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Analysis of the Glycinin *Gy2* Promoter Activity in Soybean Protoplasts and Transgenic Tobacco Plants

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Abstract : To study the regulatory expression mechanism of soybean glycinin gene, *Gy2*, the 5' upstream region of the gene was searched for the presence of putative regulatory elements by nucleotide sequencing. It revealed various kinds of regulatory sequence elements commonly found in plant storage protein genes. There were canonical promoter sequences, TATA box (TATAAT) and AGGA box (GAAT) which are common in the 5' upstream region of the plant genes. The embryo factor binding sequence, RY repeat, CACA sequences, α-conglycinin enhancer-like sequences were also found. To delineate the function of these sequences, 5' upstream deletion mutants of *Gy2* were prepared and fused to the α-glucuronidase (GUS) gene. Each chimeric construct was transferred into soybean protoplasts for transient assay, which led to the identification of the sequences between −281 and −223, −170 and −122, of *Gy2* promoter as negative regulatory elements, and the sequences between −223 and −170, −122 and −16 as positive regulatory elements. These results are consistent in transformed tobacco plants as well. The serially deleted promoter fragments fused to the *GUS* were transformed into *Nicotiana tabacum* by *Agrobacterium tumefaciens* using the binary vector system. GUS activity of *Gy2* promoter deletion constructs was detected only in seeds but not in leaves with different levels of expression as in transient assay. These results suggest that the glycinin *Gy2* promoter drives a tissue-specific expression in transgenic tobacco plants(Received September 21, 1995; accepted October 16, 1995).

Introduction

Glycinin is one of the most abundant storage protein in soybean, which accounts for 50~60% of the total proteins.[1-3] It is MW 360,000 (11S) and composed of 6 subunits of average MW 60,000.[4] Five different kinds of subunits and their genes are known.[4] Each subunit is composed of a A-polypeptide of 37~45 kDa, which has acidic pI, and a B-polypeptide of on average 20 kDa, which has basic pI. They are linked by disulfide bonds.[5] Glycinin is synthesized as a precursor of on average 60 kDa which undergoes post-translational modification by thiol protease to form A- and B-polypeptides. A- and B-polypeptides which form the glycinin subunits are, therefore, synthesized from the same mRNA.[6]

Glycinin gene family has members of the 5 gene (*Gy1*~*Gy5*) and each gene consists of 4 exons and 3 introns.[4,7-9] Glycinin is synthesized during embryogenesis and mid-maturation stage and its expression is confined to the seeds.[10,11] The tissue-specific expression of glycinin gene is known to be regulated at the level of transcription. Glycinin mRNA is synthesized during embryogenesis and reaches the maximum values at mid-maturation stage.[10-12] Spatial and temporal expression of the glycinin gene makes this gene an excellent model system for the study of the molecular mechanism underlying differentially regulated gene expression in plants.[12]

Transcriptional control of eucaryotic protein-encoding genes usually plays a major role in determining the final level of the gene product. The *cis*-acting elements, which interact with *trans*-acting factors and regulate transcription, have been found in the 5' flanking regions of various seed storage globulin genes such as the sunflower helianthinin gene,[13] the French bean phaseolin gene[14] and the soybean β-conglycinin genes.[15] Transcriptional enhancement presumably involves the interaction of enhancer sequences with specific *trans*-acting factors.[16,17]

The purpose of this experiment is to study the regulatory mechanism of glycinin gene expression. To investigate the effect of *Gy2* promoter region, a series of constructs in which 5'-deletions of the *Gy2* promoter were fused to the GUS reporter gene was made. These constructs were transferred into soybean protoplasts by polyethylene glycol method and into *Nicotiana tabacum* by *Agrobacterium*-mediated transformation method, which led to the identification of functionally distinct regulatory sequences.

Keywords : *Gy2*, promoter deletion, soybean protoplast, transgenic tobacco, *GUS*, glycinin, soybean
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Materials and Methods

Strains, plasmids, and culture media.

Escherichia coli MC1061 [*F, araD139, Δ(ara, Leu)7696, ΔlacY74, galU-, galK-, hsdR-, strA*], pUC18, and pBI221.1 were used for DNA manipulation.[18] *Agrobacterium tumefaciens* A208 and pGA482[19] for plant transformation and *Nicotiana tabacum* var. Xanthi for a recipient strain of transformation were used.[20] Soybean (*Glycine max*) samples for protoplast isolation were the seeds of cultivar Paldal. LB medium for *E. coli* culture and MS medium for tobacco cell culture were used.

Enzymes and chemicals

Restriction enzymes and DNA modifying enzymes were purchased from New England Biolab and IBI. Coomassie blue protein assay reagent was (23200X) from Pierce Co. Other chemicals were purchased from Sigma Chemical Co.

Construction of chimeric genes containing glycinin promoter

All cloning steps for construction of serial deletion mutant of glycinin promoter fused with GUS reporter gene followed standard procedures by Sambrook *et al.* (1989).[18]

Soybean protoplast isolation

Soybean protoplast isolation was performed according to Lin *et al.* (1987).[21] About 10 g of immature soybean seeds were sliced into 1 mm thick and incubated in 50 m*l* of the cell wall-digesting enzyme mixture containing 0.2% Onozuka R10, 0.01% Macerozyme, 0.01% Pectolyase Y23, 0.5 M sorbitol, 0.5 mM DTT, and Gamborg's B-5 salts at pH 5.8 for 15 hr at 26℃ with gentle shaking. Protoplasts released from the tissue were filtered through 62 μm stainless mesh. The filtrate was centrifuged for 10 min at 100 g and the supernatant was discarded. The pellet was resuspended with 10 volumes of 0.5 M sucrose, 1 mM $CaCl_2$. Two m*l* of a solution containing 0.4 M sucrose, 0.1 M sorbitol, 1 mM $CaCl_2$, 5 mM MES, pH 5.8 was layered onto this, followed by 1 m*l* of 0.5 M sorbitol, 1 mM $CaCl_2$, 5 mM MES, pH 5.8. After centrifugation for 10 min at 100 g, the protoplasts in a band between upper two layers were collected and washed 3 times with the washing buffer (0.5 M sorbitol and Gamborg's B-5 salts). Yields of protoplasts were estimated by hemocytometer and diluted to the density of 5×10^5 protoplasts/m*l* with the washing buffer.

Protoplast transformation

Protoplast transformation was performed according to the method described by Krens *et al.* (1982).[22] One m*l* (5×10^5 protoplasts) of protoplasts was taken and 0.5 m*l* of polyethylene glycol (PEG 6000, 40% w/v) dissolved in a F-medium (140 mM NaCl, 5 mM KCl, 0.75 mM Na_2HPO_4, 5 mM glucose, and 125 mM $CaCl_2$) was added, followed by addition of 10 g DNA. Protoplasts were incubated at 26℃ for 30 min with occasional shaking, after which 10 m*l* F-medium was added stepwise in 2 m*l* portions at 5 min intervals. Protoplasts were pelleted by centrifugation for 10 min at 100 g and washed 2 times with protoplast culture medium (0.4 M sorbitol, Gamborg's B-5 salts, 2% sucrose, 1 mg/m*l* NAA, and 0.1 mg/*l* 6-BAP at pH 5.8). Protoplasts were resuspended in 1 m*l* of protoplast culture medium and kept at 28℃ in the dark.

Transformation and regeneration of tobacco plants

Recombinant DNAs were transferred into *Agrobacterium* by the freeze-thaw method.[20] The chimeric gene constructs were transferred to *Nicotiana tabacum* var Xanthi cells by *Agrobacterium*-mediated leaf-disc transformation.[23] Explants were induced from callus and cultivated in MS medium in presence of kanamycin (200 μg/ml).[24]

Fluorometric assay of GUS

GUS activity was measured fluorometrically.[25] Two hundred microliter of protoplast culture was taken after 48 hr of transformation and pelleted by centrifugation and sonicated for 5 sec in the presence of 200 μ*l* GUS extraction buffer (50 mM sodium phosphate, pH 7.0, 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% Triton X-100 and 0.1% sarcosyl). After centrifugation for 5 min at 10,000 g, the supernatant was recovered. Ten microliter of extracts were incubated with 1 mM 4-methylumbelliferyl-β-D-glucuronide (4-MUG) as a substrate for 1 to 24 hr at 37℃.

Tobacco seeds of the stage of about 15 days after flowering was chosen for assay. Seeds or leaves were ground in 500 μ*l* of GUS extraction buffer on ice with prechilled mortar and pestle. Extract was transferred into cold Eppendorf tubes and centrifugated in prechilled rotor at 13,000 rpm for 5 min. After centrifugation, 100 μ*l* supernatant was removed into cold Eppendorf tubes on ice. Centrifugation was repeated again and 10 μ*l* of supernatant was incubated with 1 mM of 4-MUG as a substrate for 6 hr at 37℃. The reaction was terminated with the addition of 0.2 M Na_2CO_3. Fluorescence was measured with excitation at 365 nm, emission at 455 nm. Protein concentration of the extract was measured with Coomassie Blue protein assay reagent (Pierce Co.) and GUS activity was corrected on the basis of protein concentration.

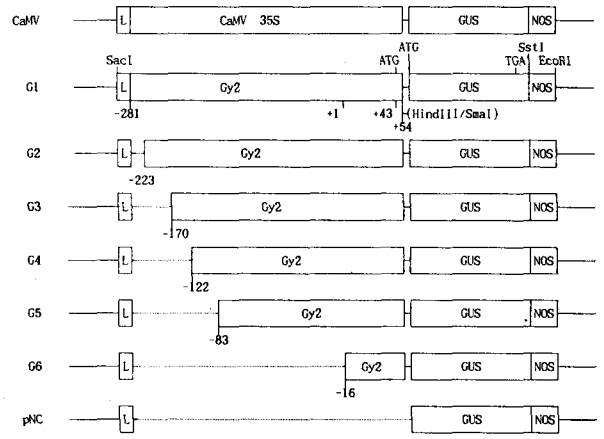

Fig. 1. Construction of serial promoter deletion mutants of *Gy2* in pGA482. Deletion mutants were made by progressive digestion of 5' upstream sequence of *Gy2* with Exo III or by restriction enzymes.

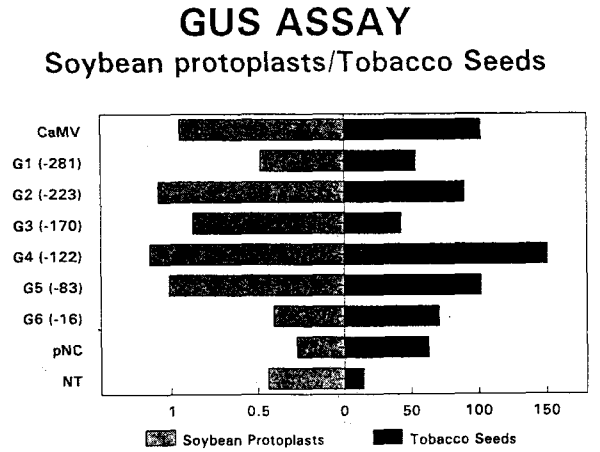

Fig. 2. Comparison of activities of α-glucuronidase by *Gy2-GUS* fusion constructs in transiently transfected soybean seed protoplasts and in seeds of transgenic tobacco plants. GUS activity was measured by fluorometric method with 4-MUG as a substrate and shown in nmole 4-MU/μg (left) and pmole 4-MU/min/mg protein (right). Mean values of 5 independent series of experiment are shown in bars.

Results

Construction of chimeric genes containing promoter deletion mutants of *Gy2* gene

To investigate the regulatory mechanism of the glycinin *Gy2* gene expression, the 5' upstream region (−328 to +55) of *Gy2* gene was searched for the presence of putative regulatory elements. There are various sets of regulatory sequence elements found in plant storage protein genes. There are canonical promoter sequences, TATA box (TATAAAT at −30)[26] and AGGA box (GAAT at −94),[27] which are common in the 5' upstream region of plant genes. The embryo factor binding sequence (AACTTAATGAG at −140),[28] the RY repeat (CATGCAT at −105),[29] CACA sequences (AACACA at −204 and −126)[30] which are found in seed protein genes. β-Conglycinin enhancer like sequences (AYCCCA at −74 and −324),[31,32] a legumin consensus sequence (TCCATAGCCATGCATACTGAAGAATGTC at −109),[33] a vicilin consensus sequence (GCCACCTC at −158),[33] and a sequence similar to the *Opaque-2* binding sequence (TGTGACGTGTC at −61)[34] are also found at the 5' upstream region.

To study the function of these sequences as *cis*-acting elements, *Gy2* promoter deletion constructs were prepared by using Exo III nuclease and restriction enzymes (Fig. 1). Construct G1 (−281) contains the 5' flanking region up to −281 bp from the transcription initiation site of *Gy2*. Constructs G2 (−223)~G9 (+43) contain various length of the 5' flanking sequence as described in Fig. 1. Construct pNC does not contain any 5' flanking region of the *Gy2* promoter. A series of promoter deletion mutants was fused to the coding sequence of *E. coli* β-glucuronidase (GUS) gene. To transform tobacco plants, all of these chimeric constructs were subcloned into pGA482 binary vector system.

Regulation of *Gy2* promoter activity in soybean seed protoplasts

To analyze the promoter activity of the *Gy2* 5' upstream sequence, each deletion mutants was tested by transforming of soybean seed protoplasts and by transferring stably into tobacco plants. For protoplast transient assay, *Gy2* promoter deletion constructs were transferred into protoplasts isolated from developing soybean seeds and the GUS activity was measured fluorometrically with 4-MUG (4-methylumbelliferyl-β-D-glucuronide) as a substrate.

Transient GUS expression from the various *Gy2* promoter constructs was compared in Fig. 2. Each dotted bar on the left represents the average GUS activity of 5 transfection experiments with independent soybean protoplasts preparations. G1 (−281) showed GUS activity of almost background level but the activity of G2 (−223) increased to almost the same as CaMV construct containing CaMV 35S promoter in protoplasts and in transgenic seeds. GUS activity decreased in G3 (−170) and G4 (−122) showed the maximal level of expression among the constructs. GUS activity decreased gradually in G5 (−83) and G6 (−16), and finally went down to background level in pNC. These results suggest the function of the −281 to −223 and −170 to −122 sequences as negative regulatory elements, whereas −223 to −170 and −122 to −16 region as positive regulatory elements. These results showed the biphasic pattern of *Gy2* expression regulation.

Tissue-specific expression of the *Gy2* promoter in transgenic tobacco plants

For stable transformation experiment, all gene con-

structs shown in Fig. 1 were introduced in tobacco plants by Ti plasmid mediated transformation. All of the *Gy2* promoter deletion mutants were introduced into *Agrobacterium tumefaciens A208* by freeze-thaw direct transformation method.[20] To test the transformation of *Agrobacterium tumefaciens A208*, plasmid DNA was isolated from transformed *Agrobacterium tumefaciens A208* and confirmed by restriction enzyme mapping (data not shown).

To introduce the *Gy2* serial deletion constructs into tobacco cells, surface-sterilized tobacco cells were transformed by cocultivation with *Agrobacterium tumefaciens* A208 containing serial deletion mutants of *Gy2* promoter. Transformed tobacco cells were selected by their ability to grow into callus on MS media containing kanamycin (200 μg/m*l*), and regenerated into mature plants. To confirm the presence of transgene in regenerated tobacco plants, genomic Southern blot analysis was carried out using GUS gene as a molecular probe. There were specific band only from the genomic DNA of transgenic tobacco plants, but not from non-transgenic control tobacco plant, as expectedly (data not shown). The GUS activity in the developing seeds of transgenic tobaccos was determined as described above. Solid bars on the right represent average GUS activity in transgenic tobacco seeds (Fig. 2). These results also showed the biphasic pattern of *Gy2* expression. These results were consistent with those in protoplast transient assay.

To study the tissue-specific expression of the *Gy2* 5' upstream region, GUS activity from seeds and leaves of transgenic tobacco plants was determined. Table 1 showed GUS activity from seeds and leaves of transgenic tobacco plants transformed with CaMV, G1 (−281), G4 (−122), G6 (−16), and pNC constructs, respectively. GUS activity was detected in both seeds and leaves of transgenic tobacco plants transformed with the CaMV construct, which means that CaMV promoter does not show tissue-specific expression in transgenic tobacco plants. GUS activity of *Gy2* promoter deletion constructs, however, was detected only in seed but not in leaves, even though expression level differed in each construct. These results suggest that the glycinin *Gy2* promoter drives tissue-specific expression of the GUS gene.

Table 1. Average GUS activities of the *Gy2* promoter deletion constructs in leaves and seeds of transgenic tobacco plants.

Construct	Average GUS activities (pmole 4-MU/min/mg protein)	
	seed	leaf
CaMV	18.9	298.7
G1 (−281)	27.2	0
G4 (−122)	87.4	0
G6 (−16)	33.1	0
pNC	0	0

Discussion

The sequence of the 5' flanking region of *Gy2* contains many regulatory sequence elements. These include the TATA box (TATAAT), AGGA box (GAAT), RY repeat (CATGCAT), and the conglycinin enhancer (AVCCCA). To study the function of these regulatory sequences as *cis*-acting elements, transient expression assay using soybean protoplasts was initially performed. These results were further evidenced by making transgenic tobacco plants with the *Gy2* promoter deletion constructs. The transient and stable expression experiments led to similar conclusion in the levels of the GUS activity. GUS activity in G1 (−281), which contains the largest 5' upstream region of the Gy2 promoter, was lower than CaMV, but increased in G2 (−223) showing almost the same activity as CaMV 35S promoter, and then reduced in G3 (−170). GUS activity reached the maximum level in G4 (−122) and then decreased when deleted to −85 in G5, suggesting biphasic mode of the gene expression.

G4(−122) contains legumin consensus sequence (leg box), TCCATAGCCATGCATACTGAAGAATGTC (−116 to −89).[33] The leg box has been proposed to be an important motif involved in the expression of the glycinin genes, because of its common presence in the 11S globulin genes so far sequenced, at very similar position, *i.e.* 45 to 65 bp upstream of the TATA box with very high sequence homology.[35,36] Chamberland *et al.*[37] reported that legumin box is involved in the transcription of the α'-subunit gene of soybean β-conglycinin, another storage protein in soybean. 'CATGCAT' conserved sequence is an important element in the legumin box in *LegB4*[38] and *Gy2*[39] gene. The deletion of the CATGCAT sequence in the leg box of the *Gy2* promoter resulted in a 10- to 15-fold reduction in β-glucuronidase activity accumulated in the seeds.[39] Our results suggest that the leg box does not act as a negative regulatory element since a promoter lacking this sequence, G5 (−83), showed lower expression than the G4 (−122). Furthermore, G5 (−83) represented higher activity than promoterless control pNC, which means the elimination of this element did not make the expression of the fusion gene impossible. The *Gy2* deletion mutants showed seed-specific expression. Surprisingly G6 (−16) also showed seed specific expression in transgenic tobacco plant. These results suggest that the leg box is not the determinant of seed-specific expression of soybean glycinin gene.

The sequence AACACA (−204 and −126), which was reported as a positive regulatory element in β-phaseolin gene expression,[40] seems to work as a negative regulatory element in the *Gy2* promoter. The fact that gene expression was reduced with progression of deletion is

assumed that nuclear factors that are required for maximal expression of the gene might interact with the deleted sequence. Conformational changes within the promoter region that are elicited by deletion of the element could cause a down-regulation of expression. G5 (−83) showed relatively high expression interestingly, in this region, ACCCAA (−75) and CACCCA (−51), which are similar to conglycinin enhancer sequence, exist. Enhancer sequence in G2 (−223) seemed to be AAACACACT (−205), which was very similar to the sequence of HAACACAMH.[30] The function of the above mentioned tentative regulatory elements of the *Gy2* promoter has to be investigated further by fine mapping through site directed mutagenesis.

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References

1. Catsimpoolas, N., D. A. Roger, S. J. Circle, and E. W. Meyer (1967) Purification and structural studies of the 11S component of soybean protein. *Cereal Chem.* **44**, 631-637.
2. Hill, J. E. and R. W. Breidenbach (1974) Proteins of soybean seeds. I. Isolation and characterization of major components. *Plant Physiol.* **53**, 742-746.
3. Nielsen, N. C. (1984) The chemistry of legume storage proteins. *Philos. Trans. R. Soc. Lond. Biol. Sci.* **304**, 287-296.
4. Nielsen, N. C., C. D. Dickenson, T. J. Cho, V. H. Thanh, B. J. Scallon, T. L. Sims, R. L. Fischer, and R. B. Goldberg (1989) Characterization of the glycinin gene family in soybean. *Plant Cell* **1**, 313-328.
5. Catsimpoolas, N., J. A. Kenney, E. W. Meyer, and B. F. Szuhaj (1972) Molecular weight and amino acid composition of glycinin subunits. *J. Sci. Food Agric.* **22**, 448-450.
6. Kim, C. H. and Y. D. Choi (1989) Molecular cloning of cDNA encoding the precursor to the glycinin A_2B_{1a} subunit of soybean. *Korean Biochem. J.* **22**, 233-241.
7. Cho, T. J., C. S. Davies, and N. C. Nielsen (1989) Inheritance and organization of glycinin genes in soybean. *Plant Cell* **1**, 329-327.
8. Sims, T. L. and R. B. Goldberg (1989) The glycinin *Gy1* gene from soybean. *Nucl. Acids Res.* **17**, 4386.
9. Thanh, V. H., N. E. Tumer, and N. C. Nielsen (1989) The glycinin *Gy2* gene from soybean. *Nucl. Acids Res.* **17**, 4387.
10. Goldberg, R. B., G. Hoschek, S. H. Tam, G. S. Ditta, and R. W. Breidenbach (1981) Abundance, diversity, and regulation of mRNA sequence sets in soybean embryogenesis. *Dev. Biol.* **83**, 201-217.
11. Goldberg, R. B., G. Hoschek, G. S. Ditta, and R. W. Breidenbach (1981) Developmental regulation of cloned superabundant embryo mRNAs in soybean. *Dev. Biol.* **83**, 218-231.
12. Goldberg, R. B., S. J. Barker, and L. Perez-Grau (1989) Regulation of gene expression during plant embryogenesis. *Cell* **56**, 149-160.
13. Walling, L., G. N. Drews, and R. B. Goldberg (1986) Transcriptional and post-transcriptional regulation of soybean seed protein mRNA leaves. *Proc. Natl. Acad. Sci. USA* **83**, 2123-2127.
14. Zenke, M., T. Grundstorm, H. Matthes, M. Wintzerith, C. Schatz, A. Wildeman, and P. Chambon (1986) Multiple sequence motifs are involved in SV40 enhancer function. *EMBO J.* **5**, 387-397.
15. Wasylyk, B., C. Wasylyk, P. Augereau, and P. Chambon (1983) The SV40 72 bp repeat preferentially potentiates transcription starting from proximal natural or substitute promoter elements. *Cell* **32**, 503-514.
16. Kandonaga, J. T., K. A. Jones, and R. Tjian (1986) Promoter-specific activation of RNA polymerase II transcription by Sp1. *Trens Biochem. Sci.* **11**, 20-23.
17. Mercola, M., J. Goverman, C. Mirell, and K. Calame (1985) Immunoglobulin heavy-chain and enhancer requires one or more tissue-specific factors. *Science* **227**, 266-270.
18. Sambrook, J., E. F. Fritsch, and T. Maniatis (1982) In "Molecular cloning," A laboratory manual. 2nd ed., Cold Spring Harbor Laboratory, New York.
19. An, J. H., P. R. Ebert, A. Mitra, and S. B. Ha (1988) Binary vector. *Plant Mol. Biol. Manual* A2, 1-19.
20. Holsters, M., D. de Waele, A. Depicker, E. Messens, M. Van Montagu, and J. Schell (1978) Transfection and transformation of *A. tumefaciens*. *Mol. Gen. Genet.* **163**, 181-187.
21. Lin, W., J. T. Odell, and R. M. Schreiner (1987) Soybean protoplast culture and direct gene uptake and expression by cultured soybean protoplasts. *Plant Physiol.* **84**, 856-861.
22. Krens, F. A., L. Molendijk, G. J. Wullems, and R. A. Schilperoort (1982) *In vitro* transformation of plant protoplasts with Ti-plasmid DNA. *Nature* **296**, 72-74.
23. Horsch, R. B., J. E. Fry, N. L. Hoffman, D. Eichholtz, S. Rogers, and R. T. Fraley (1985) A simple and general method for transferring genes into plants. *Science* **227**, 1229-1231.
24. Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol.* **15**, 473-497.
25. Jefferson, R. A., M. Klass, N. Wolf, and D. Hirsh (1987) Expression of Chimeric Genes in *Caenorhabditis elegans*. *J. Mol. Biol.* **193**, 41-46.
26. Proudfoot, N. T. (1979) Eukaryotic Promoters? *Nature* **279**, 376.
27. Messing, J., D. Geraghty, G. Heidecker, N.-T. Hu, J. Kridl, and I. Rubenstein (1983) *In Genetic Engineering of Plants* (Kosuge, T., *et al.*, eds.), pp.211-217, Plenum Press, New York.
28. Jofuku K. D., J. K. Okamuro, and R. B. Goldberg (1987) Interaction of an embryo DNA binding protein with a soybean lectin gene upstream region. *Nature* **328**, 734-737.
29. Dickinson, C. D., R. P. Evans, and N. C. Nielsen (1988)

RY repeats are conserved in the 5'-flanking regions of legume seed protein genes. *Nucl. Acids Res.* **16**, 371.
30. Goldberg, R. B. (1986) Regulation of plant gene expression. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **314**, 343-353.
31. Chen, Z. L., M. A. Schuler, and R. N. Beachy (1986) Functional analysis of regulatory elements in a plant embryo-specific gene. *Proc. Natl. Acad. Sci. USA* **83**, 8560-8564.
32. Chen, Z. L., N. S. Pan, and R. N. Beachy (1988) A DNA sequence element that confers seed-specific enhancement of a constitutive promoter. *EMBO J.* **7**, 297-301.
33. Gatehouse, J. A., I. M. Evans, R. R. D. Croy, and D. Boulter (1986) Differential expression of genes during legume seed development. *Philos. Trans. R. Soc. Lond. B. Biol.* **314**, 367-384.
34. Lohmer, S., M. Maddaloni, M. Motto, N. D. Fonzo, H. Hartings, F. Salamini, and R. D. Thomson (1991) The maize regulatory locus *Opaque-2* encodes a DNA-binding protein which contains the transcription of the *b-32* gene. *EMBO J.* **10**, 617-624.
35. Bäumlein, H., U. Wobus, J. Pustell, and F. C. Kafatos (1986) The legumin gene family: structure of a B-type gene of *Vicia faba* gene and a possible legumin gene specific regulatory element. *Nucl. Acids Res.* **14**, 2707-2720.
36. Lycett, G. W., R. R. D. Croy, A. H. Shirsat, D. M. Richards, and D. Boulter (1985) The 5'-flanking regions of three pea legumin genes: comparison of the DNA sequences. *Nucl. Acids Res.* **13**, 6733-6743.
37. Chamberland, S., N. Daigle, and F. Bernier (1992) The legumin boxes and the 3' part of a soybean β-conglycinin promoter are involved in seed gene expression in transgenic tobacco plants. *Plant Mol. Biol.* **19**, 937-949.
38. Bäumlein, H., I. Nagy, R. Villarroel, D. Inze, and U. Wobus (1992) *Cis*-analysis of a seed protein gene promoter: the conservative RY repeat CATGCATG within the legumin box is essential for tissue-specific expression of a legumin gene. *Plant J.* **2**, 233-239.
39. Lelievre, J-M., L. O. Oliveira, and N. C. Nielsen (1992) 5'-CATGCAT-3' elements modulate the expression of glycinin genes. *Plant Physiol.* **98**, 387-391.
40. Bustos, M. M., D. Begum, F. A. Kalkan, M. J. Battraw, and T. C. Hall (1991) Positive and negative *cis*-acting DNA domains are required for spatial and temporal regulation of gene expression by a seed storage protein promoter. *EMBO J.* **10**, 1469-1479.

대두 원형질체와 형질전환된 담배에서의 대두 glycinin 유전자 *Gy2* promoter의 발현조절 기작

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초록 : 대두 glycinin 유전자의 조직 특이적이고 분화 발달 특이적인 발현 조절 메카니즘을 연구하기 위하여 *Gy2* 유전자의 5' upstream 부위 염기서열을 조사한 결과, glycinin 유전자의 발현을 조절하는 인자로 여겨지는 여러가지 조절 인자들을 발견하였다. 진핵세포 유전자에 공통적으로 존재하는 TATA box와 AGGA box가 존재하고, 종자 저장 단백질에서 공통적으로 발견되는 embryo factor binding sequence, RY repeat, CACA sequence, β-conglycinin enhancer와 유사한 sequence 등이 발견되었다. 이러한 조절 요소들이 *Gy2* 유전자의 발현 조절에 미치는 영향을 알아보기 위해 *Gy2* 유전자의 5' upstream 부위를 Exo III nuclease와 여러가지 제한효소를 이용하여 일련의 deletion mutants를 제조한 후 GUS 유전자와 결합시켰다. 이들 여러가지 chimeric constructs를 대두 원형질체에 전입하고 원형질체로부터 추출물을 분리하여 GUS 활성을 조사한 결과, −281~−223 혹은 −170~−122 부위를 포함하였을 경우 활성이 감소하였고, −223~−170 혹은 −122~−16 부위를 포함하였을 경우 활성이 높게 나타났다. 이러한 *Gy2* 유전자의 이중적인 발현 양상은 glycinin 유전자의 발현조절에 음성 조절 요소와 양성 조절 요소가 관여하고있다는 사실을 제시해 주고 있다. 또한 이들 여러가지 chimeric constructs로 형질 전환된 담배의 종자와 잎에서 GUS 활성을 조사한 결과, CaMV promoter를 포함하는 chimeric construct는 종자와 잎에서 모두 활성을 나타냈으나, *Gy2* promoter를 포함하는 chimeric constructs는 종자에서만 GUS 활성을 나타내고 잎에서는 활성이 나타나지 않는 조직 특이적인 발현 양상을 나타내었다.

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