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Expression of a Maize Storage Protein Gene in Petunia Plants Is Not Restricted to Seeds[1]

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ABSTRACT

Genes encoding maize seed storage proteins, zeins, are expressed in developing endosperm tissue. To determine whether the DNA sequences controlling the developmental expression of these genes are recognized in dicots, we introduced a gene encoding a M_r 19,000 zein protein into petunia by *Agrobacterium tumefaciens* mediated transformation. Southern blot analysis of DNA from regenerated transgenic plants showed that between 1 and 12 copies of the zein gene were integrated at various locations in the petunia genome. S1 nuclease mapping with 5′ and 3′ probes for zein mRNA showed that transcription of the gene was correctly initiated and terminated in seeds of the transgenic plants. The mRNA was first detected in petunia seeds 10 days after pollination and disappeared 17 days after pollination. However, only small amounts of zein transcripts were synthesized and protein could not be detected at any stage of development. We also found low levels of zein mRNA in leaves, stems, and flowers of the transgenic plants, suggesting that DNA sequences responsible for developmental regulation are not readily recognized in petunia plants.

Zeins, the storage proteins of maize seed, are a complex group of alcohol-soluble polypeptides. The most abundant zeins are proteins of apparent M_r 22,000 and M_r 19,000 that are encoded by a complex multigene family (11, 33). The expression of zein genes is developmentally regulated and transcription occurs in endosperm tissue between 10 and 45 DAP.[4] Following their synthesis by membrane-bound polyribosomes, zeins aggregate into protein bodies within the lumen of the rough ER (20).

A number of genes encoding zein proteins have been isolated (19, 27, 32) and several were introduced into undifferentiated sunflower tissue (10, 24). Although the abundance of zein transcripts in these tissues was less than in developing maize endosperm, the genes were similarly transcribed. These results indicate that the DNA fragments containing zein genes contain sufficient information to direct their accurate transcription. Subsequent analysis has shown that DNA sequences between 70 and 350 bp upstream from the site for initiation of transcription are required for maximal expression of a gene encoding a M_r 19,000 α-zein (gZ19ab1) (29).

The development of methods for transformation and regeneration of dicotyledonous plant species (12) has made it possible to test expression of virtually any gene in a differentiated plant. We have used the Ti-plasmid of *Agrobacterium tumefaciens* to introduce a gene encoding a M_r 19,000 zein into petunia cells and analyzed its expression in regenerated plants. This gene was expressed only at very low levels and its expression was not limited to developing seed tissues.

MATERIALS AND METHODS

Restriction endonucleases and DNA modifying enzymes were purchased from New England Biolabs (Beverly, MA) and Bethesda Research Laboratory (Bethesda, MD) and used according to the suppliers instructions. ^{32}P-Labeled nucleotides and a nick translation kit were obtained from Amersham (Arlington Heights, IL). The Ti-plasmid vector, pBin 19, was a gift from M. Bevan, Plant Breeding Institute, Cambridge, England.

Construction of Transgenic Plants. A 3.0 kb *Eco*RI to *Hin*dIII DNA fragment containing a gene encoding a M_r 19,000 zein protein (gZ19ab1) (27) was subcloned between the *Eco*RI and *Hin*dIII sites to produce pBin 19.gZ19ab1 (Fig. 1). The recombinant plasmid was mobilized into the *A. tumefaciens* strain LBA 4404 by triparental mating (7). LBA 4404, containing pBin 19.gZ19ab1 was used to transform leaf discs of the diploid *Petunia hybrida* line V23xR51. Leaf disc transformation, selection of kanamycin-resistant calli, and regeneration of transgenic plants were carried out as previously described (12).

Analysis of Genes in Transgenic Plants. Isolation of DNA from petunia leaves was according to Rivin *et al.* (28). For DNA blot analysis (31), 5.2 μg of petunia genomic DNA was digested to completion with 40 units of the restriction endonuclease *Eco*RI and separated by electrophoresis in agarose gels. A plasmid containing the coding region as well as 135 bp of 5′ and 81 bp of 3′ noncoding sequence of the zein gene pSP6gZ19ab1-933 (Fig. 2) was used for gene copy reconstruction analysis. Five μg of *Eco*RI-digested calf thymus DNA was added as a carrier to the linearized plasmid DNA. DNA was separated by electrophoresis in 0.6% agarose gels at 1.5 V/cm for 36 h and transferred to nitrocellulose paper (31). The blots were incubated with the nick translated 920 bp *Eco*RI to *Xba*I fragment of pSP6gZ19ab1-933 that was isolated by a low temperature melting agarose procedure (22).

S1 Nuclease Mapping of RNA Transcripts. Total RNA was extracted from leaves, stems, flowers and seeds of transgenic petunia as previously described (2). In general, tissues above stem nodes bearing flowers were collected for extraction. For study of developing petunia seeds, about 20 ovaries were collected per plant at 5, 7, 10, 12, 14, and 17 DAP. Total RNA

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[4] Abbreviations: DAP, days after pollination; bp, base pairs.

and poly(A) RNA were extracted from membrane bound polysomes of 18-d maize endosperms of the inbred W64A as previously described (23).

Zein transcripts were detected by a sensitive S1 nuclease protection assay (3). A DNA fragment corresponding to the 5′ end of gZ19ab1 was radiolabeled with ^{32}P at the *Bam*HI site with T4 polynucleotide kinase (25). Following restriction enzyme digestion with *Hin*cII, a singly end-labeled probe (3×10^6 dpm/pmol of 5′ ends) of 597 bp was separated by electrophoresis through 5% polyacrylamide gels with subsequent isolation by electroelution (22). A probe for the 3′ end of zein transcripts was prepared from a 1440 bp *Hha*I fragment of gZ19ab1. Following digestion with exonuclease III, the 3′ ends were labeled with ^{32}P in a DNA synthesis reaction with the Klenow fragment of *Escherchia coli* polymerase I (13). A probe (1×10^6 dpm/pmol of 3′ ends) of 494 bp was recovered after cleavage at a 3′ *Hin*cII site. Fifty to 100 μg of petunia RNA was used for each S1 mapping experiment, while 0.1 to 5 μg of total RNA or 0.1 to 10 ng of poly(A) RNA from maize endosperm were used as controls. Wheat germ tRNA (Sigma, R-7876) was deproteinized before use as a carrier in S1 nuclease protection experiments. For both 5′ and 3′ end mapping about 10 ng of ^{32}P-labeled probe was hybridized at 45°C for at least 6 h with RNA. Subsequently, the samples were frozen in a dry ice-ethanol bath (−70°C). The DNA-RNA hybrids were digested with 1000 units of S1 nuclease for 30 min at 37°C. Protected DNA fragments were analyzed by electrophoresis in 5% polyacrylamide gels containing 8 M urea (25).

RESULTS

To test the expression of a zein gene in seeds of a dicot, we subcloned a genomic clone encoding a M_r 19,000 zein protein into the binary Ti-plasmid vector pBin 19 (4) (Fig. 1). In addition to the protein coding sequence, this construct contains 945 bp of 5′ flanking sequence and 1218 bp of 3′ flanking sequence. The recombinant plasmid was transferred to *A. tumefaciens* and used to transform petunia leaf discs from which transgenic plants were regenerated. Twenty-four petunia plants regenerated from kanamycin-resistant calli were analyzed by Southern hybridization and found to contain zein genes integrated into the genome (data not shown). Among these plants there were seven distinct Southern hybridization patterns that represented different transformation events (Fig. 2). The number of zein genes in these plants was estimated to range between 1 and 12 based on reconstruction hybridization analysis. Only the seven plants described in Figure 2, numbers 400, 428, 431, 434, 435, 436, and 441, were selected for further investigation.

To assay for expression of the zein gene in these plants, RNA was isolated from developing seeds 13 DAP and analyzed by 5′ S1 nuclease mapping. The petunia seed RNA was hybridized to a 597 bp *Hin*cII to *Bam*HI fragment 5′ end labeled at the *Bam*HI site (Fig. 3). When maize endosperm RNA was hybridized to this probe, protected fragments of 230 and 210 nucleotides were detected. Seed RNA from petunia plants 400, 431, and after prolonged exposure 436, protected DNA fragments of the same size as the maize RNA. This demonstrates that the zein gene(s) in these plants is transcribed in the seed and that the 5′ end of the mRNA is the same as that in developing maize seed. However, the level of transcription was variable between different transgenic plants, and in four of the plants analyzed (428, 434, 435, and 441) no zein mRNA could be detected. There was no correlation between zein gene copy number and the abundance of zein mRNA in seeds of these transgenic plants. Since plant 400 produced the largest amount of zein mRNA, it was selected for further study.

In maize, zein mRNAs are first detected in endosperm 10 DAP, are at their highest level between 18 to 25 DAP, and

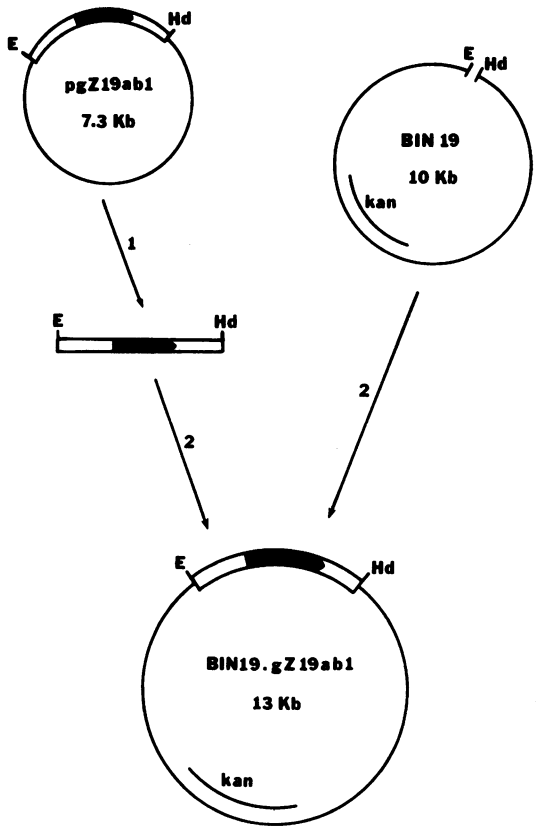

FIG. 1. Subcloning of the zein gene gZ19ab1 from pBR322 (pZ19ab1) to the binary Ti-plasmid vector pBin19. The 2938 bp *Eco*RI to *Hin*dIII insert containing gZ19ab1 was excised by restriction enzyme digestion (1) and ligated to *Eco*RI and *Hin*dIII digested pBin19 (2). Restriction endonucleases: E, *Eco*RI, Hd, *Hin*dIII. The black bar indicates the coding DNA sequences of gZ19ab1 while the open bars indicate the 5′ and 3′ flanking DNA sequences.

decrease in abundance as the seed approaches maturity at 45 DAP. In petunia, seed development takes place over 25 d. Endosperm is present at early stages of development, but by 16 DAP most growth and differentiation occurs in the expanding cotyledons of the embryo. To determine if zein mRNAs were present in petunia seeds during the period of endosperm development, we isolated RNA from self-pollinated seeds of plant 400 at various times after pollination and examined these samples for zein mRNA as described above. The earliest stage at which RNA was prepared was 10 DAP and at this time zein mRNA was clearly detectable (Fig 4A). The highest level of transcripts was found at 12 DAP and these began to decline by 14 DAP. RNA from seeds at 17 DAP contained no detectable zein mRNA (data not shown). Levels of RNA in seeds of plant 436 were so low they were not detected at any stage of development.

S1 nuclease mapping was also used to determine the 3′ end of zein mRNA transcripts in petunia seeds. For this analysis, the RNA was incubated with a 494 bp *Hha*I to *Hin*cII probe 3′ end-labeled at the *Hha*I site of the coding strand. RNA from transgenic petunia seeds protected 190 nucleotides of the 3′ end-labeled probe, which was the same size as the protected fragment detected with maize endosperm mRNA (Fig. 4B). The highest level of zein mRNA in seeds of plant 400 was at 12 DAP in agreement with the results from the 5′ S1 nuclease mapping experiment. Therefore, the mRNAs transcribed from this zein gene in both maize and petunia have the same 5′ and 3′ ends.

Having demonstrated that at least in some of the transgenic

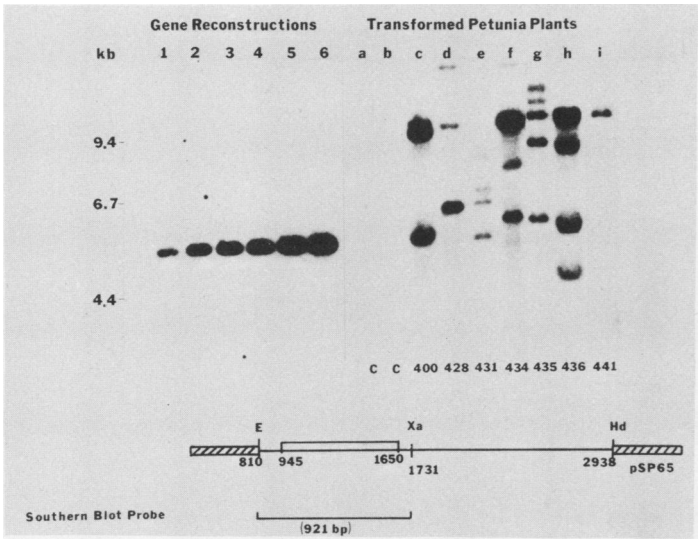

FIG. 2. Southern hybridization analysis of transgenic petunia plants. Petunia leaf DNA was digested with *Eco*RI and incubated with a 921 bp DNA fragment containing the entire coding sequence of gZ19ab1 (bottom). Lanes 1 to 6 show the hybridization intensity of one to six zein gene copies based on 1.55 pg of DNA per haploid petunia genome. Samples are as follows: a, control, nontransformed petunia; b, control petunia from regenerated leaf disks; c to i, transgenic petunia plants containing gZ19ab1. The number below each lane identifies the transformant. The restriction enzyme map below the Southern blot corresponds to a subclone of gZ19ab1 (pSP6.gZ19ab1933). The open box indicates the coding sequence and the hatched region pSP65 DNA. The restriction enzyme sites are numbered from the *Eco*RI site in the 5′ noncoding sequence of gZ19ab1. Restriction endonucleases: E, *Eco*RI, Hd, *Hin*dIII, Xa, *Xba*I.

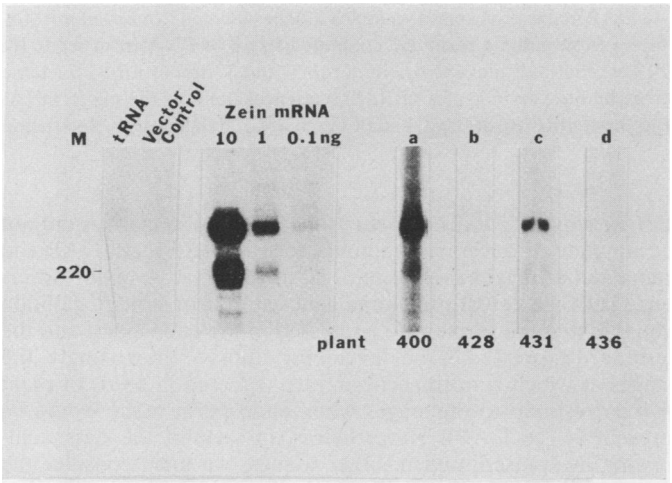

FIG. 3. S1 nuclease mapping of zein RNAs from developing seeds of transgenic petunia plants. A 5′ end-labeled probe that extended from the *Bam*HI site (radiolabeled) to a *Hin*cII site in the 5′ noncoding sequence of gZ19ab1 (see Fig. 4) was incubated with RNA from maize and petunia seeds. Lanes marked 'tRNA' and 'vector control' contained 100 μg of wheat germ tRNA or 100 μg of 13 DAP petunia seed RNA from a plant transformed with pBin19 alone. Samples in lanes marked 'zein mRNA' contained from 10 ng to 0.1 ng of poly(A) RNA from 18 DAP maize endosperm. Samples in lanes a to d contained 100 μg of RNA from seeds of transgenic petunia plants 13 DAP. The source of the RNA is indicated below the lane.

petunia plants a zein gene can be expressed in seed tissue, we wished to determine if this activity was limited exclusively to the developing embryo. We therefore isolated RNA from leaves, stems, flowers, and seeds and tested for the presence of zein transcripts. Of the seven representative plants, four (plants 428, 434, 435, and 441) did not contain detectable levels of zein mRNA in any of the tissues that were examined (data not shown). Plant 400, which contained the highest level of zein mRNA in seeds, also contained detectable levels of this transcript in leaves (Fig. 5). Plant 431 also expressed zein mRNA in leaves and flowers and at higher levels than observed in the seed. Finally, plant 436, which contained barely detectable levels of zein mRNA in the seed, had higher steady state levels of this transcript in all of the other tissues that were examined; the highest level of zein mRNA was found in the stem.

We attempted to determine if the zein mRNA present in these transgenic plants is translated into protein. Extracts of total proteins were prepared from various tissues of these plants and analyzed by a solid phase enzyme linked immunoabsorbant assay (ELISA) and by Western blotting with antibodies that react with α-zeins (21). Under optimal conditions these assays are capable of detecting as little as 10 pg and 1 to 10 ng of zein protein, respectively. However, we were unable to detect zein protein in tissues of any transgenic plants (data not shown).

DISCUSSION

Our results demonstrate that gZ19ab1 is accurately transcribed in transgenic petunia plants, but it is not properly regulated. One possible explanation is that gZ19ab1 is a pseudogene or lacks essential DNA sequences required for tissue-specific regulation. While we cannot rule out these possibilities, they are not consistent with previous analyses of this genomic sequence. The genomic clone from which gZ19ab1 was isolated came from a library constructed with DNA from a maize suspension culture (27), but several lines of evidence indicate that the gene is structurally similar to other genes encoding M_r 19,000 zeins and that it is expressed in maize endosperm. The nucleotide sequence of the coding region is very similar to cDNA clones corresponding to M_r 19,000 zein proteins (9, 23), and the protein it encodes has identical structural features of other α-zeins (1, 27). The coding sequence of the gene hybridizes to mRNA of the size expected for M_r 19,000 zeins (27), and 5′ and 3′ probes corresponding to the coding sequence are protected by endosperm mRNA in S1 nuclease mapping experiments. As has been found for several other genes encoding α-zeins, the 5′ noncoding region hybridizes to RNA transcripts that precede the initiation codon by approximately 60 nucleotides and 850 nucleotides (5, 17). We previously demonstrated that this gene is accurately transcribed by RNA polymerase in sunflower somatic tissue (10), and this was also found to be true in petunia somatic as well as seed tissue. Thus, it would appear that there are no structural defects in the gene with respect to either the coding sequence or the transcription initiation and termination signals.

It is possible the DNA sequences responsible for developmental regulation of zein genes are located more than 850 bp 5′ to the coding sequence. Although the majority of the mRNA transcripts have 5′ ends that map near the initiation codon, a small but nonetheless detectable amount of transcripts have 5′ ends that map nearly a kilobase further upstream. The origin and significance of these larger transcripts have not been demonstrated (5), but their existence implies that sequences recognized by RNA polymerase can precede the gene by an unusually far distance (19). Nevertheless, based on genomic Southern blot hybridization with probes corresponding to 5′ noncoding regions, we found that only about 850 bp of 5′ flanking sequence is conserved among the gene family (17). Probes further 5′ to this region hybridize to DNA that is moderately to highly repetitive

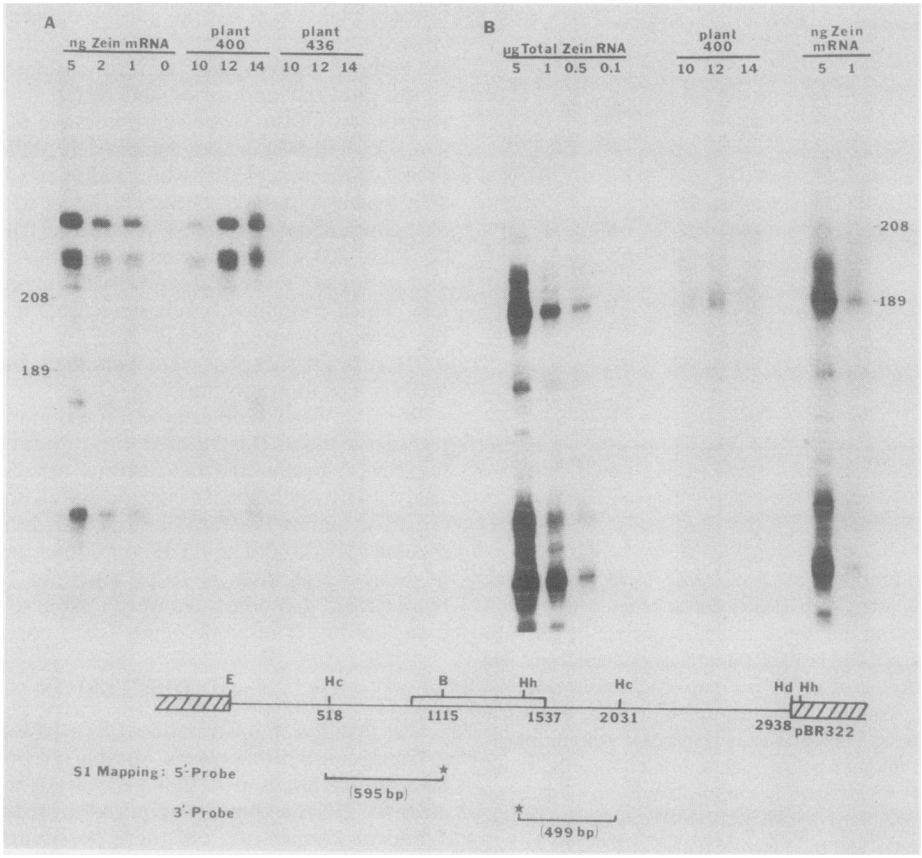

FIG. 4.. Analysis of zein transcripts in developing seeds of transgenic petunia plants 400 and 436. A, The 5′ ends of zein RNA from developing maize and petunia seeds were analyzed by S1 nuclease mapping with a DNA fragment that extended from the *Bam*HI site (radiolabeled and starred) to a *Hin*cII site in the 5′ noncoding sequence of gZ19ab1 (see diagram below the lanes). Samples in lanes labeled 'zein RNA' contained 5, 2, 1, and 0 ng of poly (A) RNA from 18 DAP maize endosperm. Samples from petunia plants 400 and 436 contained 100 μg of RNA from seeds 10, 12, and 14 DAP. B, The 3′ ends of zein RNA from developing maize and petunia seeds were analyzed by S1 nuclease mapping with a probe that extended from the *Hha*I site (radiolabeled and starred) to the Hinc II site in the 3′ noncoding region of gZ19ab1 (see diagram below). Samples in lanes labeled 'zein RNA' contained 5, 1, 0.5, and 0.1 μg of total RNA from 18 DAP maize endosperm; samples in lanes labeled 'total zein mRNA' contained 5 and 1 ng of poly(A) RNA from 18 DAP maize endosperm. Samples from petunia plant 400 contained 50 μg of RNA from seeds 10, 12, and 14 DAP. Beneath the lanes is a partial restriction enzyme map of gZ19ab1 that indicates the origin of the 5′ and 3′ probes for S1 nuclease mapping. The open box corresponds to the coding sequence of the gene; the hatched region indicates pBR322 sequences flanking the maize DNA. The positions of the restriction endonuclease sites are numbered with respect to the *Eco*RI site at the 5′ end of the maize genomic insert. Restriction endonucleases: E, *Eco*RI, B, *Bam*HI, Hc, *Hin*cII, Hd, *Hin*dIII, Hh, *Hha*I.

in the genome. Furthermore, comparisons of 5′ flanking sequences of α-zeins (17, 32) as well as other cereal prolamines (8, 16), show that the most conserved part of the 5′ noncoding sequence corresponds to a region of about 200 nucleotides that precedes the coding sequence by 300 bp.

If the DNA sequences responsible for developmental regulation of zein genes precede the coding region by more than 850 bp, the location of these sequences is significantly different from that of dicot storage globulin genes. Sengupta-Gopalan *et al.* (30) found the 7S storage globulin gene from *Phaseolus vulgaris* was properly regulated with 836 bp of 5′ flanking sequence in transgenic tobacco plants, and a 7S storage globulin gene of soybean was found to be properly regulated in transgenic petunia plants with only 257 bp of 5′ flanking sequence (6). The region responsible for tissue-specific expression of the soybean gene was contained within a 170 bp sequence that precedes the transcription start site by 100 bp (Z-L Chen, RN Beachy, personal communication).

In one plant, zein mRNA accumulated in developing petunia seeds up to 14 DAP and disappeared toward 17 DAP (Fig. 4, and data not shown). This accumulation pattern differed from that of the 7S storage globulin of soybean in petunia (Z-L Chen, RN Beachy, unpublished data.) The 7S globulin mRNA did not accumulate in developing petunia seed until 16 to 18 DAP and remained at maximal level until 20 to 22 DAP. A major difference between cereal prolamine genes and dicot storage globulin genes is that the former are expressed in the endosperm and the latter are expressed in the developing embryo. Interestingly, the stages at which zein transcripts were detected in seeds of plant 400 correspond to the stages when endosperm tissue would be present (4–16 DAP). Nevertheless, in view of the data demonstrating transcription in other tissues, we must consider the possibility that seed protein gene expression is differently regulated in dicots than monocots.

The low levels of zein mRNAs in these transgenic plants, if translated in petunia with a similar efficiency as in maize, would give rise to protein below the level of detection of our immunological assays. Our recent studies have shown that when an α-zein coding sequence is transferred into petunia plants under the control of a dicot storage protein gene promoter, high levels of mRNA and detectable levels of zein protein are produced (JD Williamson, unpublished data). The inability to detect any zein protein in the experiments described here does not reflect zein mRNA instability or translational failure in petunia seeds.

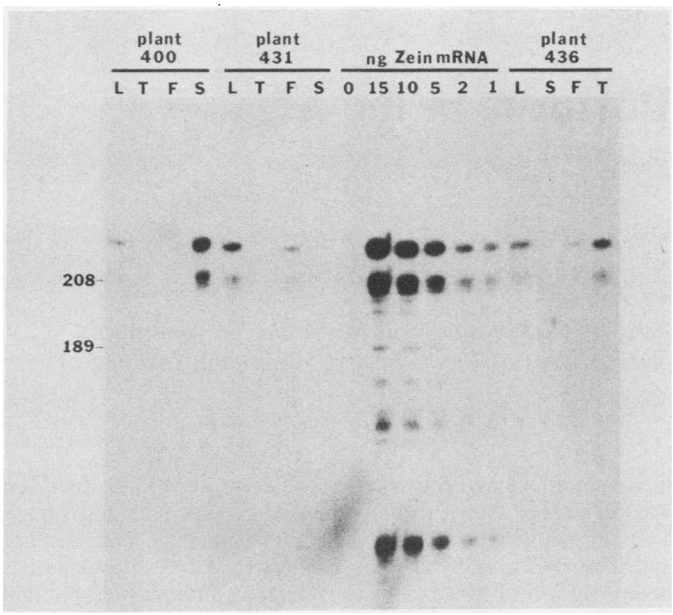

FIG. 5. Analysis of zein transcripts from various tissues of transgenic petunia plants. Total RNA was isolated from leaves (L), stems (T), flowers (F), and 12 DAP seeds (S). One hundred μg of RNA from each tissue, and 0 to 15 ng of poly(A) RNA from 18 DAP maize endosperm tissue was analyzed by S1 nuclease mapping with a 597 bp *Hin*cII to *Bam*HI probe encompassing the 5′ end of gZ19ab1 (see Fig. 4).

Instead, the low level expression of this gene indicates that the zein promoter sequences function inefficiently in this dicot species.

There have been relatively few studies on the expression of monocot genes in dicot plants. In one case, a gene encoding wheat Chl *a/b*-binding protein was shown to be both light inducible (18) and phytochrome regulated in tobacco (26). In contrast, the gene encoding the small subunit of ribulose bisphosphate carboxylase from wheat was not properly expressed in tobacco plants because of the failure to excise an intron (15). This would not appear to be a plausible explanation for the absence of regulated expression for the zein gene, since it contains no introns. The data obtained thus far indicate that some regulatory elements are conserved between monocots and dicots while others are not, and studies on additional genes will be needed to clarify this.

The variation in the level and tissue-specific expression of zein genes was not related to gene copy number. Although plant 400 contained more genes (approximately 10) and produced more RNA in the seed than some other transformants, plant 436 contained even more genes (approximately 12) and produced barely detectable levels RNA in the seed. This variability in the level of gene expression has been observed for other Ti-plasmid mediated plant transformations (14) and is presumably due to position effects of T-DNA insertions at various sites within the petunia genome rather than a developmental phenomenon. The extent to which the position effects are responsible for the low levels of nonregulated expression that occur in some plants is unclear.

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LITERATURE CITED

1. ARGOS P, K PEDERSON, MD MARKS, BA LARKINS 1982 A structural model for maize zein protein. J Biol Chem 257: 9984–9990
2. BEACHY RN, Z-L CHEN, RB HORSCH, SG ROGERS, NJ HOFFMANN, RT FRALEY 1985 Accumulation and assembly of soybean β-conglycinin in seeds of transformed petunia plants. EMBO J 4: 3047–3053
3. BERK AJ, PA SHARP 1977 Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell 12: 721–732
4. BEVAN M 1984 Binary *Agrobacterium* vector for plant transformation. Nucleic Acids Res 12: 8711–8721
5. BOSTON RS, BA LARKINS 1987 Control of maize zein gene expression. *In* JK Setlow, ed, Genetic Engineering, Principles and Methods. Plenum Press, New York, pp 61–74
6. CHEN Z-L, MA SCHULER, RN BEACHY 1986 Functional analysis of regulatory elements in a plant embryo-specific gene. Proc Natl Acad Sci USA 83: 8560–8564
7. FRALEY RT, SG ROGERS, RB HORSCH, PR SANDERS, JS FLICK, SP ADAMS, ML BITTNER, LA BRAND, CL FINK, JS FRY, GR GALLUPPI, SB GOLDBERG, NL HOFFMANN, SC WOO 1983 Expression of bacterial genes in plant cells. Proc Natl Acad Sci USA 80: 4803–4807
8. FREELING M, DC BENNET 1985 Maize Adh 1. Annu Rev Genet 19: 297–323
9. GERAGHTY DE, J MESSING, I RUBENSTEIN 1982 Sequence analysis and comparison of cDNAs of the zein multigene family. EMBO J 1: 1329–1335
10. GOLDSBROUGH PB, SB GELVIN, BA LARKINS 1986 Expression of maize zein genes in transformed sunflower cells. Mol Gen Genet 202: 374–381
11. HAGEN G, I RUBENSTEIN 1981 Complex organization of zein genes in maize. Gene 13: 239–249
12. HORSCH RB, JE FRY, NL HOFFMANN, D EICHHOLTZ, SG ROGERS, RT FRALEY 1985 A simple and general method for transferring genes into plants. Science 227: 1229–1231
13. JAMES CD, IM LEFFAK 1984 Replacement synthesis labeling of DNA molecules *in vitro* using the *Eschericia coli* exonuclease III/DNA polymerase I enzyme pair. Anal Biochem 141: 33–37
14. JONES JDG, P DUNSMUIR, J BEDBROOK 1985 High level expression of introduced chimaeric genes in regenerated transformed plants. EMBO J 4: 2411–2418
15. KEITH B, N-H CHUA 1985 Monocot and dicot pre mRNAs are processed with different efficiencies in transgenic tobacco. EMBO J 5: 2419–2425
16. KREIS M, PR SHEWRY, BG FORDE, BJ MIFLIN 1985 Structure and evolution of seed storage proteins and their genes with particular reference to those of wheat, barley, and rye. Oxford Surv Pl Mol Cell Biol 2: 253–317
17. KRIZ AL, RS BOSTON, BA LARKINS 1987 Structural and transcriptional analysis of DNA sequences flanking genes that encode 19 kilodalton zeins. Mol Gen Genet 207: 90–98
18. LAMPPA G, F NAGY, N-H CHUA 1985 Light regulated and organ specific expression of a wheat cab gene in transgenic tobacco. Nature 316: 750–752
19. LANGRIDGE P, G FELIX 1983 A zein gene of maize is transcribed from two widely separated promoter regions. Cell 34: 1015–1022
20. LARKINS BA, WJ HURKMAN 1978 Synthesis and deposition of zein proteins in maize endosperm. Plant Physiol 62: 256–263
21. LENDING CR, AL KRIZ, BA LARKINS, CE BRACKER 1988 Structure of maize protein bodies and immunocytochemical localization of zeins. Protoplasma. In press
22. MANIATIS T, FRITCH EF, J SAMBROOK 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, NY
23. MARKS MD, JS LINDELL, BA LARKINS 1985 Quantitative analysis of the accumulation of zein mRNA during maize endosperm development. J Biol Chem 260: 16451–16459
24. MATZKE MA, M SUSANI, AN BINNS, ED LEWIS, I RUBENSTEIN, AJM MATZKE 1984 Transcription of a zein gene introduced into sunflower using a Ti plasmid vector. EMBO J 3: 1525–1531
25. MAXAM AM, W GILBERT 1980 Sequencing end labelled DNA with base-specific chemical cleavages. Methods Emzymol 65: 499–560
26. NAGY F, SA KAY, M BOUTRY, M-Y HSU, N-H CHUA 1986 Phytochrome controlled expression of a wheat cab gene in transgenic tobacco seedlings. EMBO J 5: 1119–1124
27. PEDERSEN K, J DEVEREUX, DR WILSON, E SHELDON, BA LARKINS 1982 Cloning and sequence analysis reveal structural variation among related zein genes in maize. Cell 29: 1015–1026
28. RIVIN CJ, EA ZIMMER, V WALBOT 1982 Isolation of DNA and DNA recombinants from maize. *In* WF Sheridan, ed, Maize for Biological Research. Plant Molecular Biology Association, Athens, GA, pp 161–164
29. ROUSSEL DR, RS BOSTON, PB GOLDSBROUGH, BA LARKINS 1988 Deletion of DNA sequences flanking a 19 kilodalton zein gene reduces its transcriptional activity in heterologous plant tissues. Mol Gen Genet 211: 202–209
30. SENGUPTA-GOPALAN C, NA REICHERT, RF BARKER, TC HALL 1985 Developmentally regulated expression of the bean β-phaseolin gene in tobacco seed. Proc Natl Acad Sci USA 82: 3320–3324
31. SOUTHERN EM 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–518
32. SPENA A, A VIOTTI, V PIRROTTA 1983 Two adjacent genomic zein sequences: structure, organization, and tissue specific restriction pattern, J Mol Biol 169: 799–811
33. WILSON DR, BA LARKINS 1984 Zein gene organization in maize and related grasses. J Mol Evol 20: 330–340