive regulators show an increased embryogenic competence after ectopic expression. Examples are *BABY BOOM* (*BBM*) (Boutilier *et al.*, 2002), *WUSCHEL* (*WUS*) (Zuo *et al.*, 2002), and *LEAFY COTYLEDON 1 and 2* (*LEC1 and 2*) (Lotan *et al.*, 1998; Stone *et al.*, 2001; Gaj *et al.*, 2005). These positive regulators show the formation of somatic embryos or embryo-like structures originating from different organ and cell types without the application of external hormones.

The acquisition of embryogenic competence is marked by an increase in expression of the LRR receptor-like kinase *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1* (*SERK1*) gene in *Arabidopsis* (Hecht *et al.*, 2001), and its orthologues in carrot (Schmidt *et al.*, 1997), *Dactylis* (Somleva *et al.*, 2003), sunflower (Thomas *et al.*, 2004), and rice (Hu *et al.*, 2005). When ectopically expressed, *SERK1* increases embryogenic competence in culture (Hecht *et al.*, 2001). Ectopic expression of the MADS-box transcription factor *AGAMOUS-LIKE15* (*AGL15*) also results in increased embryogenic

* Present address and to whom correspondence should be sent: Max Planck Institute for Plant Breeding, Carl-von-Linne-weg 10, D-50829 Cologne, Germany. E-mail: markkw@mpiz-koeln.mpg.de

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competence in tissue culture. *AGL15* is expressed in early stage zygotic embryos and is relocalized from the cytoplasm to the nucleus at the onset of embryonic development (Perry *et al*., 1996, 1999). Ectopic expression of *AGL15* resulted in an increase in *SERK1* expression (Harding *et al*., 2003). Recently, AGL15 and SERK1 were shown to be together in protein complexes that include components of the brassinosteroid signalling pathway such as BRASSINOSTEROID-INSENSITIVE 1 (BRI1) and its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1)/SERK3 (Karlova *et al*., 2006). These results suggest that SERK1 may be involved in brassinolide signalling as well as in the acquisition of embryogenic competence. In plants, SERK1 and its closest homologue SERK2 together regulate specification of the tapetal cell layer in anthers (Albrecht *et al*., 2005; Colcombet *et al*., 2005). The SERK1 protein is found during megasporogenesis, in developing ovules from stage 1–II in ovule development onwards and in all cells of the embryo sac (Albrecht *et al*., 2005; Colcombet *et al*., 2005; Kwaaitaal *et al*., 2005). In seedlings, the SERK1 protein is found in the vascular bundles of roots, stems, leaves, and lateral roots (Hecht *et al*., 2001; Kwaaitaal *et al*., 2005). Collectively, these observations suggest that SERK1-mediated signalling is part of multiple developmental processes, most likely involving brassinosteroids and certainly including embryogenic cell formation.

In *Medicago,* both auxin and cytokinin are needed to obtain competent cells. The observed increase in *MtSERK1* expression in response to auxin and cytokinin was, however, not restricted to cells that acquired embryogenic competence, suggesting a more general effect (Nolan *et al*., 2003). In *Arabidopsis SERK1* expression increases in response to auxin at the adaxial side of cotyledons, the shoot apical meristem, and the vascular tissue of seedlings (Hecht *et al*., 2001). In *Daucus carota* explants, the vascular tissue is the origin of competent cells. After auxin treatment, cell proliferation is initiated in provascular cells (Guzzo *et al*., 1994). Procambial or provascular cells are vascular stem cells that originate from the apical meristem and generate xylem and phloem precursor cells that, in turn, differentiate into xylem and phloem, the two basic elements of the plant vascular tissue (Fukuda, 2004). In *Dactylis glomerata* L., cells giving rise to somatic embryos originate close to the vascular bundles of leaf explants and a subset of these cells express *SERK1* (Somleva *et al*., 2003).

This paper focuses on *Arabidopsis SERK1* expression as observed in vascular tissue of the primary root, stem, and hypocotyl. The results show that *SERK1* is expressed in cells of the immature xylem and the procambium. Later in vascular tissue development *SERK1* becomes restricted to procambial cells in root, stem, and hypocotyl. As embryogenic competence in *Arabidopsis* explants is usually initiated by exposure to 2,4-D, the effect of 2,4-D on *SERK1* transcription and protein localization in the vascular bundles of hypocotyls and roots was also investigated. The results show that prolonged 2,4-D exposure results in proliferation of mainly procambium cells, and to a lesser extent, pericycle cells that already expressed *SERK1* and continue to express *SERK1*, which suggested that competent cells in *Arabidopsis* tissue culture are mainly derived from *SERK1*-expressing procambial cells *in planta*.

Materials and methods

Plant lines and growth conditions

Seeds were surface-sterilized and transferred to half-strength MS medium pH 5.8 (Murashige and Skoog, 1962; Duchefa Biochemie, Haarlem, the Netherlands) agar plates containing 1% (w/v) sucrose pH 5.8. After 2 d at 4 °C, the plates were transferred to a growth chamber under fluorescent light at 22 °C with a 16/8 h light/dark cycle. After 1 week, seedlings were transferred to soil and grown to maturity under the same conditions. The *SERK1* promoter–*GUS* (*pSERK1::GUS*) line was previously described by Hecht *et al.* (2001), the *SERK1* promoter–*SERK1–YFP*, (*pSERK1::SERK1::YFP*) line by Kwaaitaal *et al.* (2005), the *DR5 auxin responsive element–GFP* (*DR5–GFP*) line by Benkova *et al*, (2003), and the *HB8* promoter–GUS (*pHB8::GUS*) line by Baima *et al.* (1995).

GUS staining

The samples were immersed in a 1 mg ml^{-1} X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid; Duchefa Biochemie, Haarlem, the Netherlands), 0.1% (v/v) Triton X100, 10 mM EDTA**,** 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide in 100 mM Na-phosphate, pH 7.2, and placed for 30 min under vacuum. Seedlings were incubated overnight at 37 °C. Stem sections just above the first cauline leaf, below the second cauline leaf, and from the base of the stem of 4-week-old plants were cut to a suitable size before immersion and incubated at 37 °C for 2 d. Seedlings were mounted in chloral hydrate:glycerol:water (8:1:2 by vol.) and imaged with Nikon Optiphot-2 microscope with Normarski optics (Tokyo, Japan). Photographs were taken with a Nikon Coolpix 990 digital camera. Alternatively, seedlings were embedded and sectioned as described below.

Plastic embedding and sectioning

For root and hypocotyl sectioning, seedlings were first imbedded in 1% (w/v) agarose in 100 mM Na-phosphate solution pH 7.2 and cut to the right size with a razor blade. Samples were vacuum-infiltrated for 15 min in 5% (w/v) glutaraldehyde in 100 mM phosphate pH 7.2 and fixed overnight at 4 °C. The material was washed twice with 100 mM phosphate buffer pH 7.2 and once with water for 15 min at room temperature. The samples were dehydrated through an ethanol range (10/30/50/70/96/100%, v/v). Next, the samples were infiltrated with Technovit 7100 (Hereaus Kulzer, Wehrheim, Germany) in the following range: 3:1, 1:1, and 1:3 ethanol: Technovit, for 45 min each. Finally, the samples were incubated overnight in pure Technovit at room temperature. The samples were positioned in a mould for plastic embedding. The Technovit solutions were prepared and the plastic polymerization was done according to manufacturer's protocol. With a microtome (Biocut, Reickert and Jung, Leica, Rijswijk, the Netherlands) 7 μm sections were cut and dried on slides. Sections were stained in 0.05% (w/v) Ruthenium Red in water for 10 min, dried, and imaged using a Zeiss Axioplan 2 microscope (Jena, Germany).

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Agar embedding and sectioning

Plant tissue was embedded in 1.5% (w/v) agarose in 50 mM Na-phosphate at pH 7.2 and 2–3 mm sections were cut manually with a razor blade, placed in a drop of 50 mM Na-phosphate at pH 7.2 on a cover slip and imaged directly as described below.

Treatment of seedlings with 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-N-naphthylphthalamic acid (NPA)

Seedlings were germinated and grown on a plate according to the conditions described above, but the plates were oriented vertically. Four days after germination (DAG) the seedlings were transferred to plates containing 0.1 μM or 5 μM 2,4-D (Sigma) or 10 μM or 50 μM NPA (Duchefa Biochemie, Haarlem, the Netherlands). Seedlings expressing the described reporter constructs were followed and harvested at different time points for GUS staining, fluorescence microscopy or sectioning, and subsequent microscopy.

Fluorescence microscopy

Seedlings or plants expressing fluorescent reporter constructs were imaged with a confocal laser scanning microscope (Zeiss Confocor 2-LSM 510 combination setup, Jena, Germany). A ×40 Plan-Neofluar oil immersion objective with a numerical aperture of 1.3 was used. CFP was excited with a 458 nm argon laser line controlled by an acousto-optical-tunable filter (AOTF). The excitation and emission light was separated by a dichroic beam splitter (HFT 458/514). The CFP fluorescence was filtered through a 475–525 nm band pass filter. GFP was excited with the 488 nm argon laser line controlled by the AOTF, and excitation and emission light were separated through a HTF488 beam splitter. The GFP fluorescence was filtered through a 505–550 nm bandpass filter. YFP was excited with the 514 nm argon laser line controlled by the AOTF, and excitation and emission light were separated through a HTF458/514 beam splitter. The YFP fluorescence was filtered through a 530–600 nm bandpass filter. Chlorophyll and red auto fluorescence were detected using a NFT635 filter combined with a 650 nm longpass filter. For comparison of treatments, microscope settings were kept the same for all samples.

Results

SERK1 *is expressed in the procambial cells of the vascular bundle in all organs studied*

SERK1 is expressed in the reproductive tissues and the vascular bundles of seedlings (Albrecht *et al*., 2005; Kwaaitaal *et al*., 2005). Because the provascular tissue is an origin of embryogenic competent cells (Guzzo *et al*., 1994; Schmidt *et al*., 1997; Somleva *et al*., 2000; Raghavan, 2004) *SERK1* expression as observed in the vascular bundle was investigated in more detail. This detailed investigation will make it the possible to determine the relationship between *SERK1* as a marker for single competent cells in culture and its vascular localization. *Arabidopsis* plants expressing either a *SERK1* promoter–*GUS* fusion (*pSERK1::GUS*) (Hecht *et al*., 2001) construct or a *SERK1* promoter *SERK1 cDNA–Yellow Fluorescent Protein cDNA* (*YFP*) (*pSERK1::SERK1::YFP*) construct (Kwaaitaal *et al*., 2005) were used to investigate the vascular localization of *SERK1* in seedlings and inflorescence stems. Figure 1A shows a longitudinal view of the primary root of an *Arabidopsis* seedling expressing *pSERK1::GUS*. GUS staining was observed in the vascular bundle, commencing directly proximal to the quiescent centre (QC) in cells referred to as stem cells (Xu *et al*., 2006) or vascular initials (Kwaaitaal *et al*., 2005). Figure 1B to E show transverse sections through the root tip of a *pSERK1::GUS*-expressing plant at the positions marked in Fig. 1A. Figure 1B shows a section at 250 μm from the tip. To help assign the *SERK1*-expressing cells in Fig. 1B, the different cell types in the same section as shown in this figure are assigned according to Mahonen *et al*. (2000) and colour-coded (Fig. 1C). GUS staining was observed in the procambium, protoxylem, metaphloem, and phloem companion cells and was also discernible in metaxylem cells. About 60 μm from the quiescent centre (Fig. 1D), most cells of the immature vascular bundle show GUS staining. GUS staining seemed higher in the metaxylem and was not observed of the protophloem initial cells. Immediately proximal to the QC, one of the vascular stem cells showed GUS staining (Fig. 1E). The SERK1 protein was first visible in the vascular stem cells. YFP fluorescence was higher in the first vascular descendants of the stem cells and became reduced in more mature parts of the vascular bundle (Fig. 1F). In cross-sections at ~250 μm from the QC in the *pSERK1::SERK1::YFP* line, low YFP fluorescence was detected in the complete vascular bundle except for the protophloem (Fig. 1G). Some yellow autofluorescence was also observed in the metaxylem in cross-sections through wild-type roots, suggesting that most of the YFP signal in the root metaxylem of the *pSERK1::SERK1::YFP* line is caused by autofluorescence (data not shown). In cross-sections through the immature vascular bundle of the *pSERK1::SERK1::YFP* line, ~60 μm proximal to the QC, the YFP fluorescence increased and was found in membranes of the metaxylem, protoxylem, and procambium (Fig. 1H). The YFP fluorescence seemed higher in the metaxylem and appeared absent from the protophloem initial cells.

To summarize, *SERK1* expression in roots is initially seen in stem cells of the root vasculature and most cells of the immature vascular bundle. Later *SERK1* expression becomes restricted to the procambium, protoxylem, and phloem companion cells. The SERK1 protein localization pattern closely followed the gene expression pattern.

Next, *SERK1* expression in the hypocotyl vasculature was investigated. Figure 2A shows GUS staining in a cross-section about 70 μm below the apical meristem of a hypocotyl from a 4-d-old seedling containing the *pSERK1::GUS* construct. Cell and tissue types were assigned according to Busse and Evert (1999*a*, *b*). *SERK1* expression was confined to the procambium cells (Fig. 2A). In a younger part of the hypocotyl at 50 μm from the apical meristem (Fig. 2B) *SERK1* procambium expression increased, was higher and remained visible in the adjacent

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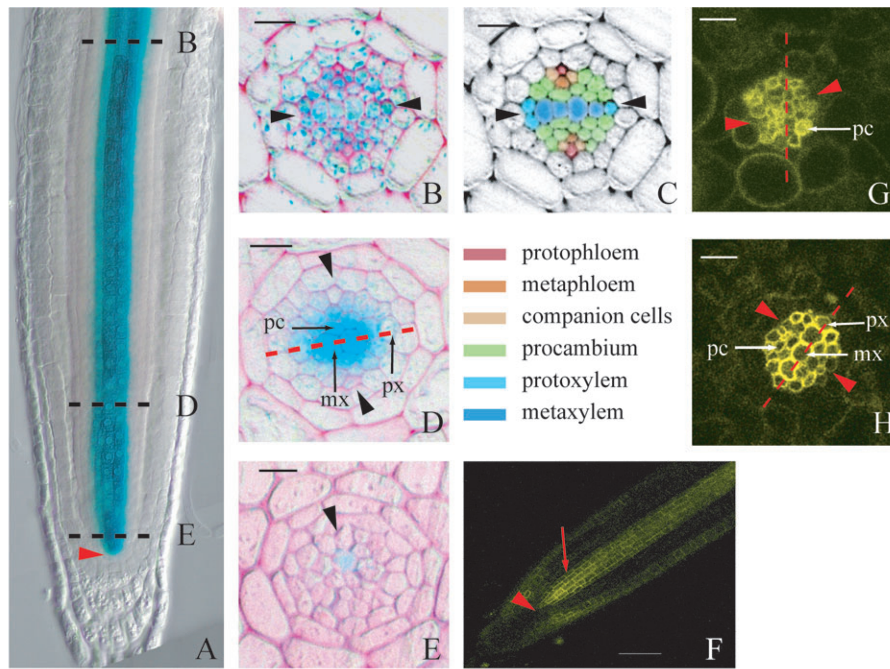

Fig. 1. *SERK1* expression in primary root tips. (A) Longitudinal view of a GUS-stained primary root of a *pSERK1::GUS* expressing seedling. Arrowhead indicates the QC. (B), (D) and (E) mark positions of sections shown in (B), (D) and (E). (B) GUS and Ruthenium red-stained 7 μm cross-section through a *pSERK1::GUS*-expressing root tip at 120–150 μm from QC. (C) The same as section shown in (B), but with cell types marked according to Mahonen *et al.* (2000). The arrowheads mark the xylem plate. (D) GUS and Ruthenium red-stained 7 μm cross-sections through a pSERK1::GUS-expressing root tip at 50–60 μm from the QC. Arrowheads mark the phloem initials. (E) GUS and Ruthenium red-stained 7 μm cross-sections through a *pSERK1::GUS*-expressing root tip at 10–15 μM from the QC. Arrowhead marks a single cell with GUS staining. (F) Longitudinal view of a *pSERK1::SERK1::YFP*-expressing primary root. Arrowhead indicates the QC and the red arrow marks cells with increased SERK1-YFP fluorescence. (G, H) YFP fluorescence in hand-cut section of a *pSERK1::SERK1::YFP*-expressing root tip at (G) 60 μm from the QC and (H) ~250 μm from the QC. mx, Metaxylem; pc, procambium; px, protoxylem. Scale bar = 20 μm.

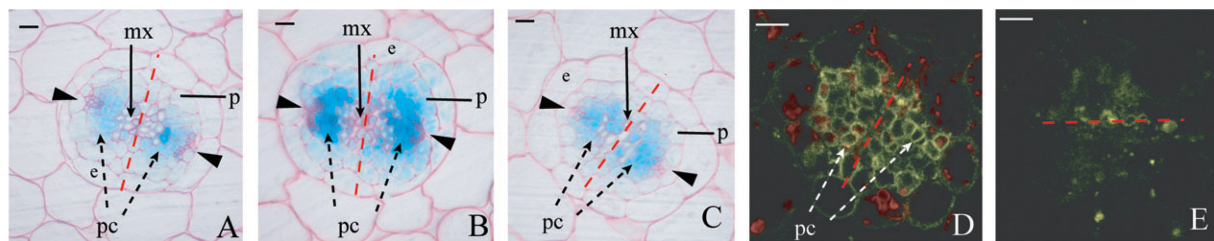

Fig. 2. *SERK1* expression in the hypocotyl. (A, B) GUS and Ruthenium red-stained 7 μm cross-sections through a *pSERK1::GUS*-expressing hypocotyl at (A) 70 μm from the apical meristem and (B) 50 μm from the apical meristem. (C) GUS and Ruthenium red-stained 7 μm cross-sections through a *pHB8-GUS* (Baima *et al*., 1995) expressing hypocotyl 70 μm from the apical meristem. (D) YFP and red chlorophyll fluorescence in hand-cut sections through a *pSERK1::SERK1::YFP*-expressing hypocotyl about 60 μm below the apical meristem. (E) YFP fluorescence in a hand-cut section through the hypocotyl of a wild-type plant. Dashed red line marks the xylem plate. e, Endodermis; p, pericycle; pc, procambium; mx, metaxylem; and arrowheads mark the phloem sieve elements. Scale bar = 20 μm.

vascular cells of the metaxylem and phloem and the pericycle. A hand-cut section through the hypocotyl of seedlings containing the *pSERK1::SERK1::YFP* construct showed a pattern similar to the GUS staining (Fig. 2D); YFP fluorescence was observed in the procambium cells. Figure 2E shows the yellow autofluorescence in a comparable section of a wild-type hypocotyl. Autofluorescence was visible in the metaxylem and in a few procambial cells. The YFP signal in the wild-type sections suggests that the remaining signal in the metaxylem seen in Fig. 2D is caused by autofluorescence. In the hypocotyl *SERK1* expression appears to be more restricted to the procambium and also here the protein localization follows the gene expression pattern closely. The HD-ZIPIII transcription factor HB8 has been reported as a regulator of procambium proliferation as well as a marker for procambium development (Baima et al., 1995, 2001). Figure 2C shows that *HB8* expression is indistinguishable from that of *SERK1* and also marks the procambium and metaxylem cells.

Subsequently, different positions of the inflorescence stem, representing a range of developmental stages of the

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inflorescence stem vascular tissue were studied. Sections were made of GUS-stained and plastic-embedded inflorescence stems of 4-week-old *Arabidopsis* plants containing the *pSERK1::GUS* fusion. Figure 3A shows a section through the vascular bundle at the base of the stem. The protoxylem, the metaxylem; procambium, and phloem tissues can be distinguished. Procambium cells are seen here as an arch of two to three cell layers between the xylem and phloem. Individual cells in the procambium seem to have an increased *SERK1* expression based on the GUS staining intensity. A section through the stem just below the second cauline leaf showed a similar pattern (Fig. 3B). Also here a limited amount of cells within the procambial layer showed GUS staining. Sections just above the first cauline leaf showed GUS staining in all procambial cells and in the immature metaxylem cells (Fig. 3C). Figure 3D shows a GUS-stained section of the HB8 promoter GUS marker line at the base of the inflorescence stem. *HB8* is transcribed in the protoxylem and procambium (Fig. 3D). At position 1 in the inflorescence stem (Fig. 3E), *HB8* was expressed in immature metaxylem, procambium, and single phloem cap cells. Figure 3F–H show hand-cut sections through the stems of 4-week-old *Arabidopsis* plants containing the *pSERK1::SERK1::YFP* construct. The red fluorescence in all images originates from chlorophyll. Figure 3F shows a section through a vascular bundle at the base of the stem. Similar to the GUS expression data (Fig. 3A), only a subset of procambium cells shows YFP fluorescence. Figure 3G shows a section just below the second cauline leaf. The brightest YFP fluorescence is seen in only a subset of the procambium cells. Yellow fluorescence was also observed in some phloem and phloem companion cells. Figure 3H shows a section just above the first cauline leaf of the *pSERK1::SERK1::YFP* line where yellow fluorescence is strongest in all procambial cells and immature metaxylem cells (imx), but also visible in cells in the interfascicular region. Autofluorescence in wild-type plants was restricted mainly to mature xylem elements (data not shown). In inflorescence stems, the SERK1 protein localization pattern closely follows the gene expression pattern. To summarize, *SERK1* appears to be expressed in procambium cells in the vasculature in all organs investigated. In roots, *SERK1* expression commences in all stem cells of the vascular tissue and continues in the first descendants of these stem cells. In roots, hypocotyls, and inflorescence stems, metaxylem cells in the immature vascular bundle also express *SERK1*.

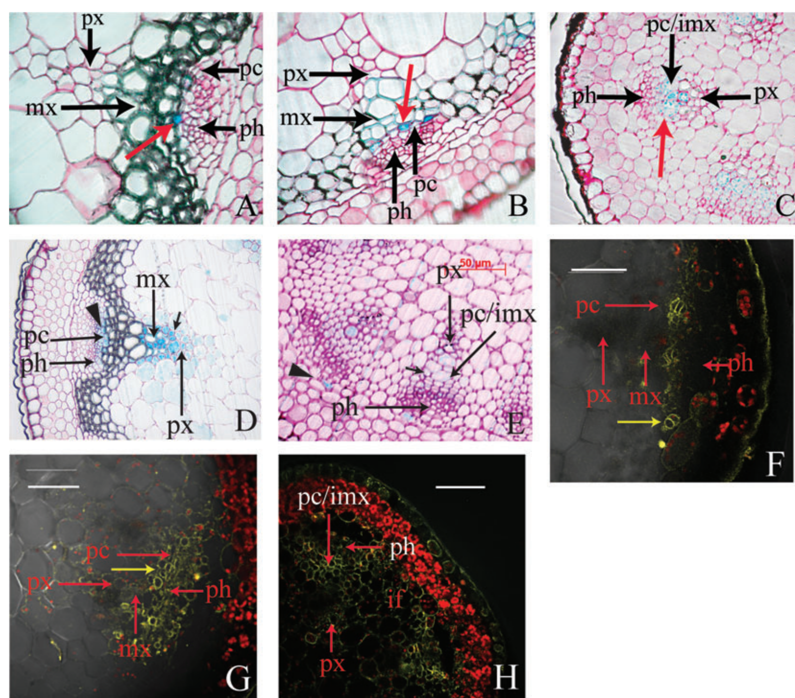

Fig. 3. *SERK1* expression in the inflorescence stem. (A–C) GUS and Ruthenium red-stained 7 μm cross-sections through the inflorescence stem of a 4-week-old *pSERK1::GUS*-expressing plant. (A) Base of the stem. (B) Just below the second cauline leaf. (C) Just above the first cauline leaf. Blue arrows mark cells with GUS staining. (D, E) GUS and Ruthenium red-stained 7 μm cross-sections through the inflorescence stem of a 4-week-old *pHB8::GUS*-expressing plant (Baima *et al*., 1995). (D) At position 4. The arrow marks GUS-stained protoxylem. The arrowhead marks GUS-stained procambium. (E) At position 1. The arrowhead marks a single phloem cap cell with GUS staining. The arrow marks procambium and immature metaxylem with GUS staining. (F–H) YFP fluorescence in hand-cut sections of stems from a *pSERK1::SERK1::YFP*-expressing plant. (F) Base of the stem. (G) Just below the second cauline leaf. (H) Just above the first cauline leaf. Yellow arrows mark cells with YFP fluorescence. px, Protoxylem; mx, metaxylem; imx, immature metaxylem; pc, procambium; ph, phloem. Scale bar = 50 μm.

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Prolonged exposure to 2,4-D induces the proliferation of SERK1 *expressing cells*

Given the findings that SERK1 marks and promotes the formation of cells competent to initiate somatic embryogenesis and the presence of SERK1 in the procambium, the response of the *SERK1* gene under conditions that allow formation of embryogenic cells was investigated. Embryogenic cell formation in *Arabidopsis* explant tissue requires a prolonged incubation with 2,4-dichlorophenoxyacetic acid (2,4-D) (Mordhorst *et al.*, 1997). *pSERK1::GUS*-expressing seedlings were transferred 4 DAG to plates containing 5 μm 2,4-D and were embedded and sectioned 2, 7, and 10 d after transfer. Representative sections are shown taken about 70 μm below the apical meristem. Cell and tissue types were assigned according to Busse and Evert (1999*a*, *b*). Figure 4A shows a section of a non-treated hypocotyl after 2 d. Procambium cells flanking the xylem seem to have more GUS staining. After 2 d of 2,4-D treatment there is a clear increase in the number of pericycle cells (compare bracket in Fig. 4A with bracket in Fig. 4D) that also begin to express *SERK1* (Fig. 4D). A slight increase in GUS staining was observed in the endodermal cell layer. Figure 4B shows a non-treated hypocotyl after 7 d. The vascular bundle is enlarged, due to an increase in the number of cells in all vascular tissues (e.g. xylem, phloem, pericycle, and procambium). The procambium cells flanking the mature xylem seem to have more GUS staining than the adjacent cells. At 7 d of 2,4-D treatment (Fig. 4E), the first procambium cells begin to proliferate, while the number of pericycle cells outside the phloem poles increased (compare bracket in Fig. 4B with bracket in Fig. 4E). The GUS staining is more even throughout the vascular bundle. The endodermis is flattened due to the enlargement of the vascular bundle. No expression of *SERK1* in the endodermis or tissue layers other than the vascular bundle was observed after 7 d of incubation with 2,4-D. Figure 4C shows a non-treated hypocotyl after 10 d. The *SERK1* expression pattern was similar to that shown in Fig. 4B; procambium cells flanking the mature xylem seemed to have more GUS staining compared with the surrounding tissues. After 10 d of 2,4-D treatment (Fig. 4F), a disorganized mass of cells is visible in the centre of the vascular bundle expressing *SERK1*. This mass of cells appears to have originated from the procambium cells rather than from the pericycle, because cells similar to the enlarged pericycle cells (bracket in Fig. 4F) observed after 2 d (bracket in Fig. 4D) and 7 d (bracket in Fig. 4E) of treatment with 2,4-D were still present and surrounding the mass of cells in the centre. The vascular bundle is completely disorganized due to the massive proliferation of cells from a procambial origin. Even after this prolonged incubation, cortical and epidermal cells do not express *SERK1* (Fig. 4F).

In roots, 2,4-D induces SERK1 *expression only in procambium cells*

The response of *SERK1* to 2,4-D in roots was investigated next. In roots, the different vascular cell types can more easily be recognized, so observed changes in *SERK1*

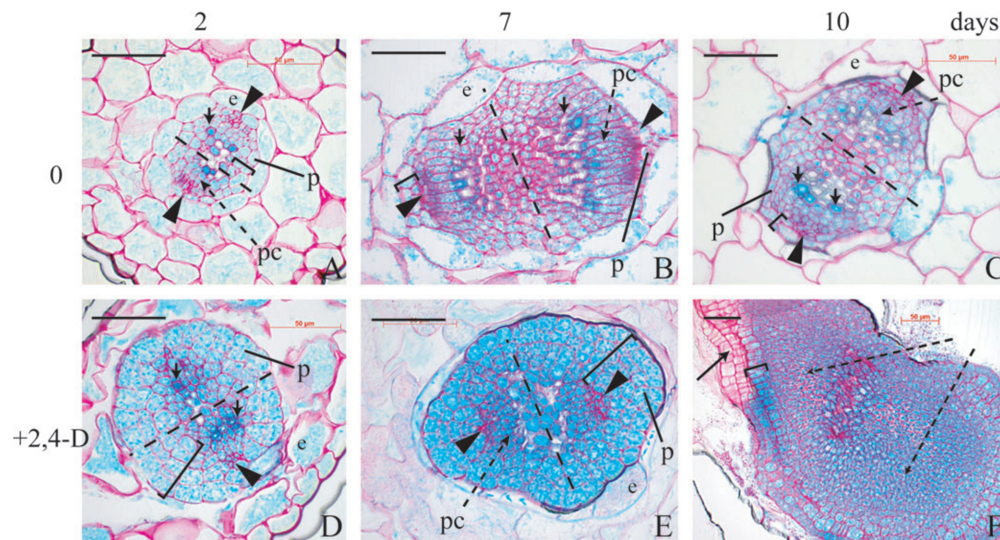

Fig. 4. Proliferation and *SERK1* expression in seedlings after prolonged 2,4-D treatment. (A–C) 7 μm cross-sections through the hypocotyl of GUS and Ruthenium red-stained *pSERK1::GUS*-expressing seedlings about 70 μm from the apical meristem. (A) Non-treated seedling after 2 d (6 DAG), (B) after 7 d (11 DAG), and (C) after 10 d (14 DAG). (D–F) Hand-cut sections from seedlings transferred to plates containing 5 μM 2,4-D 4 DAG: (D) after 2 d of treatment (6 DAG), (E) after 7 d of treatment (11 DAG), (F) after 10 d of treatment (14 DAG). Dashed arrows mark proliferating cells with *SERK1* expression. Arrows mark cells without *SERK1* expression. Arrowheads mark phloem. Small arrows mark *SERK1*-expressing procambium. Brackets mark pericycle cell layers and the dashed red line marks the xylem plate. pc, Procambium; e, endodermis; p, pericycle. Scale bar = 50 μm.

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expression in response to 2,4-D can be better assigned to specific cell types. Figure 5A shows GFP fluorescence in the root tip of the *DR5 element–GFP* marker line (Benkova *et al.*, 2003). An auxin maximum, visualized by high GFP fluorescence, is present in the QC, the columella initial cells, and the mature columella cells. A 2 d incubation of the same line with 0.1 μM 2,4-D strongly increased the GFP fluorescence in all root tissues (Fig. 5B). Higher concentrations (0.5 μM and 2 μM) of 2,4-D gave a similar response (data not shown). These results confirm that the application of 2,4-D results in an elevated auxin response in all root tissues. Figure 5C shows GUS staining in a non-treated root containing the *pSERK1::GUS* construct and the root shown in Fig. 5D was treated for 2 d with 0.1 μM 2,4-D. Higher concentrations of 2,4-D gave similar results (data not shown). SERK1 expression gradually increased, but remained localized to cells in the vascular bundle. Figure 5E shows a control root of a line containing a *pSERK1::SERK1::YFP* construct. Figure 5I shows SERK1–YFP fluorescence after 2 d of incubation with 0.1 μM 2,4-D. SERK1–YFP fluorescence increased slightly and the region with increased SERK1-YFP fluorescence was longer and wider compared to non-treated roots. In addition, the incubation of roots with 2,4-D resulted in an increase in autofluorescence in the epidermis (Fig. 5F) Higher concentrations of 2,4-D showed similar results (data not shown). In response to the 2,4-D treatment, *SERK1* transcription was clearly increased, but only resulted in a slight increase of SERK1–YFP protein. No induction of *SERK1* was seen in cells outside the vascular tissue. To determine which cells actually showed a change in SERK–YFP protein, cross-sections were made about 60 μm from the QC. Figure 5G shows a section through a non-treated seedling. Figure 5H shows a section of a seedling treated with 0.1 μM 2,4-D for 2 d. YFP fluorescence is elevated in all procambium cells of the 2,4-D-treated seedlings, while the amount of cells present at this stage was similar to non-treated seedlings. Similar experiments in the hypocotyl did not show an increase in SERK1–YFP protein. Taken together these observations suggest that the observed limited increase in SERK1–YFP protein after 2,4-D treatment is due to an increase in transcription of the *SERK1* gene in procambium cells and not due to the increased proliferation of cells expressing *SERK1*. A comparable result was obtained after treatment of roots containing the *pSERK1::GUS and pSERK1::SERK::YFP* constructs with NPA (data not shown).

Discussion

Earlier observations suggested that the *Arabidopsis SERK1* gene is expressed in sporophytic tissues, during lateral root outgrowth and in the vascular bundles (Hecht *et al.*, 2001; Kwaaitaal *et al.*, 2005). Here it is shown that the SERK1 gene is expressed in the procambium of the vascular bundles in roots, hypocotyls, and inflorescence stems. In younger parts of roots and hypocotyls, *SERK1* expression was less restricted and was also observed in protoxylem cells, immature metaxylem cells, and phloem companion cells. In roots, *SERK1* expression was first detected in root vascular stem cells and was notably

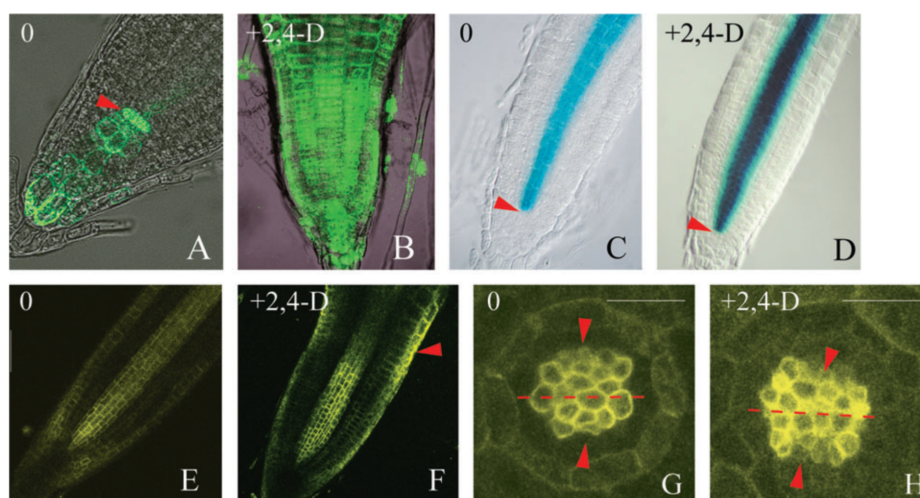

Fig. 5. *SERK1* gene expression and protein level in roots after 2,4-D treatment. (A, B) GFP fluorescence in primary root tips of *DR5-element::GFP*-expressing seedlings. (A) Non-treated, (B) 2 d of treatment with 0.1 μM 2,4-D. (C) Primary root tip of a *pSERK1::GUS*-expressing and GUS-stained seedling. (D) Primary root tip of a *pSERK1::GUS*-expressing seedling treated with 0.1 μM 2,4-D for 2 d and GUS stained. (E) YFP fluorescence in primary root tips of a *pSERK1::SERK1::YFP*-expressing seedling. (F) YFP fluorescence in primary root tips of a *pSERK1::SERK1::YFP*-expressing seedling after 2 d of treatment with 0.1 μM. 2,4-D. Arrowhead marks 2,4-D induced autofluorescence in the epidermis. (G, H) YFP fluorescence in hand-cut sections ~60 μm from the QC of *pSERK1::SERK1::YFP*-expressing seedlings. (G) Non-treated, (H) treated for 2 d with 0.1 μM 2,4-D. The dashed red line marks the xylem plate and the arrowheads in (G) and (H) mark the phloem poles. Scale bar = 20 μm.

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absent from the QC. In hypocotyls, prolonged application of 2,4-D resulted in extensive unorganized proliferation of *SERK1*-expressing cells originating from the procambium and pericycle. In roots, 2,4-D treatment results in an increase in *SERK1* transcription that results in a moderate increase in the amount of SERK1–YFP fusion protein. The restricted vascular pattern of *SERK1* expression in roots remains unaffected after 2,4-D treatment.

SERK1 *as a marker of transit amplifying cells?*

To help answer this question it is essential to determine the identity of the *SERK1*-expressing cells *in planta*. Because the *SERK1* gene was originally identified as a marker for embryogenically competent single cells in tissue culture (Schmidt *et al*., 1997), an important remaining question is whether this competence was newly acquired or already present in the organized explant tissue. Procambium is regarded as a primary meristematic tissue with the task to provide xylem and phloem cells (Steeves and Sussex, 1989; Mahonen *et al*., 2000). Procambium cells fulfil the criteria for being stem cells since they have the capacity for long-term self renewal and being able to differentiate into one or more specialized cell types (Alison *et al*., 2002; Rippon and Bishop, 2004). Procambium cells can, therefore, be regarded as pluripotent stem cells (Mahonen *et al*., 2006) and these pluripotent cells express *SERK1*. Both plant and animal stem cell pools are maintained in niches by signals from surrounding cells. Stem cell divisions are intrinsically asymmetric, due to the ability to generate one cell that remains as a stem cell and another that exits the niche and differentiates to become a Transit Amplifying (TA) cell. The main task of TA cells is to increase the population of cells originating from a single stem cell division. These cells have a limited proliferative capacity and a limited differentiation potential (Stahl and Simon, 2005; Singh and Bhalla, 2006). The first TA cells maintain many of the characteristics of the stem cells and gradually lose these properties with continued division and differentiation. The progressive differentiation of the TA cells, will coincide with activation of genes marking the differentiated state (Potten and Loeffler, 1990). When this TA cell model is superimposed on the plant vasculature, the immature vascular cells proximal to the root stem cells can be regarded as TA cells (Stahl and Simon, 2005). *SERK1* is expressed in most immature vascular cells and, upon maturation of the organ, becomes confined to the procambium. It is therefore proposed that *SERK1* expression marks the vascular TA cell population.

In *Arabidopsis*, the SAM and the cotyledon axils of embryos are reported as origins of somatic embryos (Mordhorst *et al*., 1998, 2002), while *SERK1* gene expression was reported to be increased by 2,4-D in both the SAM and at the base of the cotyledons (Hecht *et al*., 2001). However, neither the meristem itself nor the stem cells present in the meristem appear to be required because mutants lacking a functional SAM like *shoot meristemless* (*stm*), *wuschel* (*wus*), and *zwille/pinhead* (*zll/pnh*) did not show a reduction in embryogenic competence (Mordhorst *et al*., 2002). In a cytological study where *Arabidopsis* embryos were followed over time in the presence of 2,4-D, it was found that the shoot apical meristem and the hypocotyl did not participate in somatic embryogenesis as long as cells in the cotyledons were actively dividing. Instead, cell divisions were initiated in procambial cells eventually leading to the formation of somatic embryos on the cotyledons (Raghavan, 2004). Also in carrot (Guzzo *et al*., 1994, 1995) and in *Dactylis glomerata* L., cells close to the vascular bundles of leafs are thought to be the origin of somatic embryos (Somleva *et al*., 2000). So, apparently the property to form embryogenic cells in tissue culture is restricted to the vascular stem cell or TA cell population and is not shared with the stem cells in the apical meristem.

In carrot tissue culture, *DcSERK* marks a single embryogenic competent cell and somatic embryos only originate from *DcSERK*-expressing cells (Schmidt *et al*., 1997). However, not all *SERK*-expressing cells develop into embryos. *Medicago SERK1* is induced by 2,4-D in both embryogenic and non-embryogenic lines (Nolan *et al*., 2003), which also indicates that not all *SERK*-expressing cells are embryogenically competent. Most likely other factors present in a subpopulation of the *SERK1*-expressing cells are needed to specify embryogenic competence fully.

SERK1 *expression remains tissue-specific in the presence of 2,4-D*

Prior to the initiation of proliferation in both root and hypocotyl, 2,4-D treatment induced *SERK1* transcription and SERK1 protein levels only in the vascular bundles. In the hypocotyl, 2,4-D resulted in an increase in cell size and limited proliferation of the hypocotyl pericycle. This is similar to what was observed in carrot hypocotyl explants, where, as a response to 2,4-D, epidermis and cortical parenchyma cells increased in size while provascular cells increased in size and divided (Guzzo *et al*., 1994, 1995). In cultures prepared from carrot hypocotyl explants, the enlarged cells originating from the provascular tissue, are the ones that have become competent for embryogenesis and express the *SERK1* gene (Schmidt *et al*., 1997). Competent enlarged cells undergo an asymmetric division, which is considered to be the earliest event in both zygotic and somatic embryogenesis. From this asymmetric division small, isodiametric cells are generated, which proliferate into so-called pro-embryonic masses that generate somatic embryos after the removal of 2,4-D (Guzzo *et al*., 1994). One unanswered question in these complex and sequential events in the pathway to

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somatic embryos is whether exogenous application of 2,4-D acts via modification of endogenous IAA or that competent cell formation is a direct response to 2,4-D. Due to the scarcity of markers available it is difficult to distinguish between these possibilities during the actual formation of competent cells. However, in root tissues with their precise developmental history it was possible to determine whether *SERK1* expression responded directly to changes in endogenous auxin. The results clearly showed that, in the presence of 2,4-D, *SERK1* transcription is not initiated in cells other than the vascular cells, suggesting that *SERK1* expression remains under tight tissue-specific control. If this control persists in tissue culture, it is proposed that the single *SERK*-expressing embryogenic carrot cells as well as their *Arabidopsis* counterparts (Hecht *et al.*, 2001) represent a small population of cells retaining their procambium fate. Unfortunately, the HB8 promoter strongly responds to auxin (Baima *et al.*, 1995) and could not be used to verify this hypothesis in tissue culture (data not shown). As often noted in tissue culture experiments, only after the prolonged exposure of explant tissue to 2,4-D were somatic embryos formed, while shorter incubations only resulted in root and shoot formation. In this study, 2,4-D treatment initiated divisions in the pericycle after 2 d, whereas incubation of seedlings longer than 7 d resulted in the mass proliferation of procambium cells that express *SERK1*. In carrot, a similar period of incubation with 2,4-D is needed to obtain embryogenically competent cells (Guzzo *et al.*, 1995) that express *DcSERK* (Schmidt *et al.*, 1997). These experiments suggest that induction of *SERK* gene expression by exogenous growth regulators such as 2,4-D is not regulated via modulation of endogenous IAA levels.

The continued presence of *SERK1* in the procambium TA cell population makes it tempting to speculate that *SERK1* functions in maintaining the pluripotent fate of these cells. The observations that *SERK1* overexpression increases the competency in culture (Hecht *et al.*, 2001) supports this theory and would argue for an increase in the population of procambium/TA cells or possibly increased *SERK1* levels keep these TA cells closer to a pluripotent fate, which would make the transition to a totipotent fate easier. The *serk1* mutant described by Albrecht *et al.* (2005) did not show a reduction in competency (MACJ Kwaaitaal and SC de Vries, unpublished results), suggesting that either SERK1 is not essential for, or functions redundantly in, the acquisition of a competent state or in the maintenance of a pluripotent state. The recent discovery that SERK1 is part of a protein complex including BRI1 and SERK3 and also genetically together with BRI1 functions in BR signalling (Karlova *et al.*, 2006) makes it tempting to speculate that BRs are the long sought ligands of the SERK1 signalling complex that regulates the acquisition of a competent state. The existence of a BRI1–SERK1 signalling complex in competent cells still needs to be demonstrated. However, preliminary evidence showing that BR synthesis and *bri1* receptor mutants show a reduced competence in culture (MACJ Kwaaitaal and SC de Vries, unpublished results) support a role for BR signalling in somatic embryogenesis.

In summary, it is proposed that *SERK1* is expressed in the procambium and the TA cell population. Upon treatment with 2,4-D some of these TA or procambium cells are released and revert to a totipotent state in the absence of their normal 'vascular' cues. In this scenario, *SERK1* expression marks and *SERK1* possibly maintains a pluripotent cell population residing in plant vascular tissues.

Acknowledgements

We thank Simona Baima and Giorgio Morelli for providing us with the *HB8 promoter–GUS* seeds (Baima *et al.*, 1995). Jiří Friml for providing us with the *DR5–GFP reporter* line (Benkova *et al.*, 2003). We thank Boudewijn van Veen for help on the digital art. We thank Dolf Weijers for critical reading of the manuscript. This work was supported by grant ERBIO4-CT96-0689 from the EU Biotechnology program, grant QLG2-2000-00602 from the EU Quality of Life and Management of Living Resources program, and Wageningen University, Department of Agrotechnology and Food Sciences to MK and SdV.

References

Albrecht C, Russinova E, Hecht V, Baaijens E, de Vries S. 2005. The *Arabidopsis thaliana* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES 1 and 2 control male sporogenesis. *The Plant Cell* **17,** 3337–3349.

Alison MR, Poulsom R, Forbes S, Wright NA. 2002. An introduction to stem cells. *Journal of Pathology* **197,** 419–423.

Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I, Morelli G. 1995. The expression of the *Athb-8* homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*. *Development* **121,** 4171–4182.

Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G. 2001. The *Arabidopsis* ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiology* **126,** 643–655.

Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jurgens G, Friml J. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115,** 591–602.

Boutilier K, Offringa R, Sharma VK, *et al.* 2002. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *The Plant Cell* **14,** 1737–1749.

Busse JS, Evert RF. 1999*a*. Pattern of differentiation of the first vascular elements in the embryo and seedling of *Arabidopsis thaliana*. *International Journal of Plant Sciences* **160,** 1–13.

Busse JS, Evert RF. 1999*b*. Vascular differentiation and transition in the seedling of Arabidopsis thaliana (Brassicaceae). *International Journal of Plant Sciences* **160,** 241–251.

Colcombet J, Boisson-Dernier A, Ros-Palau R, Vera CE, Schroeder JI. 2005. *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASES 1 and 2 are essential for tapetum development and microspore maturation. *The Plant Cell* **17,** 3350–3361.

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Dean Rider Jr S, Henderson JT, Jerome RE, Edenberg HJ, Romero-Severson J, Ogas J. 2003. Coordinate repression of regulators of embryonic identity by PICKLE during germination in *Arabidopsis*. *The Plant Journal* **35,** 33–43.

Feher A, Pasternak TP, Dudits D. 2003. Transition of somatic plant cells to an embryogenic state. *Plant Cell Tissue and Organ Culture* **74,** 201–228.

Feher A, Pasternak T, Otvos K, Miskolczi P, Dudits D. 2002. Induction of embryogenic competence in somatic plant cells: a review. *Biologia* **57,** 5–12.

Fukuda H. 2004. Signals that control plant vascular cell differentiation. *Nature Reviews Molecular Cell Biology* **5,** 379–391.

Gaj MD, Zhang S, Harada JJ, Lemaux PG. 2005. Leafy cotyledon genes are essential for induction of somatic embryogenesis of *Arabidopsis*. *Planta* **222,** 977–988.

Guzzo F, Baldan B, Levi M, Sparvoli E, Loschiavo F, Terzi M, Mariani P. 1995. Early cellular events during induction of carrot explants with 2,4-D. *Protoplasma* **185,** 28–36.

Guzzo F, Baldan B, Mariani P, Loschiavo F, Terzi M. 1994. Studies on the origin of totipotent cells in explants of *Daucus carota* L. *Journal of Experimental Botany* **45,** 1427–1432.

Harding EW, Tang WN, Nichols KW, Fernandez DE, Perry SE. 2003. Expression and maintenance of embryogenic potential is enhanced through constitutive expression of AGAMOUS-Like 15. *Plant Physiology* **133,** 653–663.

Hecht V, Vielle-Calzada JP, Hartog MV, Schmidt EDL, Boutilier K, Grossniklaus U, de Vries SC. 2001. The *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiology* **127,** 803–816.

Hu H, Xiong L, Yang Y. 2005. Rice SERK1 gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. *Planta* **222,** 107–117.

Karlova R, Boeren S, Russinova E, Aker J, Vervoort J, de Vries S. 2006. The *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1 protein complex includes BRASSINOSTEROID-INSENSITIVE 1. *The Plant Cell* **18,** 626–638.

Kwaaitaal MA, de Vries SC, Russinova E. 2005. *Arabidopsis thaliana* somatic embryogenesis receptor kinase 1 protein is present in sporophytic and gametophytic cells and undergoes endocytosis. *Protoplasma* **226,** 55–65.

Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ. 1998. *Arabidopsis* LEAFY COTYLEDON 1 is sufficient to induce embryo development in vegetative cells. *Cell* **93,** 1195–1205.

Mahonen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, Tormakangas K, Ikeda Y, Oka A, Kakimoto T, Helariutta Y. 2006. Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* **311,** 94–98.

Mahonen AP, Bonke M, Kauppinen L, Riikonen M, Benfey PN, Helariutta Y. 2000. A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root. *Genes and Development* **14,** 2938–2943.

Mordhorst AP, Hartog MV, El Tamer MK, Laux T, de Vries SC. 2002. Somatic embryogenesis from *Arabidopsis* shoot apical meristem mutants. *Planta* **214,** 829–836.

Mordhorst AP, Toonen MA, De Vries S. 1997. Plant embryogenesis. *Critical Reviews in Plant Science* **16,** 535–576.

Mordhorst AP, Voerman KJ, Hartog MV, Meijer EA, van Went J, Koornneef M, de Vries SC. 1998. Somatic embryogenesis in *Arabidopsis thaliana* is facilitated by mutations in genes repressing meristematic cell divisions. *Genetics* **149,** 549–563.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15,** 473–497.

Nolan KE, Irwanto RR, Rose RJ. 2003. Auxin up-regulates MtSERK1 expression in both *Medicago truncatula* root-forming and embryogenic cultures. *Plant Physiology* **133,** 218–230.

Nomura K, Komamine A. 1985. Identification and isolation of single cells that produce somatic embryos at a high-frequency in a carrot suspension-culture. *Plant Physiology* **79,** 988–991.

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

Ogas J, Kaufmann S, Henderson J, Somerville C. 1999. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **96,** 13839–13844.

Perry SE, Lehti MD, Fernandez DE. 1999. The MADS-domain protein AGAMOUS-like 15 accumulates in embryonic tissues with diverse origins. *Plant Physiology* **120,** 121–129.

Perry SE, Nichols KW, Fernandez DE. 1996. The MADS domain protein AGL15 localizes to the nucleus during early stages of seed development. *The Plant Cell* **8,** 1977–1989.

Potten CS, Loeffler M. 1990. Stem cells: attributes, cycles, spirals, pitfalls, and uncertainties. Lessons for and from the crypt. *Development* **110,** 1001–1020.

Raghavan V. 2004. Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of *Arabidopsis*: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. *American Journal of Botany* **91,** 1743–1756.

Rippon HJ, Bishop AE. 2004. Embryonic stem cells. *Cell Proliferation* **37,** 23–34.

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

Singh MB, Bhalla PL. 2006. Plant stem cells carve their own niche. *Trends in Plant Science* **11,** 241–246.

Somleva MN, De Vries SC, Schmidt ED. 2003. Embryogenic cells in *Dactylis glomerata* L. (Poaceae) explants indentified by cell tracking and by SERK expression. *Plant Cell Reports* **19,** 718–726.

Stahl Y, Simon R. 2005. Plant stem cell niches. *International Journal of Developmental Biology* **49,** 479–489.

Steeves TA, Sussex M. 1989. *Patterns in plant development*. Cambridge: Cambridge University Press.

Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ. 2001. LEAFY COTYLEDON 2 encodes a B3 domain transcription factor that induces embryo development. *Proceedings of the National Academy of Sciences, USA* **98,** 11806–11811.

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

Toonen MAJ, Hendriks T, Schmidt EDL, Verhoeven HA, Vankammen A, De Vries SC. 1994. Description of somatic-embryo-forming single cells in carrot suspension-cultures employing video cell tracking. *Planta* **194,** 565–572.

Xu J, Hofhuis H, Heidstra R, Sauer M, Friml J, Scheres B. 2006. A molecular framework for plant regeneration. *Science* **311,** 385–388.

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

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