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Soybean seed lectin gene and flanking nonseed protein genes are developmentally regulated in transformed tobacco plants

(gene regulation/transformation)

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ABSTRACT **We introduced a 17.1-kilobase soybean DNA fragment containing the lectin gene and at least four nonseed protein genes into the tobacco genome. As in soybean plants, lectin mRNA is present in tobacco seeds, accumulates and decays during tobacco seed development, and is translated into a protein that accumulates prior to dormancy. Each soybean nonseed protein mRNA is present in tobacco leaves, roots, stems, and seeds at levels similar to that found in soybean plants. We conclude that a differentially expressed soybean gene cluster is correctly regulated in transformed tobacco plants and that sequences controlling their expression are recognized by regulatory factors present in tobacco cells.**

The lectin gene represents one of the repertoire of seed protein genes that is highly regulated during the soybean life cycle (1). This gene does not contain introns, directs the synthesis of a 1.1-kilobase (kb) mRNA that accumulates and decays during embryogenesis, and is regulated in part at the transcriptional level (1–3). Transcription studies demonstrated that the lectin gene is embedded in a domain containing other transcription units that are differentially regulated (1, 3). Experimental analysis of the lectin gene in a non-lectin-producing (Le^-) soybean line showed that the mutant lectin gene is transcriptionally inactivated by a 3.5-kb insertion sequence and that this mutation has no detectable effect on the transcription of surrounding regions (1). This finding, and the observation that there are several differentially regulated genes within the lectin gene region, suggests that each gene may have a closely linked sequence that is responsible for controlling its expression.

To begin to identify DNA sequences that regulate seed protein gene expression, we introduced a 17.1-kb DNA fragment containing the lectin gene and at least four nonseed protein genes into tobacco plants. Our experiments showed that the expression program for each soybean gene in transformed tobacco plants is similar to that which occurs during soybean development. We conclude that regulatory factors contained within tobacco cells recognize sequences required for soybean seed protein and nonseed protein gene expression.

MATERIALS AND METHODS

Isolation of Lectin Phages and Plasmids. Recombinant λ Charon 4 phages containing the L1 and L2 lectin genes and plasmids containing recloned λL9-4 restriction fragments were described earlier (1). The isolation and characteristics of the lectin cDNA plasmid L-9 were outlined in previous studies (1, 4).

DNA Isolation and Labeling. Phage and plasmid DNAs were isolated as described (5). Tobacco leaf nuclear DNA was isolated according to Fischer and Goldberg (5). DNAs were labeled by nick-translation (6).

Polysomal mRNA Isolation. Soybean polysomal poly(A) mRNAs were isolated as described (7). Tobacco polysomal poly(A) mRNAs were isolated according to Goldberg *et al.* (8), except that the polysome extraction buffer was adjusted to 400 mM Tris·HCl, pH 9/100 mM KCl/25 mM 2-mercaptoethanol (K. Cox and R.B.G., unpublished results).

Seed Protein Isolation. Tobacco seed proteins were extracted according to the procedure of Sano and Kawashima (9).

Gel Blot Studies. DNA and RNA gel blot experiments were carried out according to the procedures of Wahl *et al.* (10) and Thomas (11), respectively. Protein gel blot studies were performed according to Johnson *et al.* (12).

Transformation of Tobacco Cells and Regeneration of Tobacco Plants. The strategy of Zambryski *et al.* (13) was used to transform tobacco cells with the λL9-4 lectin 17.1-kb *Eco*RI DNA fragment. In brief, the 17.1-kb DNA fragment was recloned into plasmid pLGVneo2103 provided by L. Herrera-Estrella (14). The 17.1-kb DNA fragment was transferred from pLGVneo2103 into the disarmed Agrobacterium pGV3850 Ti-plasmid vector by the procedure of Van Haute *et al.* (15). Tobacco cells were transformed with the pGV3850:pLGVneo2103Eco17.1 cointegrate plasmid by cocultivation, kanamycin-resistant (Kn^R) cells were selected, and transformed tobacco plants were regenerated (16).

S1 Nuclease Protection Studies. S1 nuclease protection assays were carried out by using the procedure of Favaloro *et al.* (17).

RESULTS

Lectin Gene Is Expressed in Soybean Roots. We showed previously that the lectin gene is highly expressed during soybean embryogenesis and that at its highest level lectin mRNA constitutes 0.75% of the embryo mRNA mass, or ≈4000 molecules per cell (1). We hybridized a lectin cDNA plasmid, designated L-9 (1), with gel blots containing leaf, stem, and root mRNAs to determine whether lectin gene expression occurred in cells of the mature plant. As shown in Fig. 1, no detectable signals were obtained with leaf and stem mRNAs. In contrast, L-9 hybridized with a 1.1-kb root mRNA. We estimated that lectin sequences constitute only 4×10^{-5}% of the root mRNA mass, or about 0.2 molecules per cell (Fig. 1).

The Same Lectin Gene Is Expressed in Embryo and Roots. Previous studies showed that there are two lectin genes, designated L1 and L2, in the soybean genome (1). Maps of phages containing these genes are shown in Fig. 2*A*. We hybridized embryo and root mRNAs with L1 and L2 gene probes (Fig. 2*A*) and then measured the size of S1 nuclease-resistant DNA fragments to determine which lectin gene was expressed in the root. As shown in Fig. 2 *B* and *C*, both

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Abbreviation: kb, kilobase(s).

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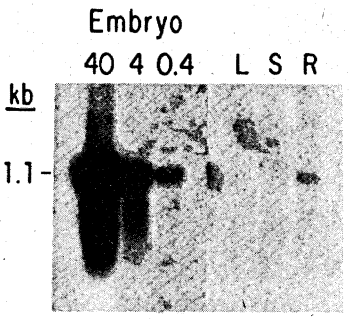

FIG. 1. Lectin gene expression in soybean organ systems. Ten micrograms of leaf (L), stem (S), and root (R) mRNA were fractionated on methylmercuric hydroxide gels, transferred to nitrocellulose, and hybridized with labeled L-9 DNA. Embryo lanes contained 0.1 μg, 0.01 μg, and 0.001 μg of mid-maturation stage mRNA, or the equivalent of 40, 4, and 0.4 molecules per cell of lectin mRNA, respectively (1).

embryo and root mRNAs protected a 1-kb DNA fragment using the L1 probe. In contrast, embryo mRNA protected a 0.2-kb DNA fragment with the L2 probe (Fig. 2*B*), whereas root mRNA did not detectably protect any L2 DNA fragment. These findings indicate that the L1 lectin gene is expressed at different levels in embryos and roots and that the L2 gene is probably an unexpressed lectin pseudogene.

Differentially Expressed Nonseed Protein Genes Are Present Within the Lectin Gene Region. Transcription studies indicated that there are RNA polymerase II transcription units located 5′ and 3′ to the L1 lectin gene (1, 3). In contrast to the lectin gene, these transcription units are active in leaf nuclei, suggesting that they represent differentially expressed nonseed protein genes (3). To test this possibility, we hybridized DNA segments representing individual regions of the 17.1-kb *Eco*RI fragment containing the lectin gene with embryo, leaf, stem, and root mRNA gel blots. As shown in Fig. 3*A*, each DNA segment hybridized with one or more RNAs in all mRNA populations tested. This differed from the results obtained with plasmid L-9, which produced signals with only embryo and root mRNAs (Fig. 1). We presume that the nonseed protein transcripts represent mRNAs because identical results were obtained with EDTA-released polysomal mRNAs (data not shown). We estimated the transcript prevalence by comparing the autoradiogram signals to those obtained with known lectin mRNA equivalents (Fig. 1). The

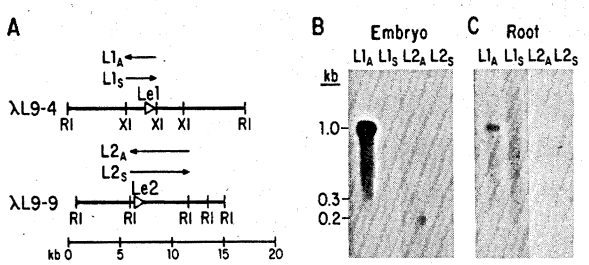

FIG. 2. S1-nuclease analysis of lectin gene expression in the soybean embryo and root. (*A*) Maps of relevant restriction endonuclease sites within the L1 lectin (λL9-4) and L2 lectin (λL9-9) gene regions (1). Arrows indicate the location and orientation of regions recloned into the M13 phages used for the S1 nuclease protection studies. (*B*) L1 and L2 lectin gene expression in soybean embryos. Excess, unlabeled single-stranded M13 phage DNAs, containing either the sense (S) or antisense (A) strands of the L1 and L2 lectin genes, were hybridized separately with 0.1 μg (L1 lanes) and 1 μg (L2 lanes) of embryo mRNA and treated with S1 nuclease. S1 nuclease-resistant hybrids were subjected to electrophoresis, transferred to nitrocellulose, and hybridized with labeled L-9 DNA. (*C*) L1 and L2 gene expression in the soybean root. The experiment was identical to that described in *B* except that 20 μg of root mRNA was used.

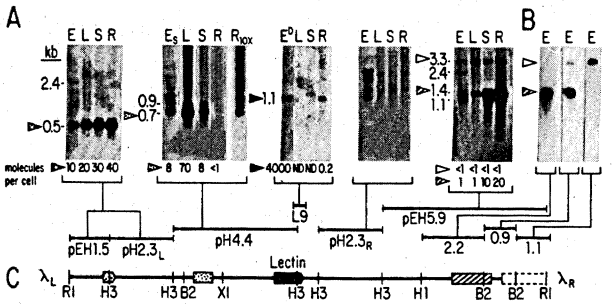

FIG. 3. Organization of the L1 lectin gene region. (*A*) Hybridization of lectin gene region probes with soybean mRNAs. Ten micrograms of soybean embryo (E), leaf (L), stem (S), and root (R) mRNA were fractionated on methylmercuric hydroxide gels, transferred to nitrocellulose, and hybridized with labeled DNA representing the relevant lectin gene regions. E, L, S, and R lanes contained mRNAs from a lectin-producing (Le^+) soybean line. E_S lane contained mRNA from a mutant, non-lectin-producing (Le^-) soybean line (1). E^D lane contained 1×10^{-3} μg of Le^+ embryo mRNA, which represents a 0.4 molecule per cell equivalent of lectin mRNA (see Fig. 1). $R_{10\times}$ is a 10-fold longer exposure of the autoradiogram shown in the contiguous R lane. Molecules per cell were estimated by densitometric analysis of the autoradiogram relative to known equivalents of lectin mRNA. (*B*) Localization of the 3.3-kb and 1.4-kb mRNA regions. Experiments were similar to that shown in *A* except that gel-purified restriction fragments from the right end of the lectin gene region were used as hybridization probes. (*C*) Map of restriction endonuclease sites and locations of transcription units within the lectin gene region. The bracketed lines above the restriction map represent probes used in RNA gel blot studies. The numbers indicate restriction fragment sizes in kb. R1, H3, B2, X1, and H1 refer to *Eco*RI, *Hin*dIII, *Bgl* II, *Xba* I, and *Hpa* I restriction endonucleases, respectively. Boxes represent approximate gene locations and arrows show transcriptional orientations. mRNAs corresponding to these genes are designated by similarly coded arrows. Nonseed protein gene boxes represent minimum gene lengths.

results indicate that the nonseed protein transcripts represent low-prevalence mRNAs, varying from <1 to ≈50 molecules per cell (Fig. 3*A*).

To localize the nonseed protein genes, we hybridized shorter DNA segments with embryo, leaf, stem, and root mRNA gel blots. Fig. 3*B* shows that the 5.9-kb *Eco*RI/*Hin*dIII fragment, representing the extreme right of the lectin region (Fig. 3*C*), contains at least two independent transcription units. In contrast, plasmids pEH1.5 and pH2.3L produced identical gel blot patterns. This suggests that 3.8 kb at the left end of the lectin region contains only one nonseed protein gene and that the multiple embryo RNAs that hybridized with these plasmids are due to other factors (e.g., repeated sequences, alternative processing, etc.). Together, these findings show that there are at least four nonseed protein genes in the lectin gene region and that these genes are differentially expressed relative to the lectin gene.

Transformed Tobacco Plants Contain One Copy of the Lectin Gene Region. We transformed tobacco plants with the 17.1-kb soybean lectin DNA fragment to determine whether all genes present within this region would retain their expression characteristics in tobacco development (13). Seeds obtained from one self-fertilized transformed plant segregated 3:1 relative to the kanamycin resistance phenotype (3 Kn^R:1 Kn^S), indicating the presence of a single soybean locus in the tobacco genome. To show directly that this was the case, we hybridized the 17.1-kb lectin DNA fragment with tobacco DNA gel blots. As shown in Fig. 4*A*, no detectable signal was observed with untransformed tobacco DNA (C_{RI} lane). In contrast, a low-copy 17.1-kb DNA fragment was detected in transformed tobacco DNA (Fig. 4*A*). To show whether the soybean DNA fragment was intact and not

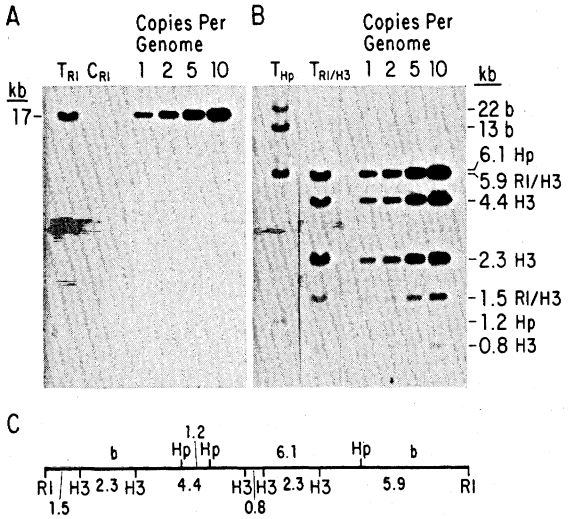

FIG. 4. Representation of the soybean lectin gene region in transformed tobacco plants. Seven micrograms of leaf DNA from a single transformed tobacco plant was digested with the indicated restriction endonucleases, fractionated by electrophoresis on agarose gels, transferred to nitrocellulose, and hybridized with the 17.1-kb *Eco*RI DNA fragment purified from λL9-4 lectin phage DNA. (*A*) Digestion of tobacco DNAs with *Eco*RI. T_{RI} and C_{RI} refer to transformed and untransformed tobacco DNAs, respectively. (*B*) Digestion of tobacco DNAs with *Hpa* I and *Hin*dIII/*Eco*RI. T_{RI}, T_{Hp}, and $T_{RI/H3}$ refer to transformed DNAs digested with *Eco*RI, *Hpa* I, and *Eco*RI/*Hin*dIII, respectively. C_{RI} refers to *Eco*RI-digested untransformed DNA. Sizes of restriction fragments are shown to the right of the autoradiogram. b, H3, RI, Hp, and RI/H3 refer to border, *Hin*dIII, *Eco*RI, *Hpa* I, and *Eco*RI/*Hin*dIII DNA fragments, respectively. (*C*) Map of relevant restriction endonuclease sites within the lectin gene region (1).

rearranged in the tobacco genome, we hybridized the 17.1-kb *Eco*RI fragment with *Eco*RI/*Hin*dIII-digested transformed tobacco DNA. Fig. 4*B* shows that the lectin gene region is not detectably rearranged in the tobacco genome because identical banding patterns were produced with λL9-4 and tobacco DNAs.

We hybridized the 17.1-kb *Eco*RI fragment with *Hpa* I-digested tobacco DNA to establish whether only a single-copy soybean DNA fragment was present in the transformed tobacco genome. The lectin gene region contains three *Hpa* I sites (Fig. 4*C*), whereas the vector DNA contains none. Thus, a single-copy lectin insert is expected to produce two internal *Hpa* I fragments plus two border fragments. As shown in Fig. 4*B*, the results are consistent with this prediction.

Soybean Nonseed Protein Genes Are Expressed in Transformed Tobacco Plants. We hybridized plasmids representing different soybean nonseed protein genes with gel blots containing transformed tobacco seed, leaf, stem, and root mRNAs. Parallel gel blot experiments were also carried out with analogous untransformed tobacco and soybean mRNAs. As shown in Fig. 5, each plasmid reacted with a normal-sized soybean mRNA in all transformed tobacco mRNA populations. We estimated the extent to which the soybean genes were expressed in transformed tobacco plants by comparing the autoradiographic signals produced with parallel soybean and tobacco RNA gel blots (Fig. 5). Fig. 6 shows that most soybean gene transcripts are represented at slightly reduced levels in transformed tobacco mRNA populations. We conclude that soybean nonseed protein genes are expressed in the correct tobacco developmental states but at different quantitative levels.

Soybean Lectin Gene Is Developmentally Regulated in Transformed Tobacco Plants. Figs. 3*C* and 5*C* show that the

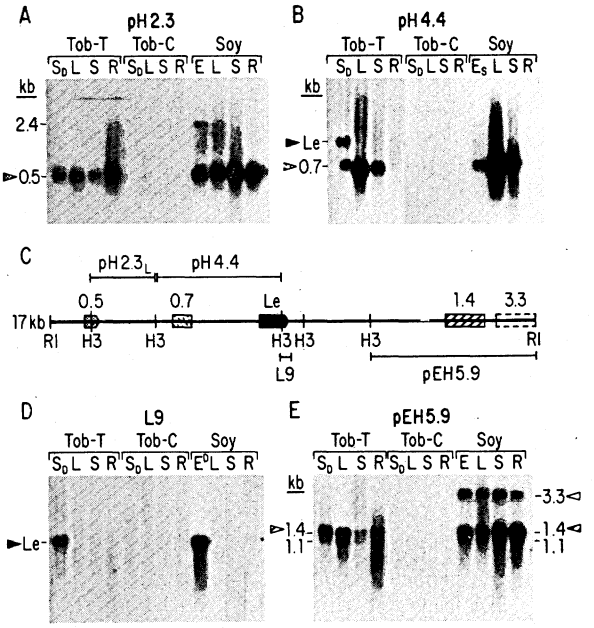

FIG. 5. Expression of soybean genes in transformed tobacco plants. Ten micrograms of soybean and tobacco mRNAs were subjected to electrophoresis on methylmercuric hydroxide agarose gels, transferred to nitrocellulose, and hybridized with the plasmid DNAs indicated above each autoradiogram. Tob-T, Tob-C, and Soy refer to transformed tobacco, untransformed tobacco, and soybean mRNAs, respectively. S_D, L, S, R, and E refer to seed, leaf, stem, root, and Le^+ embryo mRNAs, respectively. E_S refers to Le^- embryo mRNA, whereas E_D refers to a 40-copy lectin mRNA equivalent in Le^+ embryo mRNA (0.1 μg). (*A*) Hybridization with the plasmid pH2.3. (*B*) Hybridization with the plasmid pH4.4. (*C*) Restriction endonuclease sites and transcription unit positions within the lectin gene region. Taken from the data presented in Fig. 2. Bracketed lines above and below the restriction map represent probes used in the mRNA gel blot studies. (*D*) Hybridization with the lectin cDNA plasmid L-9. Dots represent weak root lectin mRNA signals. (*E*) Hybridization with plasmid pEH5.9.

4.4-kb *Hin*dIII fragment present in plasmid pH4.4 contains the 0.7-kb nonseed protein transcription unit and most of the lectin gene. As shown in Fig. 5*B*, this plasmid hybridized with a 1.1-kb lectin-sized transcript in transformed tobacco seed mRNA (Tob-T, S_D lane). No comparable transcript was observed in soybean embryo mRNA (Soy, lane E^S) because Le^- embryo mRNA was used (1). We hybridized the L-9 lectin cDNA plasmid with transformed tobacco mRNA gel

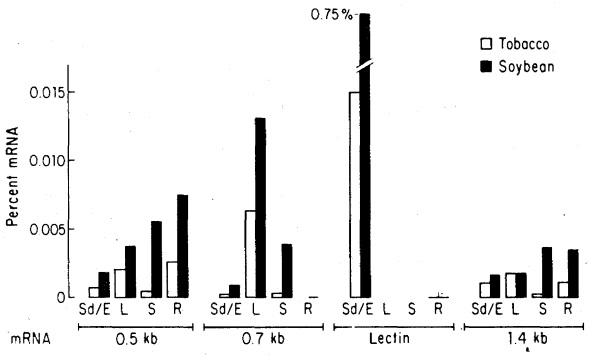

FIG. 6. Representation of soybean mRNAs in transformed tobacco plants. Percent mRNA was estimated by densitometric analysis of RNA gel blot autoradiograms relative to known equivalents of lectin mRNA. Sd, E, L. S, and R refer to seed, Le^+ embryo, leaf, stem, and root mRNAs, respectively. Absence of a bar indicates that there was no detectable hybridization signal.

blots to show directly that the 1.1-kb transcript was lectin mRNA. Fig. 5*A* shows that L-9 produced a strong 1.1-kb signal with transformed mRNA from 2-week tobacco seeds. No discrete transcript was detected with leaf and stem mRNAs, but a weak 1.1-kb signal above a heterodisperse background was observed with root mRNA. This signal was stable at a 57°C washing criterion. In contrast, L-9 failed to hybridize with untransformed tobacco leaf, stem, and root mRNAs; however, a weak high molecular weight signal was detected in untransformed seed mRNA. This signal was eliminated by raising the gel blot wash temperature to 57°C, suggesting that it may be due to an endogenous tobacco message related to soybean lectin mRNA. Densitometric analysis of the autoradiograms presented in Fig. 5*D* indicated that the lectin mRNA in 2-week tobacco seeds was lower in prevalence by a factor of ≈200 than that observed in soybean at mid-embryogenesis. On the other hand, the lectin mRNA level in tobacco root was approximately the same as that observed in soybean.

We isolated mRNAs from transformed tobacco seeds at weekly intervals after pollination and then hybridized plasmid L-9 with mRNA gel blots to test whether the soybean lectin gene was temporally regulated during tobacco seed development. Fig. 7 *A* and *B* show that soybean lectin mRNA accumulates and then diminishes in tobacco seed development. The quantitative fluctuation in lectin mRNA level is similar to that which occurs in soybean development but takes place over a shorter span of time (Fig. 7*B*). At its peak prevalence (3 weeks), lectin mRNA constitutes 0.014% of tobacco seed mRNA mass or approximately 1/50 of that observed at the peak period in soybean embryogenesis (Fig. 6). The reduced prevalence could be due to position effect, tissue differences between tobacco and soybean seeds, or both. We conclude that the soybean lectin gene is expressed in the correct differentiated states and is temporally regulated during tobacco seed development.

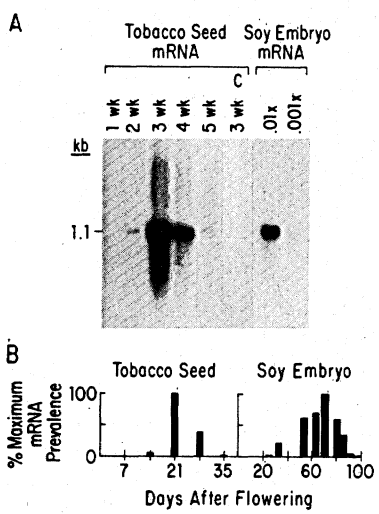

FIG. 7. Soybean lectin gene expression in developing tobacco seeds. (*A*) Hybridization of a lectin cDNA plasmid with transformed tobacco seed mRNA gel blots. Two micrograms of tobacco seed mRNAs from different developmental stages were fractionated on methylmercuric hydroxide agarose gels, transferred to nitrocellulose, and hybridized with labeled L-9 DNA. wk refers to weeks after flowering. 3 wk C refers to mRNA from untransformed 3-week tobacco seeds. The .01× and .001× soybean embryo mRNA lanes contained 0.02 μg and 0.002 μg of Le^+ embryo mRNA, respectively. These reconstructions correspond to lectin mRNA prevalences of 7.5×10^{-3}% and 7.5×10^{-4}%, respectively. (*B*) Changes in lectin mRNA concentration during transformed tobacco seed development. Data taken from *A* were plotted as a percentage of the maximum mRNA prevalence (week 3). For comparison, we also show the changes in lectin mRNA prevalence that occurs during soybean embryogenesis (3).

Soybean Lectin Is Present in Transformed Tobacco Seeds. We isolated proteins from mature seeds and then allowed lectin antibodies to react with protein gel blots to determine whether the lectin gene was expressed at the protein level in transformed tobacco plants. As shown in Fig. 8, the antibodies reacted with 29- and 31-kDa proteins from transformed seeds (Tob-T lane) and did not detectably react with proteins from untransformed seeds (Tob-C lane). The transformed protein bands were comparable in size with those obtained with purified soybean lectin (Le lane). Relative to known lectin protein equivalents, we estimated that soybean lectin constitutes ≈0.2% of the tobacco seed protein mass or about 1/10 of that found in soybean.

DISCUSSION

A Single Lectin Gene Is Differentially Expressed During the Soybean Life Cycle. The soybean lectin gene represents an excellent example of a plant gene under strict developmental control. Lectin gene transcription is activated at a precise point during early embryogenesis, accelerates during mid-embryogenesis, and is repressed prior to dormancy (3). Changes in lectin gene transcriptional activity are paralleled by the accumulation and decay of lectin mRNA, indicating that lectin gene expression is controlled, in part, at the transcriptional level (1, 3). The same lectin gene (L1) encoding a prevalent embryo mRNA is expressed at a low level in the mature plant root system (Figs. 1 and 2). The root lectin mRNA level is lower than that observed at mid-embryogenesis by a factor of ≈20,000 (Fig. 1). In contrast, lectin gene transcriptional activity differs by only 10-fold in the embryo and root, suggesting that posttranscriptional processes also play a major role in regulating lectin gene expression (3).

Lectin Gene Is Flanked by Differentially Expressed Nonseed Protein Genes. A significant aspect of our results is the observation that the lectin gene is embedded in a genomic region containing several differentially expressed nonseed protein genes. These genes encode low-prevalence messages that are present on leaf, stem, root, and embryo polysomes (Fig. 3). Although quantitative differences exist in specific transcript levels from one mRNA population to another, the nonseed protein genes appear to have expression programs similar to a rare class gene set shown to be expressed in all plant organ systems (18). Restriction mapping data indicate that the lectin gene region contains at least four distinct transcriptional units in addition to the lectin gene itself (Fig. 3*C*). The minor transcripts shown in Fig. 3 could be due to

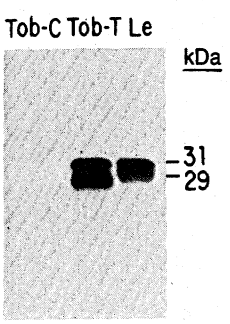

FIG. 8. Presence of lectin protein in transformed tobacco seeds. One hundred micrograms of tobacco seed protein (20 seed equivalents) was subjected to electrophoresis on polyacrylamide gels, transferred to nitrocellulose, and allowed to react with lectin antibodies. Lectin protein was detected by using a horseradish peroxidase immunoassay. Tob-C and Tob-T lanes contained untransformed and transformed tobacco seed protein, respectively. The Le lane contained 100 ng of purified soybean seed lectin.

differential transcription start and stop sites, alternative mRNA processing, homology with repeats within the region, or all of the above factors. We favor repeat homology as a major factor in generating the multiple gel blot signals because each sequence within the lectin gene region is present in at least two other locations in the soybean genome (J.K.O. and R.B.G., unpublished results) and because minor transcripts were not detected in transformed tobacco mRNA populations (Fig. 5).

The presence of five differentially expressed genes within a 20-kb chromosomal region is not unexpected if we consider the soybean genome size (1×10^6 kb; ref. 7), the percentage of soybean single-copy DNA and interspersed repetitive elements (50%; ref. 19), and the estimated number of transcription units active during the entire plant life cycle (75,000; ref. 18). These values predict that there should be approximately one gene per 6–7 kb in the soybean genome, a number close to that obtained in the present study. Our finding that there are differentially expressed genes in close proximity to each other strongly suggests that each gene is regulated independently of each other and contains tightly linked, cis-control elements programming their expression. Recent transformation studies with regulated plant genes containing only a few hundred base pairs of 5′ flanking region indicate that this is indeed the case (20–24).

All Genes Within the Lectin Region Are Expressed in Transformed Tobacco Plants. A major finding presented here is that all genes within a 17.1-kb soybean chromosomal domain retain their expression programs in tobacco development. As seen in Figs. 5 and 6, each nonseed protein gene is expressed constitutively, whereas the lectin gene is expressed only in seed development and in roots of the mature tobacco plant. Recently, Sengupta-Gopalan *et al.* (21) and Beachy *et al.* (24) showed that legume storage protein genes are also expressed correctly during tobacco and petunia seed development. In addition, we have observed that soybean Kunitz trypsin inhibitor genes are regulated correctly in tobacco (K.D.J. and R.B.G., unpublished results). Considering that the Solanaceae and Leguminoseae diverged >100 million years ago (25–27), these findings indicate that DNA sequence elements and cellular factors required for seed protein and nonseed protein gene expression have been conserved over large evolutionary distances.

To our knowledge, the correct expression of a large plant gene cluster in a foreign cell environment has not been reported previously. Presently, no upper limit has been placed on the amount of DNA capable of being transferred by Ti-plasmid vectors from one plant to another, although 50-kb inserts have been used successfully (28). This result, and the observations presented here, imply that plant cells can be transformed with large, active chromosomal regions for either practical molecular genetic engineering purposes or for investigating the developmental regulation of large polygenic units.

Soybean Lectin Gene Is Temporally Regulated During Tobacco Seed Development. The soybean lectin gene is expressed at a precise time during tobacco seed development (Fig. 7). Lectin mRNA accumulates and decays within the 5-week tobacco seed maturation period (Fig. 7*B*) in contrast to the 15-week interval required for lectin mRNA accumulation and decay in soybean (Fig. 7*B*). We infer from these observations that factors required for regulating lectin gene transcription in the nucleus as well as those responsible for modulating lectin mRNA levels in the cytoplasm are conserved between soybean and tobacco plants. The temporal production and/or utilization of these factors, however, are specific for each plant. In other words, soybean lectin gene expression adapts to physiological processes inherent in tobacco seed development. Clearly, the tobacco transformation system should facilitate the identification of cellular factors and DNA sequences required for the control of soybean seed protein gene expression.

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