Differential expression of the *Arabidopsis* genes coding for Em-like proteins

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Abstract

Late embryogenesis abundant (*lea*) genes are a large and diverse group of genes highly expressed during late stages of seed development. Five major groups of LEA proteins have been described. Two *Em* genes (group I *lea* genes) are present in the genome of *Arabidopsis thaliana* L., *AtEm1* and *AtEm6*. Both genes encode for very similar proteins which differ basically in the number of repetitions of a highly hydrophilic amino acid motif. The spatial patterns of expression of the two *Arabidopsis* *Em* genes have been studied using *in situ* hybridization and transgenic plants transformed with the promoters of the genes fused to the β-glucuronidase reporter gene (*uidA*). In the embryo, *AtEm1* is preferentially expressed in the pro-vascular tissues and in meristems. In contrast, *AtEm6* is expressed throughout the embryo. The activity of both promoters disappears rapidly after germination, but is ABA-inducible in roots of young seedlings, although in different cells: the *AtEm1* promoter is active in the internal tissues (vasculature and pericycle) whereas the *AtEm6* promoter is active in the external tissues (cortex, epidermis and root hairs). The *AtEm1* promoter, but not *AtEm6*, is also active in mature pollen grains and collapsed nectaries of young siliques. These data indicate that the two *Em* proteins could carry out at least slightly different functions and that the expression of *AtEm1* and *AtEm6* is controlled at, at least, three different levels: temporal, spatial and hormonal (ABA).

Key words: Embryogenesis, LEA, promoter, transgenic.

Introduction

Seed maturation is characterized by a desiccation process. During desiccation, a number of specific proteins referred to as LEA (Late Embryogenesis Abundant proteins) accumulate in the embryo (Baker *et al.*, 1988; Hughes and Galau, 1989). According to their accumulation pattern and physico-chemical proprieties, it has been suggested that LEA proteins could be involved in seed desiccation tolerance (Dure *et al.*, 1989; Hughes and Galau, 1989; Dure 1993; Leprince *et al.*, 1993; Xu *et al.*, 1996; Kermode, 1997). LEA proteins have a widespread distribution among plant species. Five major groups of LEA proteins have been described on the basis of their amino acid sequence homologies (Dure *et al.*, 1989; Delseny *et al.*, 1993). Expression of many *lea* genes can be precociously induced in immature seeds or in vegetative tissues upon ABA treatment or by osmotic or water-deficit stress (Skriver and Mundy, 1990; Jakobsen *et al.*, 1994). The first LEA protein was identified in wheat, and corresponded to the so-called Em protein (Cuming and Lane, 1979). Since then, several *Em-like* coding genes have been isolated and characterized in many monocot and dicot species (Vicient *et al.*, 1998), all of them encoding proteins having the highly conserved hydrophilic 20 amino acid motif (Dure *et al.*, 1989). In some species, the *Em* genes are encoded by multigene families, sometimes encoding proteins with different numbers of the 20 amino acid motif arranged in tandem, as for example in cotton (Galau *et al.*, 1992), maize (Williams and Tsang, 1992), barley (Espelund *et al.*, 1992; Stacy *et al.*, 1995), *Arabidopsis* (Gaubier *et al.*, 1993), mung bean (Manickam *et al.*, 1996) or soybean (Calvo *et al.*, 1997). *Em* genes...
are physiologically expressed only during the later steps of seed maturation (Hughes and Galau, 1989, 1991; Raynal et al., 1989; Almoguera and Jordano, 1992; Espelund et al., 1992; Gaubier et al., 1993; Hollung et al., 1994; Parcy et al., 1994) and are not inducible in adult vegetative tissues. However, the expression of these genes can be induced by ABA application in immature seeds and young seedlings (Williamson and Quatrano, 1988; Morris et al., 1990, Williams and Tsang, 1991; Almoguera and Jordano, 1992; Espelund et al., 1992; Gaubier et al., 1993; Hollung et al., 1994; Manickam et al., 1996). Their expression is reduced in ABA-deficient mutants and suppressed in some ABA-insensitive mutants (McCarty et al., 1991; Butler and Cuming, 1993; Parcy et al., 1994; Vasil et al., 1995). There are few examples in which the expression of the various members of an Em gene family has been characterized. The members of the barley B19 (Em-like) gene family accumulate to different levels in the developing embryo and respond differentially to salt-stress (Espelund et al., 1992).

Despite this information on Em gene expression, very little information is known about the spatial patterns of expression of the Em genes in any species. In situ hybridization using a carrot EMB-1 probe showed that most of this mRNA is localized in the meristematic regions, particularly in the procambium (Wurtele et al., 1993). GUS activity in transgenic tobacco seeds transformed with the promoter of the wheat Em gene fused to the uidA reporter gene showed uniform GUS activity throughout the embryo (Marcotte et al., 1989). The Arabidopsis AtEm1 promoter directs GUS activity uniformly throughout the embryo of transgenic tobacco (Hull et al., 1996).

During recent years, this laboratory has focused on the analysis of expression of Em genes in Arabidopsis thaliana. The genome of Arabidopsis thaliana contains two Em genes, AtEm1 and AtEm6 (Gaubier et al., 1993). The AtEm6 protein has one copy of the 20-amino-acid motif, whereas AtEm1 has four copies arranged in tandem. Although the AtEm1 and AtEm6 mRNAs accumulate to high levels in dry seeds, they appear to be differentially regulated. AtEm1 is expressed less abundantly and at a slightly earlier stage in seed development than AtEm6 mRNA (Gaubier et al., 1993; Bies et al., 1998) and AtEm1 and AtEm6 are differentially regulated by the ABI3 protein (Parcy et al., 1994). The promoter sequences of these genes do not reveal any similarity (Gaubier et al., 1993). As a part of the efforts to understand the differential regulation of the two Em genes in Arabidopsis thaliana, the spatial patterns of expression of both genes have been studied. In situ RNA hybridization and transgenic Arabidopsis plants containing the promoters of these genes fused to the β-glucuronidase reporter gene were used. This study shows that AtEm1 and AtEm6 genes are subjected to different tissue-specific control and that the AtEm1 promoter, but not AtEm6, drives GUS activity in pollen and collapsed nectaries.

**Materials and methods**

**In situ hybridization**

Mature Arabidopsis seeds were fixed in 4% paraformaldehyde, 0.25% glutaraldehyde, 0.1 M NaCl, 10 mM sodium phosphate pH 7.2. Seeds were embedded in 1% agarose in sodium phosphate buffer and washed twice in the same buffer. Fixed samples were dehydrated by a passage through ethanol/water-solutions. The dehydrated samples were then incubated with xylene. The seeds were embedded in paraffin at 60 °C for 2 d replacing paraffin four times. The embedded material was cut into 7 μm thick slices with a rotatory microtome and placed onto poly-o-lysine coated slides. The RNA probes were prepared using pBluescriptII constructs (Stratagene) and the T7 or T3 RNA polymerases in the presence of digoxigenine-uridine 5′ triphosphate (Boehringer). Final probe concentration was 100 ng ml⁻¹ in hybridization buffer (0.3 M NaCl, 50% formamide, 1 × Denhardt’s solution, 10% dextran sulphate, 10 mM TRIS-HCl pH 7.5, 1 mM EDTA, 60 mM DTT, 150 μg ml⁻¹ yeast tRNA, and 300 μg ml⁻¹ poly(A) RNA). Labelled probes were heated at 80 °C for 5 min and added to the hybridization buffer. Hybridization controls with sense probes were systematically carried out. Prior to hybridization, paraffin was removed and sections rehydrated by passage through ethanol/water solutions containing increasing amounts of water. The sections were subjected to proteinase K digestion in 0.5 M NaCl, 100 mM TRIS-HCl pH 7.5, 1 mM EDTA at 37 °C for 30 min. Samples then were incubated in 0.2% glycerol in PBS buffer (10 mM sodium phosphate pH 7.5, 150 mM NaCl), rinsed in PBS, treated with 0.5% acetic anhydride in 0.1 M triethanolamidine pH 8.0 for 10 min., rinsed again in PBS and then in 2 × SSC. Hybridization was carried out at 42 °C overnight in a humid chamber. After hybridization, the samples were washed with 2 × SSC at 37 °C. Digestion of non-specifically bound probe was done with 25 μg ml⁻¹ RNase in 0.5 M NaCl, 100 mM TRIS-HCl pH 7.5, EDTA 1 mM at 37 °C for 30 min, and then washed three times in the same buffer and conditions. After additional washes (once for 10 min, 2 × SSC at 55 °C, twice for 20 min, 0.2 × SSC at room temperature and once for 5 min in PBS), sections were blocked for 1 h at room temperature with 1% BSA, 0.5% blocking reagent (Boehringer) in TTBS (0.15% Triton X100, 150 mM NaCl, 100 mM TRIS-HCl pH 7.5), and then incubated for 1 h at room temperature with antidigoxigenin antibodies coupled with alkaline phosphatase in blocking buffer. Unbound antibody was removed by several 5 min washes in TTBS. Alkaline phosphatase activity was revealed with 5-bromo-4-chloro-3-indolyl-phosphate and mRNA (Gaubier et al., 1993). Their samples were dehydrated by a passage through ethanol and then incubated for 1 h at room temperature with antidigoxigenin antibodies coupled with alkaline phosphatase in blocking buffer. Unbound antibody was removed by several 5 min washes in TTBS. Alkaline phosphatase activity was revealed with 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium. Sections were examined in a differential interference contrast optic microscope (Zeiss axioplan).

**DNA constructs and transformation**

The construction pEm1 containing the promoter region of the AtEm1 gene is described elsewhere (pEm1.1443 in Hull et al., 1996). pEm6 contains the entire cloned promoter region of AtEm6 gene (Gaubier et al., 1993). A fragment extending from −1188 bp to +85 bp (relative to start of transcription) was excised by digestion with SpelI and AhaII, and ligated into pH101.2 digested with SpelI and AccI. AhaII cuts 1 bp 3′ of the initiation codon of AtEm6 and ligation into the AccI site of pBl101.2 generates a transcriptional fusion with a 13 amino acid Em-like gene family accumulate to different levels in the developing embryo and respond differentially to salt-stress (Espelund et al., 1992).
acid N-terminal extension. The sequences of the GUS fusion junctions were checked by sequencing.

pEm1 and pEm6 constructs were transferred to Agrobacterium tumefaciens LBA4404 by triparental mating. Roots of Arabidopsis thaliana cv. C24 were transformed as described previously (Clarke et al., 1992). Transformants were transferred to soil and grown in a growth room at 25 °C, 16 h day. Most analyses were performed with plants of three lines per construct homozygous for the T-DNA insertion with medium to high levels of GUS activity in seeds. All transgenic plants were grown according to the French safety guidelines and the French Genetic Engineering Commission approved experiments with these plants.

Fluorometric measurement and histochemical localization of β-glucuronidase (GUS) activity

GUS assays were carried out essentially as described previously (Jefferson, 1987). Fluorometric GUS assays based on the enzymatic conversion of 4-methylumbelliferyl glucuronide (4-MUG) to 4-methylumbelliferone (4-MU) were carried out in microtitre plates using a Fluoroskan II automated fluorometer (Labsystems, Helsinki) in conjunction with computer software Deltasoft™ (BioMetallics Inc. Princeton). Plant samples for GUS assays were ground up in 50 mM TRIS-HCl pH 7.0, 0.1% Triton, 10 mM EDTA, 3 mM DTT. Protein content of the extracts was determined according to Bradford (Bradford, 1976). Histochemical staining of GUS activity was carried out as described previously (Hull and Devic, 1995) using X-GLUC (5-bromo-4-chloro-3-indolyl-β-D-glucuronide) as substrate. Transverse sections of stained tissues were prepared using cleared and dehydrated tissues embedded in Historesin™ (Leica Instruments GmbH) according to manufacturer’s protocols. 10 micrometer sections were made with a microtome and mounted on a glass slide.

Abscisic acid treatments

For ABA treatment, (+)-ABA (Sigma) was added to water from a stock solution. The stock solution consisted in ABA dissolved in ethanol to a final concentration of 50 mM. Seeds were germinated and at 7 d or 21 d after germination sprayed with a 100 μM ABA. Control plants were sprayed with water supplemented with the equivalent concentration of ethanol. Both groups of plants were grown alongside each other in a growth chamber (25 °C, 16 h day) for 36 h.

Results

Localization of the expression of AtEm1 and AtEm6 mRNAs in the embryo of Arabidopsis thaliana

RNA slot blot analysis showed that the Arabidopsis Em genes are strongly expressed in the mature seed (Gaubier et al., 1993). To compare the distribution of AtEm1 and AtEm6 transcripts in the mature embryo, mRNA in situ hybridizations were performed. Dry Arabidopsis seeds were embedded in paraffin, sectioned, and hybridized with antisense digoxigenin-labelled RNA probes specific for each of the Arabidopsis Em genes. These probes correspond to the 3’ untranslated region of the genes and control experiments demonstrated that there was no cross-hybridization (Gaubier et al., 1993). A different spatial pattern of hybridization was observed using AtEm1 or AtEm6 probes. AtEm1 is preferentially expressed in the provascular tissue of the embryonic axis and in the root tip (Fig. 1A). Slight hybridization signals were also detected in the shoot apical meristem and in the cotyledon provascular bundles. The specificity of the hybridization was confirmed by the lack of signal in the hybridization controls using sense RNA probes that had been labelled to the same specific activity as the antisense ones (Fig. 1B). The AtEm6 antisense probe (Fig. 1C) hybridized throughout the entire embryo with a stronger signal in provascular tissues and shoot apical meristem. As in the case of the AtEm1 gene, the hybridization controls using the sense RNA probe labelled to the same specific activity as the antisense one, no hybridization signal was detected (data not shown). AtEm1 and AtEm6 transcripts did not accumulate in the seed coat.

Fig. 1. Expression of AtEm1 and AtEm6 mRNAs in longitudinal sections of Arabidopsis mature seeds visualized by in situ hybridization. Paraffin embedded sections of mature seeds were hybridized with (A) DIG-labelled AtEm1 antisense probe, (B) AtEm1 sense probe and (C) AtEm6 antisense probe. In (A), the small arrows indicate the provascular tissue in the cotyledons and the large arrow indicates the shoot apical meristem. Scale bar represents 50 μm.
**AtEm1 and AtEm6 promoter-driven expression in seeds of transgenic Arabidopsis**

The putative full-length promoters of *AtEm1* and *AtEm6* genes were fused to the *uidA* reporter gene (pEm1 and pEm6 constructs) and introduced into *Arabidopsis thaliana*. Eight independently transformed transgenic plants carrying pEm1 and 28 carrying pEm6 were generated. GUS activity was assayed fluorimetrically in mature seeds of the T₀ plants (Fig. 2). 50 seeds were pooled and assayed for each of the independently transformed transgenic plants. A wide range of GUS activity was observed among the plants transformed with the same construct, which is not unusual for promoter analysis using the *uidA* reporter gene (Peach and Velten, 1991). A similar variation was previously observed for the *AtEm1* promoter in transgenic tobacco (Hull *et al.*, 1996). Despite the interclonal variation, the average GUS activity in seeds of transgenic plants transformed with pEm6 was 4.6-fold higher (39.6 nmol 4-MU min⁻¹ mg⁻¹ protein) than in seeds transformed with pEm1 (8.7). Seeds of T₂ transgenic plants were collected and plated on selective medium. Lines homozygous for the T-DNA insertion were initiated from T₂ plants that gave 100% resistant seedlings after selfing. Three independent insertions per construct with medium to high levels of GUS activity in seeds were fixed in this way and used for further analysis (Fig. 2). The homozygosity for the T-DNA insertion was confirmed by the 100% of GUS positive seeds in histochemical assays. In all of the histochemical and fluorometric GUS analyses, all three lines transformed with the same construct presented a similar pattern of promoter activity.

The developmental regulation of the *AtEm1* and *AtEm6* promoters during the course of embryogenesis was analysed. Seeds of transgenic *Arabidopsis* were sampled at intervals of 2 d and assayed for GUS activity (Fig. 3). In the lines transformed with pEm1 the promoter activity remained null or very low in seeds before 11 d after pollination (d.a.p.), increased until 17 d.a.p. and then decreased in mature seeds. The time-course of GUS induction in plants transformed with pEm6 was slightly different. The *AtEm6* promoter remained inactive in immature transgenic seeds until about 13 d.a.p., then GUS activity increased rapidly until a maximum level at 19 d.a.p. and finally decreased a bit in mature seeds.

The spatial patterns of expression were examined by quantitative analysis of GUS activity in various organs of the plant (Fig. 4). Both promoters directed the highest GUS activities in mature seeds. Vegetative tissues (leaves, caulinar leaves, stems, and roots) did not show GUS expression in any of the transgenic plants. The *AtEm1* promoter also directed *uidA* expression in flowers. On the contrary, the pEm6 construct did not show activity in flowers. GUS activity was also analysed in transgenic plants different times after germination. A rapid decrease in the GUS activity was observed after germination in both groups of transgenic plants.

It was tested whether *AtEm1* and *AtEm6* promoters were ABA-inducible in transgenic *Arabidopsis* seedlings (Fig. 5). Seeds were germinated and at 7 d after germination were sprayed with 100 μM ABA. Control plants were sprayed with water plus the equivalent amount of ethanol. Both groups of plants were grown alongside each other for 36 h. Application of exogenous ABA induced, on average, a 1.41-fold higher GUS activity in the seedlings transformed with pEm1 and 4.49-fold higher GUS activity in the seedlings transformed with pEm6.

Histochemical staining of GUS activity was used for a higher resolution determination of tissue-specificity in *Em* expression. These analyses were done with the three transgenic lines selected per construct with similar results. Although the level of GUS activity varied from one line to another, the overall pattern of GUS activity was qualitatively the same in all the plants transformed with

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**Fig. 2.** GUS activity in seeds of transgenic *Arabidopsis* plants transformed with the pEm1 or the pEm6 constructs. Each column represents the GUS activity in a pool of 50 seeds from one independent T₀ transformant. GUS activities have been measured as the production of 4-MU (4-methylumbelliferone) per minute and normalized on the basis of the protein content of the sample. The mean GUS activity value for each construct is represented by a solid line and the standard error by two dashed lines. Dark columns indicate the transformant lines selected for further analysis. Their identity number is indicated.
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Fig. 3. GUS activity during the course of seed development in transgenic plants transformed with the pEm1 or the pEm6 construct. Data of three independently transformed lines per construct are presented. The average of three samples obtained from different plants of the same transgenic line is shown. Bars indicate the standard errors. GUS activities have been measured as production of 4-MU (4-methylumbelliferone) per minute and normalized on the basis of protein content of the sample. d.a.p., days after pollination. 21 d.a.p. corresponds to dry seed in our conditions.

the same construct. Seeds of plants transformed with pEm1 or pEm6 were dissected and the embryos were assayed for GUS activity. In embryos from plants transformed with pEm1, GUS activity was localized predominantly in the provascular tissues and in the root tip and apical meristem (Fig. 6A). In embryos from plants transformed with pEm6, GUS activity was distributed uniformly throughout the embryo (Fig. 6B). The seed coat did not show GUS activity in any of the lines. The spatial patterns of GUS activity in seedlings upon ABA treatment were determined using 21 d.a.g. seedlings and the previously used treatment conditions. Untreated 21 d.a.g. seedlings did not show X-GLUC staining (data not shown). After ABA treatment, GUS activity was detected in the roots of either seedlings transformed with pEm1 or with pEm6 (Fig. 6C, D). Transverse sections of the ABA treated roots showed that AtEm1 promoter activity was located in the central cylinder (vascular cells and pericycle) (Fig. 6E) whereas the activity of the AtEm6 promoter was located in the external tissues of the root (cortex, epidermis and root hairs) (Fig. 6F). Staining was also detected after ABA treatment in the base of the first true leaves in plants transformed with pEm1 (Fig. 6G), but in plants transformed with pEm6 the staining was located in the base of the cotyledons (Fig. 6H). Histochemical staining of detached flowers of plants transformed with the pEm1 construct showed that GUS activity was localized in the anthers (Fig. 6I). A closer view demonstrated that anthers-walls were not stained (Fig. 6J) and that all the GUS activity was located exclusively in the pollen grains (Fig. 6K). This GUS activity was still visible in the stigma of the fertilized flowers due to the attached pollen grains (Fig. 6L). In plants transformed with the pEm1 construct, GUS activity was also detected in the collapsed nectaries at early stages of silique development (less than 1 cm long) (Fig. 6M). In order to determine whether GUS expression in pollen grains was under developmental control, flowers of the same inflorescence at different stages of development were assayed fluorometrically for GUS activity (Fig. 7). No GUS activity was detected in floral buds, but high GUS expression was detected in the flowers
containing mature pollen grains with higher activities during anthesis. GUS activity decreased after pollination.

**Discussion**

The expression patterns of the two *Arabidopsis Em-like* genes (*AtEm1* and *AtEm6*) have been studied using in situ hybridization and *Arabidopsis* transgenic plants containing the promoters of these genes fused to the *uidA* reporter gene. The spatial pattern of promoter activity observed in the embryos were very similar to the patterns of mRNA accumulation obtained. Moreover, the patterns of promoter activity during seed development were similar to the patterns of mRNA accumulation previously observed (Gaubier *et al*., 1993; Parcy *et al*., 1994). These data suggest that the promoter fragments used in the pEm1 and pEm6 constructs contain the specific cis elements required for their correct regulation in *Arabidopsis*.

The experiments presented here demonstrate that the expression of the two *Em* genes of *Arabidopsis* is subject to differential, tissue-specific control. Although the expression of both genes is highest in the mature embryo (Gaubier *et al*., 1993), *AtEm1* mRNA accumulates mainly in the provascular tissues of the embryo axis and in meristems, while *AtEm6* mRNA is distributed throughout the embryo. Previous data, based on RNA blot analyses, indicated that *AtEm1* mRNA accumulates at a lower level in seeds than does *AtEm6* mRNA (Gaubier *et al*., 1993). This observation is probably due to their different spatial patterns of expression, since *AtEm1* is expressed mainly in vascular tissues and meristems, and these tissues represent only a small portion of the whole seed. A differential pattern of promoter activity was also detected in the roots of ABA-treated young seedlings; *AtEm1* promoter is active in the vascular tissues whereas *AtEm6* is active in the external tissues. *AtEm1* and *AtEm6* promoter activities disappeared rapidly after germination. A rapid disappearance of the *AtEm1* and *AtEm6* transcripts has also been observed during the first steps of germination (Bies *et al*., 1998). A similar situation has been observed in several *lea* genes (Hughes and Galau, 1989; Raynal *et al*., 1989; Goday *et al*., 1988; Harada *et al*., 1989; Espelund *et al*., 1992). However, the accumulation of some *lea* mRNAs can be re-induced in seedlings by treatment with ABA (Berge *et al*., 1989; Vilardeil *et al*., 1991; Hong *et al*., 1992; Finkelstein, 1993). *AtEm1* and *AtEm6* promoters are also ABA inducible in seedlings. The presence of some putative ABA regulatory elements in the promoters of the *AtEm1* and *AtEm6* genes has been reported previously (Gaubier *et al*., 1993; Hull *et al*., 1996). In addition to these elements, a sequence (tCTCCGTaa) similar to the binding site of the VSF1 protein (GCTCCGTTG) located at position −200 in the promoter of the French bean (*Phaseolus vulgaris* L.) *grp1.8* gene (Ringli and Keller,
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Fig. 6. Histochemical analyses of GUS activity in Arabidopsis plants expressing the β-glucuronidase gene under the control of the AtEm1 or the AtEm6 promoter. Data for the pEm1.1 and pEm6.56 lines are presented. (A) Embryo dissected from a seed of a plant transformed with pEm1 construct. (B) Embryo dissected from a seed of a plant transformed with pEm6. (C) Seedling 21 d.a.g. (days after germination) transformed with pEm1 and treated with 100 μM ABA. (D) Seedling 21 d.a.g. transformed with pEm6 and treated with 100 μM ABA. (E) Longitudinal section of the proximal part of the root of a 21 d.a.g. seedling transformed with pEm1 and treated with 100 μM ABA. (F) Longitudinal section of the proximal part of the root of a 21 d.a.g. seedling transformed with pEm6 and treated with 100 μM ABA. (G) Apex of a 21 d.a.g. seedling transformed with pEm1 and treated with 100 μM ABA. (H) Apex of a 21 d.a.g. seedling transformed with pEm6 and treated with 100 μM ABA. (I) Flower at anthesis of a transgenic plant transformed with the pEm1 construct. (J) Mature stamen after dehiscence of a transgenic plant transformed with the pEm1 construct. (K) Pollen grain of a transgenic plant transformed with the pEm1 construct. (L) Papillar tissue of a stigma with pollen grains of a transgenic plant transformed with the pEm1 construct. (M) Collapsed nectaries of a transgenic plant transformed with pEm1. Scale bars are 1000 μm in (C) and (D), 500 μm in (I), 200 μm in (G) and (H), 100 μm in (L) and (M), 50 μm in (A), (B) and (J), 20 μm in (E) and (F), and 10 μm in (K).
factors which recognize elements in the promoter of may be absent from tobacco, or transcription of the two genes remain to be elucidated. Arabidopsis et al. AtEm1 promoter in the embryos of L.) (Hull tabacum) fi in the vascular speci

uidA activity assay in pollen have been reported (Mascarenhas et al., 1996). These results temporal, spatial and hormonal (ABA). The exact naturesuggest that at least some of the transcription factors of the factors determining this regulation and the func-

Fig. 7. GUS activity during the course of flower development in transgenic plants transformed with the pEm1 construct. GUS activity was measured in the whole flower. The approximate floral stages were determined according to Bowman (Bowman, 1994) and corresponded to: 1, flower buttress arises; 7, long stamen primordia stalked at base; 13, bud opens, anthesis; 14, long anthers extend above the stigma; 16, Petals and sepals withering; 17, all organs fall from green siliques. The average of three samples obtained from different plants of the same transgenic line is shown. Bars are the standard errors. GUS activities have been measured as production of 4-MU (4-methylumbelliferone) per minute and normalized on the basis of protein content of the sample.

1998) has been found in the AtEm1 promoter, at position −139 bp. This fragment is sufficient and necessary to confer vascular specific expression to a minimal promoter. Interestingly, preliminary results indicate that the first 182 bp of the AtEm1 promoter are sufficient to direct GUS activity in provascular tissues of the embryo (G Hull, unpublished data). Further analysis will be necessary to determine which promoter sequences are involved in the vascular specific expression of AtEm1.

The pattern of AtEm1 promoter activity has been previously determined in transgenic tobacco (Nicotiana tabacum L.) (Hull et al., 1996). In this specie, the AtEm1 promoter directed the same organ and development specific activity as in Arabidopsis, with higher expression in mature embryos and some expression in pollen grains. However, the predominantly vascular expression of the AtEm1 promoter in the embryos of Arabidopsis was not observed in tobacco (Hull et al., 1996). These results suggest that at least some of the transcription factors required for the vascular expression of AtEm1 in Arabidopsis may be absent from tobacco, or transcription factors which recognize elements in the promoter of tobacco. The AtEm1 promoter-directed vascular predominant expression is present in the embryos of Brassica napus L. (C Vicent, unpublished data) indicating that the vascular regulatory factors are conserved in this species. Phylogenetically, B. napus is very much more closely related to Arabidopsis than tobacco.

The AtEm1 promoter is also active in pollen grains. GUS artefacts associated with the histochemical GUS activity assay in pollen have been reported (Mascarenhas and Hamilton, 1992). Uknes et al. have proposed that GUS expression in pollen may be due more to the coding region of the uidA gene than to the fused promoter (Uknes et al., 1993). However, the pEm1 and pEm6 constructs contain the same uidA gene coding region but only plants transformed with pEm1 show GUS activity in pollen grains. It is interesting that high levels of GUS activity were also driven in pollen by the promoters of some other ABA- or dehydration-inducible genes such as Tas14 (Parra et al., 1996), CDeT6–19 (Michel et al., 1994), CDeT27–45 (Michel et al., 1993), cor15a (Baker et al., 1994), kin1, cor6.6 (Wang and Cutler, 1995), osmotin (Kononowicz et al., 1992), and Xero2/lit30 (Rouse et al., 1996). The pEm1 construct also showed expression in pollen grains when it was introduced in tobacco (Hull et al., 1996) and B. napus (C Vicent, unpublished results). Pollen grains are highly desiccated tissues (Heslop-Harrison, 1987), suggesting that the expression of the AtEm1 promoter in these tissues could be due to desiccation. Interestingly, collapsed nectaries, in which the AtEm1 promoter is also active, are also highly desiccated tissues.

Although the exact function of the Em proteins is not known, they may be involved in seed desiccation tolerance (Dure et al., 1989; Hughes and Galau, 1989; Dure 1993; Leprince et al., 1993; Xu et al., 1996; Kermode, 1997). The expression of the Em protein in yeast cells attenuates Interestingly, preliminary results indicate that the two Em proteins could confer vascular specific expression to a minimal promoter. Interestingly, preliminary results indicate that the two Em proteins could confer vascular specific expression to a minimal promoter. Interestingly, preliminary results indicate that the two Em proteins could confer vascular specific expression to a minimal promoter. Interestingly, preliminary results indicate that the two Em proteins could confer vascular specific expression to a minimal promoter.
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