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Accumulation and assembly of soybean β-conglycinin in seeds of transformed petunia plants

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A gene encoding the α′-subunit of β-conglycinin, a seed storage protein of soybean (*Glycine max*), was transformed into petunia cells on a disarmed Ti-plasmid of *Agrobacterium tumefaciens*, and plants were regenerated. Transcripts of the introduced gene accumulated in immature embryos but not in leaves of the transformed plants. Soybean protein was first detected immunologically in proteins extracted from embryos at 10 days post pollination (d.p.p.), concurrent with the accumulation of subunits of the major petunia seed proteins. Between 10 and 16 d.p.p. the primary soybean protein detected had an apparent mol. wt. of 55 kd. The 76-kd α′-subunit and several smaller polypeptides accumulated between 16 and 24 d.p.p., when seeds had matured. Polypeptides <76 kd probably resulted from specific proteolytic cleavage of the α′-subunit. The α′-subunit and the smaller polypeptides assembled into multimeric proteins with sedimentation coefficients of 7–9S, similar to the sedimentation coefficients of β-conglycinins isolated from soybean seeds. This transformation and expression system should be ideally suited for testing gene mutations to alter the amino acid composition of these seed storage proteins.

Key words: β-conglycinin/soybean/seed storage protein/petunia

Introduction

Storage proteins accumulate to high levels in many types of seeds as a means of storing nitrogen for use during germination and early seedling growth. Because the genes encoding seed storage proteins are expressed at a specific period in the life cycle of the plant, seed proteins represent excellent model systems for studies of the regulated expression of genes in plants.

Legume seeds contain proportionately larger amounts of proteins than most cereal grains, with soybean seeds containing as much as 50% protein by weight. Soybeans contain two major storage proteins, the glycinins or 11S proteins, and the β-conglycinins, or 7S proteins. The latter are trimeric molecules of approximate mol. wt. 200 000, and are produced by assembly of three subunits, α′ (76 kd), α (72 kd) and β (53 kd) subunits. The expression of the genes encoding the subunits of β-conglycinin and the accumulation of their respective mRNAs has been studied in several laboratories (Goldberg *et al.*, 1981; Meinke *et al.*, 1981). More recent studies of the β-conglycinin genes have included *in vitro* run-off transcription experiments (Walling, Harada and Goldberg, in preparation), micrococcal nuclease and DNase I sensitivity of gene sequences in nuclei isolated from embryos (Tierney and Beachy, unpublished), and studies of the role of abscisic acid in regulating the expression of genes in embryos (Bray and Beachy, 1985). Studies of the biosynthesis and assembly of the subunits to form β-conglycinin (Beachy *et al.*, 1981; Shattuck-Eidens and Beachy, 1985), and degradation of β-conglycinin during seed germination (Bryant, Hosangadi and Beachy, in preparation) have also been completed. These and other experiments have resulted in a good understanding of the biology of the β-conglycinins, and the types of regulation that control their accumulation during seed development.

Soybean seed proteins, in particular the β-conglycinin, contain low amounts of sulfur-amino acids (Holowach, 1984; Koshiyama, 1983), preventing the use of soy protein as a fully balanced source of protein. It may be possible to alter the amino acid composition of the storage protein genes to improve the nutritional quality of the encoded protein. Each mutation made, however, must be tested for effects of the mutation on the structure and function of the protein product. In this paper we report the results of experiments to introduce a gene encoding the α′-subunit β-conglycinin into petunia cells *via* a disarmed Ti-plasmid in *Agrobacterium tumefaciens*. Seeds produced on plants regenerated from transformed cells produced high levels of the α′-subunit and a 55-kd breakdown product of the α′-subunit. Soybean proteins isolated from petunia seeds have sedimentation coefficients of 7–9S, consistent with the hypothesis that they assemble into a multimeric form similar to the assembly of β-conglycinin in soybeans. This system should provide a convenient assay for testing *in vivo* the effect of mutations that alter the amino acid composition of a soybean protein on its structure and function.

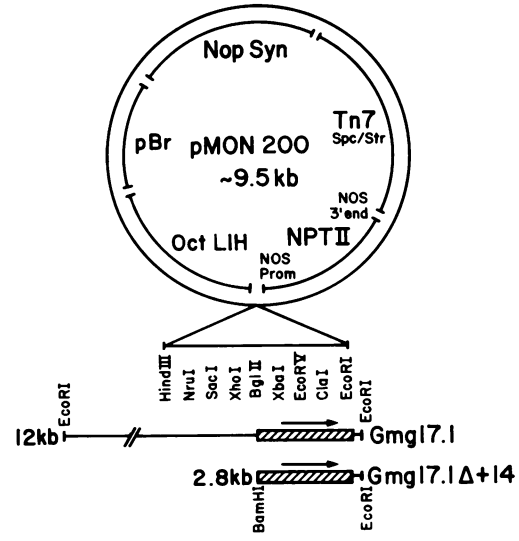

Fig. 1. Intermediate plasmid used to deliver target genes to *A. tumefaciens* Ti-plasmid. pMON200 (Fraley *et al.*, 1985) was linearized with *Eco*RI, or *Eco*RI and *Bgl*II, and ligated with Gmg 17.1 or Gmg 17.1 Δ + 14. Gmg 17.1 contains the α′-subunit gene (boxed area; arrows indicate direction of transcription) flanked by 11.5 kb of DNA 5′ of the gene. Gmg 17.1 Δ + 14 lacks 5′ flanking sequences, and begins at nucleotide +14.

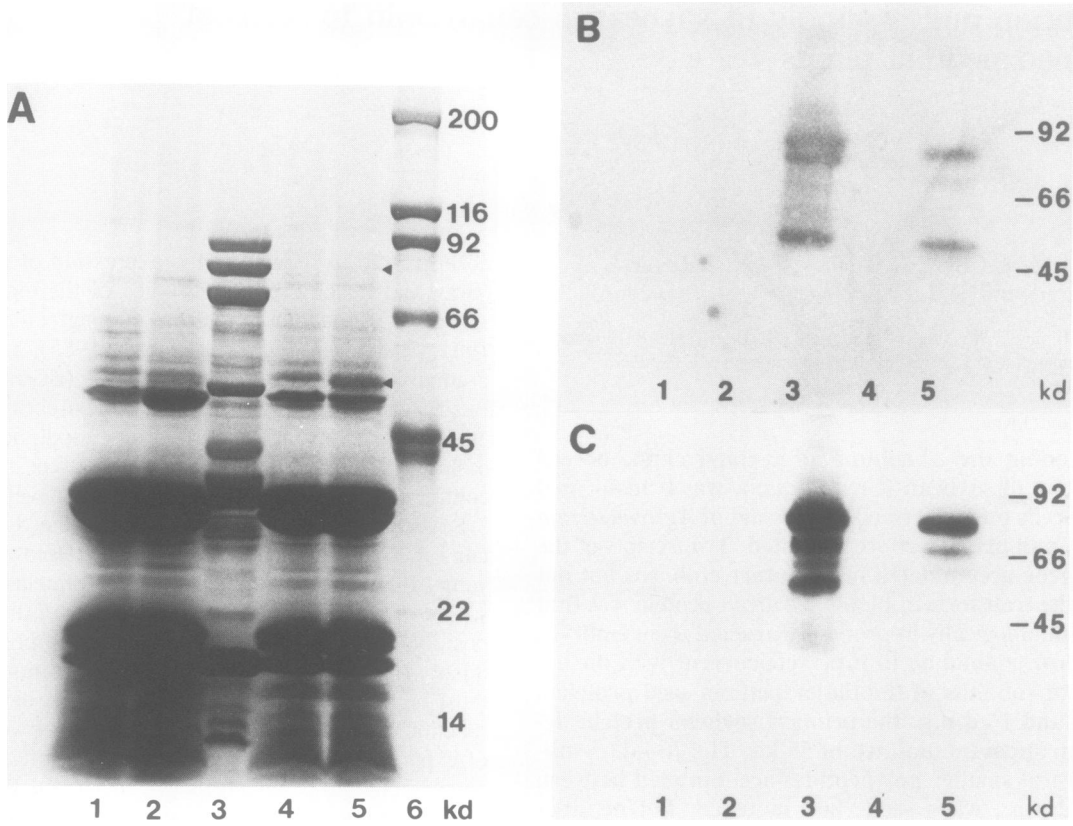

Fig. 2. Accumulation of the soybean α′-subunit in petunia seeds. 50 μg of protein extracted from petunia seeds or 10 μg of soybean protein were subjected to SDS-PAGE and stained with Coomassie blue **(A)** or immunoblot analysis **(B,C)**. **Lane 1:** protein from seeds of normal petunia plants; **lane 2:** protein from seeds of petunia plants transformed to contain vector pMON200; **lane 3:** protein from soybean seeds; **lane 4:** protein from seeds of petunia plants transformed to contain Gmg 17.1 on pMON200; **lane 5:** protein from seeds of petunia plants transformed to contain Gmg 17.1 Δ + 14 on pMON200. **(A)** is a 5–25% gradient gel. In **B** and **C**, proteins were separated on 10% acrylamide gels and then electroblotted to nitrocellulose. Blotted proteins were reacted with a rabbit polyclonal antibody raised against soybean β-conglycinin followed by ^{125}I-donkey anti-rabbit antibodies **(B)** or a mouse monoclonal antibody that reacts to the α′-subunit followed by ^{125}I-rabbit anti-mouse antibodies **(C)**. The positions of mol. wt. markers are indicated.

Results

Accumulation of soybean proteins and mRNA in seeds of transformed petunia plants

Plants were regenerated from transformed cells selected on medium containing kanamycin as described by Horsch *et al.* (1985). To confirm that regenerated plants were transformed, leaf segments were removed periodically and assayed for the presence of nopaline by paper electrophoresis using purified nopaline as standard. Kan^r nop$^+$ plants were grown in the greenhouse, the flowers were self-pollinated and mature seeds were collected. 50 μg of seed protein was extracted and subjected to SDS-PAGE on gels containing a gradient of 5–25% polyacrylamide. Proteins that stained with Coomassie blue are shown in Figure 2A. Included as controls in these experiments were proteins extracted from non-transformed petunia seed (lane 1), transformed petunias carrying pMON200 (lane 2), and transformed petunias carrying Gmg 17.1 Δ + 14 (lane 4). These proteins are compared with proteins extracted from transformed petunias carrying Gmg 17.1 (lane 5) and with proteins extracted from soybean seeds (lane 3). The arrows to the left indicate the position of new protein bands found in plants containing Gmg 17.1 that are not present in the controls. The intensely stained proteins in lanes 1, 2, 4 and 5 with apparent mol. wts. of 30–35 kd and 17–20 kd are subunits of the major petunia seed protein, a legumin-like protein with a sedimentation coefficient of ~12S.

Seed proteins were also subjected to analysis by immunoblot assays using polyclonal antibodies against total soybean β-conglycinin raised in rabbits, or to a monoclonal antibody AG3.4 that is specific for the α′-subunit. As shown in Figure 2B and C, only transformed petunia plants carrying Gmg 17.1 contain pro-

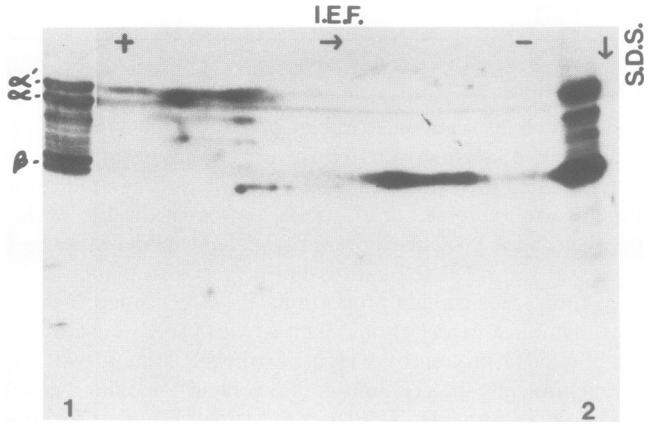

Fig. 3. Immunoblot analysis of petunia seed proteins separated on a two-dimensional gel. Petunia seed proteins were separated in the first dimension by isoelectric focussing and in the second dimension by SDS-PAGE using the methods of O'Farrell (1975). The direction of the first dimension is indicated at the top. **Lane 1** contains soybean seed proteins; **lane 2** contains petunia seed proteins. Electroblotted proteins were reacted with the polyclonal antibody as described in Figure 2.

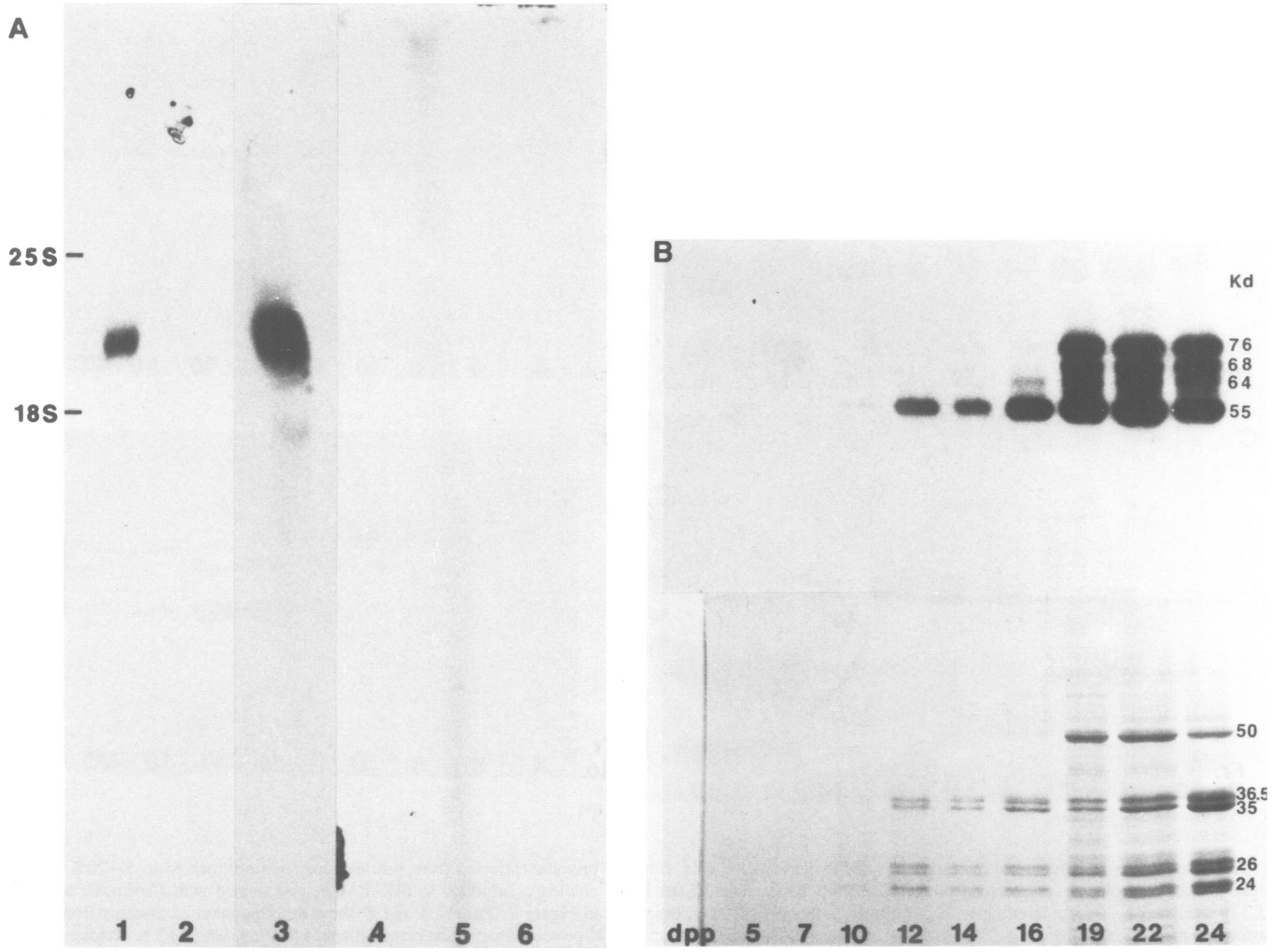

Fig. 4. Regulated expression of the α′-subunit genes in transformed petunia plants. **(A)** Northern blot analysis of 10 μg of total RNA extracted from immature embryos of plants transformed to contain Gmg 17.1 **(lane 1)** or Gmg 17.1 Δ + 14 **(lane 2)**. **Lane 3** contains RNA from immature soybean seeds; **lane 4**, poly(A)$^+$ RNA from leaves of normal petunia; **lane 5**, poly(A)$^+$ RNA from leaves transformed to contain Gmg 17.1, **lane 6**, poly(A)$^+$ RNA from leaves of plants transformed to contain Gmg 17.1 Δ + 14. **(B)** Analysis of proteins extracted from embryos collected from transformed plants containing Gmg 17.1 at increasing d.p.p. Each lane contained 20 μg of protein. **Upper:** electroblotted proteins were reacted with the rabbit polyclonal antibody against soybean β-conglycinin, followed by ^{125}I-donkey anti-rabbit antiserum. **Lower:** proteins were stained with Coomassie blue. The apparent mol. wt. of the most abundant seed proteins is indicated.

teins which reacted with the antibodies used in these experiments. The 76 kd protein has electrophoretic mobility similar to the α′-subunit of soybean, and is immunoreactive with the α′-specific monoclonal antibody. The major immunoreactive protein has an apparent mol. wt. of 55 kd, slightly greater than the soybean β-subunit (53 kd), and was not immunoreactive with Ag3.4. In addition, other polypeptides, with apparent mol. wts. of 68, 64 and 20 kd, were also detected in the protein samples extracted from the plants transformed with Gmg 17.1 (Figure 4B).

To characterize further the soybean proteins that accumulated in petunia seeds, total seed proteins were separated in two-dimensional gels and analyzed by immunoblots. As shown in Figure 3, the α′-subunit from petunia seeds is made up of at least two charged isomeric forms, while the 55 kd polypeptide exhibits a wide range of charge heterogeneity. Each of the other polypeptides that are antigenically related to the α′-subunit are also made up of multiple charged isomers. Heterogeneity in charge of α′-subunits was also observed in proteins extracted from soybean seeds (Ladin *et al.*, 1983).

Since multiple polypeptides related to β-conglycinin accumulated in petunia seeds it was important to determine whether single or multiple soybean mRNA(s) was produced in transformed petunias. Poly(A)$^+$ RNA from leaves and total RNA from seeds were hybridized on Northern blots with pMON200 containing Gmg 17.1 Δ + 14 as the probe. As shown in Figure 4A only seeds of petunia plants carrying Gmg 17.1 (lane 1) contained an RNA with mobility equivalent to the α′-mRNA extracted from the soybean seeds (lane 3). This mRNA is ~2400 nucleotides in length, the size of the α′-mRNA as determined by Beachy *et al.* (1981), and by DNA sequence analysis of the α′-gene on Gmg 17.1 (Doyle *et al.*, in preparation) and a cloned cDNA corresponding to the α′-mRNA (Schuler *et al.*, 1982). Leaves of transformed plants did not contain the 2.4-kb α′-mRNA.

The results of these experiments indicated that the α′-subunit gene on Gmg 17.1 is expressed in a tissue-specific manner in transformed petunias, producing the expected 2.4-kb mRNA. The product of the translation of this mRNA is the α′-subunit. The other antigenically related polypeptides found in seeds of plants

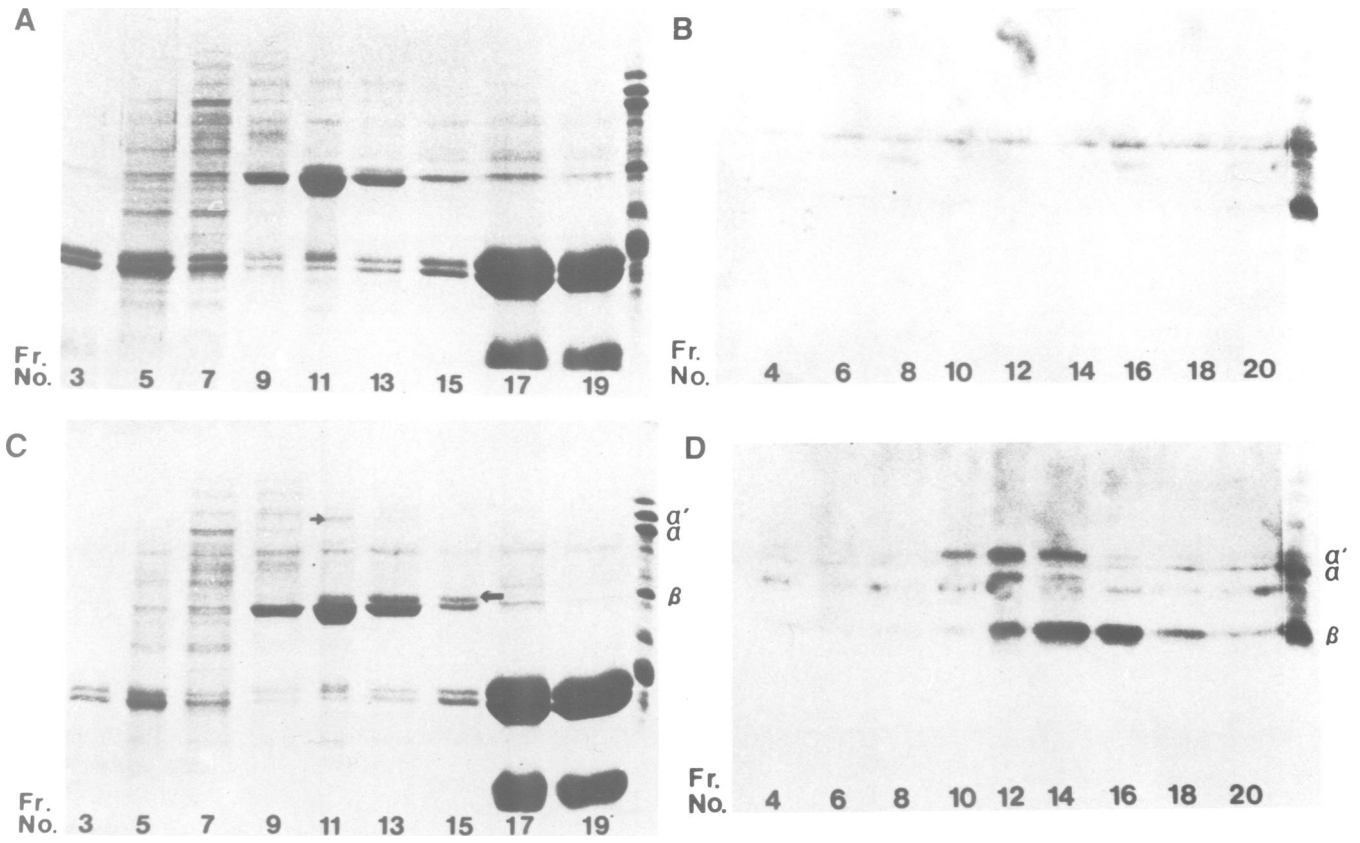

Fig. 5. Sedimentation of petunia seed proteins in sucrose density gradients. 5 mg of proteins extracted from petunia seeds was sedimented in 5–20% linear sucrose gradients. Proteins were acetone precipitated from 20 fractions of the 14 ml gradients, subjected to SDS-PAGE, and stained with Coomassie blue **(A,C)** or analyzed by immunoblotting with polyclonal antibodies **(B,D)** as described in Figure 2. **Panels A** and **B** show the separation of proteins from transformed plants containing Gmg 17.1 Δ + 14; **(C)** and **(D)** show the separation of proteins from transformed plants containing Gmg 17.1. Arrows in **(C)** indicate proteins in seeds of plants carrying Gmg 17.1 that are not present in seeds of plants carrying Gmg 17.1 Δ + 14. The right-most lanes in each panel contain soybean seed proteins. β-Conglycinin from soybean seed sediments in fractions 10–14 (not shown).

containing the α′-gene are presumed to result from limited proteolysis of the α′-subunit, but further characterization of the related polypeptides remains to be done.

Accumulation of soybean protein during development of petunia seeds

The β-conglycinins are temporally regulated throughout soybean embyrogenesis (Meinke *et al.*, 1981; Goldberg *et al.*, 1981; Gayler and Sykes, 1981). To determine if the α′-subunit gene present on Gmg 17.1 was temporally regulated in petunia embryos, proteins were extracted from petunia seeds taken at various days post pollination (d.p.p.) and subjected to SDS-PAGE and immunoblot analyses. In these experiments the seed capsule was mature 22 d.p.p. The 55 kd polypeptide related to the α′-subunit was first detected at 10 d.p.p. and the 76 kd α′-subunit and the other smaller polypeptides were detected at later times in seed development (Figure 4B, upper panel). The petunia seed storage proteins were also first detected at 10 d.p.p. (Figure 4B, lower panel), indicating that accumulation of the soybean protein is temporally regulated during petunia seed development in a manner similar to that of the major petunia storage proteins.

Assembly of the soybean polypeptides into multimeric proteins in petunia seeds

In soybean seeds α′-subunits are assembled with α- and β-subunits to produce multimeric proteins with sedimentation coefficients of 7–9S (Thanh and Shibasaki, 1978; reviewed by Koshiyama, 1983). To determine whether the α′-subunit in petunia seeds was assembled into multimers, 5 mg of protein from mature petunia seeds collected from transformed plants containing Gmg 17.1 or Gmg 17.1 Δ + 14 were subjected to sedimentation in sucrose gradients. Proteins present in 0.6-ml fractions were collected and resolved by SDS-PAGE and were either stained with Coomassie blue or transferred to nitrocellulose for immunoblot analyses. As shown in Figure 5A and C the stained protein patterns are similar in the two samples except for the presence of the 76 and 55 kd polypeptides (indicated by arrows) in fractions 10–16. These proteins are immunologically related to β-conglycinin (Figure 5D). The 76 kd protein was present in fractions 10–13 and has sedimentation characteristics similar to those of β-conglycinin isolated from soybeans (data not shown; see Beachy *et al.*, 1979). The 55 kd polypeptide was, however, present in protein complexes that sedimented between 7S and ~9S (fractions 10–18) indicating that under these experimental conditions the multimeric forms of the protein containing primarily the 55 kd protein are physically different from proteins that contain primarily the α′-subunit.

Quantitation of soybean proteins in petunia seeds and plant-to-plant variability

We examined the seed proteins of four independently transformed

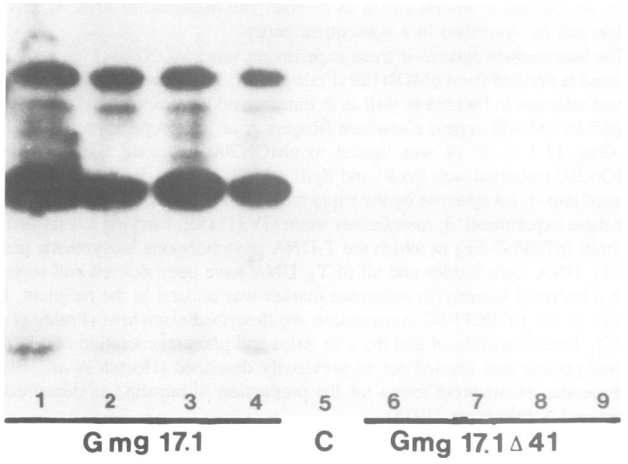

Fig. 6. Immunoblot analysis of proteins from four different transformants containing Gmg 17.1 or Gmg 17.1 Δ + 14. Each lane contains equivalent amounts of protein. Electroblotted proteins were allowed to react with the polyclonal antibody as described in Figure 2. Position of the 76-kd α′-subunit and the 55-kd polypeptide are indicated.

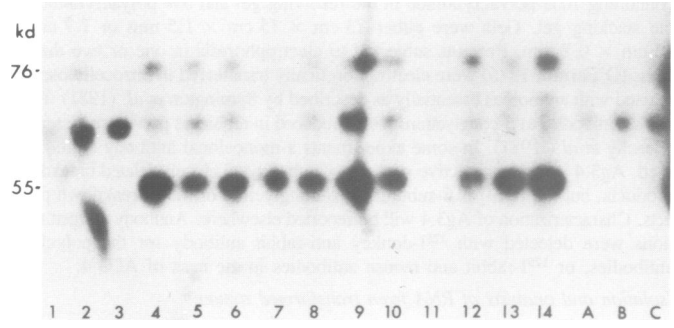

Fig. 7. Immunoblot analysis of the segregation of Gmg 17.1 in single seeds of transformed petunia plants. Proteins extracted from single seeds of a transformed petunia plant containing Gmg 17.1 were reacted with the polyclonal antibody as described in Figure 2 (lanes 1 – 14). **Lanes A, B** and **C** contain proteins of seeds from untransformed petunia, petunia transformed with pMON200, and petunia transformed to contain Gmg 17.1 Δ + 14, respectively.

plants carrying Gmg 17.1 to determine whether or not there was marked variability in the amount of accumulated soybean protein in different plants. In seeds of each of the four plants there was a nearly identical amount of soybean protein (Figure 6). The amount of soybean protein in the seeds was estimated to be between 0.1 and 1% of the total seed proteins extracted in an SDS-containing buffer. We also examined the amount of protein produced in plants that contained Gmg 17.1 in opposite orientation (relative to the NPTII gene; Figure 1) to that in these four plants. The level of protein in these plants was identical to that shown in Figure 6 (data not shown). Lanes 6 – 9 in Figure 6 contain proteins extracted from seeds of four different plants containing Gmg 17.1 Δ + 14, none of which produced a protein that was antigenically related to the α′-subunit.

To study the inheritance and segregation of Gmg 17.1 Western blot analyses were carried out on protein extracts prepared from single seeds from one transformed plant. In these experiments 58 of 77 seeds (75.3%) examined contained soybean protein, closely approximating the expected segregation ratios of 3:1. Figure 7 presents the results of one experiment in which 14 such seeds were examined. Lanes 1, 3 and 11 were scored as negative since they do not contain either the 76 kd or the 55 kd polypeptides. Lanes A, B and C contain extracts of seeds from various control plants (as indicated in the figure legend). We were unable to distinguish unequivocally homozygous seeds from heterozygous seeds on the basis of these experiments, probably due to the unequal extraction of proteins from individual seeds.

Discussion

The gene encoding the α′-subunit of the soybean seed storage protein β-conglycinin was introduced into petunia cells *via* a modified Ti-plasmid. The intermediate plasmid pMON200, which contains several unique restriction sites to facilitate the introduction of genes, and a strain of *A. tumefaciens* carrying a disarmed Ti-plasmid (Fraley *et al.*, 1985) were used throughout. The soybean proteins that accumulated in petunia seeds have physical characteristics similar to β-conglycinins isolated from soybean seeds. First, there are multiple isomeric forms of the 76-kd α′-subunit both in petunia seeds (Figure 3) and in soybean seeds (Ladin *et al.*, 1983, Figure 2). This is an important observation since it indicates that the number of charged isomers of the subunits does not necessarily reflect the number of different genes or alleles that encode these subunits.

Second, the 76-kd α′-subunit and the antigenically related 68, 64, 55, 20 and 18 kd polypeptides produced in petunia seeds assemble into multimeric proteins with sedimentation coefficients similar to those of the β-conglycinins from soybean seeds. The majority of the β-conglycinins from soybean and petunia seeds have sedimentation coefficients of 7 – 8S under the conditions used in these experiments (Figure 5). However, other β-conglycinins have sedimentation coefficients of ~9 – 11S. This is especially apparent in the case of the 55 kd polypeptide that accumulates in petunia seeds (fractions 14 – 18, Figure 5C,D). It is well known that the sedimentation properties of the β-conglycinins are variable in solutions containing different molarities of the NaCl (Koshiyama, 1983). However, it is unknown whether sedimentation of the 55 kd polypeptide at ~9S reflects an altered assembly of the protein, perhaps into a hexameric molecule (Koshiyama, 1983), or assembly with other petunia proteins to produce novel multimeric proteins.

Thanh and Shibasaki (1978) reported that β-conglycinin oligomers containing three α′-subunits were not present in soybean seeds. In petunia seeds which contain only the α′-subunit, oligomers comprised of the α′-subunit and polypeptides derived therefrom were found. These results clearly demonstrated that the product of the α′-subunit gene can assemble into a multimeric protein, and may indicate that the conclusions of Thanh and Shibaski (1978) should be re-examined. On the other hand, our conclusion that the α′-subunit self-reassmbles in petunia seeds is tentative until it is demonstrated that β-conglycinin produced in petunia seeds does not contain petunia polypeptides.

A third similarity between soybean and petunia β-conglycinins is that each contains numerous polypeptides that are antigenically related to, and probably derived from, the α′-subunit. These polypeptides in soybean β-conglycinins are collectively designated the γ-subunits (Ladin *et al.*, in preparation), and generally have mol. wts. between 68 and 55 kd. Several of these polypeptides have electrophoretic mobilities similar to the 68, 60 and 55 kd polypeptides found in petunia seeds (Figure 2). Whether the polypeptides found in petunia seeds are in fact identical to those found in soybean seed remains to be determined.

The 55-kd polypeptide is the first soybean protein that ac-

cumulates in petunia seeds during embryogenesis, followed by the 76-kd α′-subunit (Figure 3) and the 68 and 64 kd polypeptides. This suggests that the 76 kd protein is initially unstable in the immature embryos, but becomes increasingly stable as the seed matures. Recently we reported that the α′- and α-subunits are also unstable during early stages of soybean seed development (Shattuck-Eidens and Beachy, 1985). Taken together, these results lend support to the hypothesis that accumulation of β-conglycinin is, to some degree, dependent upon the stability of the protein in the protein body. Protein bodies in soybean seeds are derived from the cell vacuole (Beachy *et al.*, 1979; Yoo and Chrispeels, 1979), and it appears that storage proteins are inherently resistant to the activity of hydrolases in vacuolar protein bodies, or that the hydrolases are less active in mature seeds than in immature seeds. Either or both conditions would result in accumulation of seed storage proteins as the seed matures. Sengupta *et al.* (1985) recently reported the expression of a gene encoding the β-subunit of phaseolin in seeds of transformed tobacco plants. In this study the 48 kd phaseolin β-subunit was the first protein that accumulated while smaller polypeptides derived from the β-subunit accumulated late in seed development. These authors suggested that the phaseolin β-subunit was less protected from proteolysis as the seed matures. Thus, the results of the experiments reported by Sengupta *et al.* (1985) differ significantly from those reported here.

Plant transformation experiments have previously been used to study the expression of genes under control of promoters taken from the T-DNA region of the Ti-plasmid (Fraley *et al.*, 1983; Bevan *et al.*, 1983; Herrera-Estrella *et al.*, 1983), from cauliflower mosaic virus (Koziel *et al.*, 1984), and from the small subunit of ribulose bisphosphate carboxylase (RuBisCo; Broglie *et al.*, 1984; Herrera-Estrella *et al.*, 1984). In the latter case, transcription was induced by exposing transformed tissues to light. We have studied the expression of a gene which is under stringent developmental control and is expressed primarily in developing embryos. We have found that Gmg 17.1 is under tight control in plants regenerated from transformed petunia cells, and is expressed in much higher levels in developing seeds than in other parts of the plant (to be reported elsewhere). Similar results have recently been reported for the phaseolin gene (Sengupta *et al.*, 1985). We are currently identifying the DNA sequences which are required for developmental regulation of the Gmg 17.1 gene.

Since the protein product of the α′-gene Gmg 17.1 assembles into a multimeric form in petunia, this system represents an excellent model system for testing proteins with modified amino acid composition. Each modified gene can be tested in petunia plants where the expression and stability of the modified protein can be determined. Such studies may make it possible to improve the nutritional quality of these proteins with the anticipation of eventually returning the modified genes into agronomically valuable seed grains.

Materials and methods

Construction of the intermediate plasmid and conjugation into A. tumefaciens

The isolation and partial characterization of a 12-kb *Eco*RI fragment of soybean genomic DNA containing an α′-subunit gene has been previously reported (Schuler *et al.*, 1982). On this fragment, Gmg 17.1, the α′-gene is flanked on the 5′ end by ~9 kb of DNA and by 0.4 kb of DNA on the 3′ end. No other storage protein genes are located on this DNA fragment. A second gene fragment used in these experiments, referred to as Gmg 17.1 Δ + 14, was produced by restriction digestion and *Bal*31 exonuclease digestion to remove all sequences 5′ of the gene, and ending at nucleotide +14 (+1 is the first nucleotide transcribed). Gmg 17.1 Δ + 14 contains a *Bam*HI site at its 5′ end. The methods for creating this deletion will be described in a subsequent paper.

The intermediate plasmid in these experiments was pMON200 (Figure 1). This plasmid is derived from pMON128 (Fraley *et al.*, 1983) and contains genes that permit selection in bacteria as well as in transformed plant cells. A full description of pMON200 will appear elsewhere (Rogers *et al.*, in preparation). Gmg 17.1 or Gmg 17.1 Δ + 14 was ligated to pMON200 restricted with *Eco*RI, or pMON200 restricted with *Eco*RI and *Bgl*II. pMON200 and derivatives were conjugated into *A. tumefaciens* by the triple mating procedure previously described. For these experiments *A. tumefaciens* strain GV3111SE, carrying a disarmed Ti-plasmid (pTiB6S3-SE) in which the T-DNA phytohormone biosynthetic genes, the T_L DNA right border and all of T_R DNA have been deleted and replaced with a bacterial kanamycin resistance marker was utilized as the recipient. Full details of the pTiB6S3-SE construction are described elsewhere (Fraley *et al.*, 1985). Transformation of leaf discs or strips and plant regeneration of a diploid hybrid petunia was carried out as previously described (Horsch *et al.*, 1985). Regenerated plants were tested for the production of nopaline as described by Otten and Schilperoort (1978).

Extraction and analysis of proteins from petunia seeds

Proteins were extracted from immature seeds and from single mature seeds by grinding in Laemmli (1970) sample buffer (2% SDS, 0.285 M Tris-HCl, pH 6.8, 20% glycerol, 4% β-mercaptoethanol and 0.0025% bromophenol blue). Proteins for two-dimensional gel analysis and sucrose gradient centrifugation were extracted by grinding seeds in the high salt buffer used to extract globulin seed proteins, i.e., 0.4 M NaCl, 0.035 M $NaPO_4^-$, 0.01 M β-mercaptoethanol, pH 7.2. Extracted proteins were quantitated by the Coomassie blue assay (Bradford, 1976) using bovine serum albumin as standard.

SDS-PAGE was carried out essentially as described by Laemmli (1970) in gels containing 10% polyacrylamide in the resolving gel and 5% polyacrylamide in the stacking gel. Gels were either 23 cm × 15 cm × 1.5 mm or 7.7 cm × 13 cm × 0.7 mm. Proteins subjected to electrophoresis in one or two dimensions (O'Farrell, 1975) were electrophoretically transferred to nitrocellulose and reacted with antibodies essentially as described by Symington *et al.* (1981). Polyclonal antibodies to β-conglycinins were produced in rabbits as previously described (Beachy *et al.*, 1981). In some experiments a monoclonal antibody Ag3.4 was used. Ag3.4 is immunoreactive with the α′-subunit and closely related breakdown products, but not to α- or β-subunits of β-conglycinin or their breakdown products. Characterization of Ag3.4 will be reported elsewhere. Antibody:antigen reactions were detected with ^{125}I-donkey anti-rabbit antibody for the polyclonal antibodies, or ^{125}I-rabbit anti-mouse antibodies in the case of AG3.4.

Isolation and analysis of RNA from transformed tissues

RNA was isolated from leaves by multiple phenol extraction essentially as described by Lane and Tumaitis-Kennedy (1981). RNA was isolated from immature seeds removed from seed capsules collected between 15 and 20 d.p.p. Immature fresh seeds were immersed in phenol and homogenized with an equal volume of 1 M Tris-HCl, pH 9.0, with a ground glass pestle in a microfuge tube. The phenol phase was re-extracted with 0.1 M Tris, pH 9.0, and combined aqueous phases were re-extracted with phenol. RNAs that were precipitated with 2.0 M NaCl were collected and subjected to oligo(dT) cellulose chromatography. Poly(A)$^+$ RNA or total RNA [not selected on oligo(dT) cellulose] was resolved by electrophoresis in 1.2% agarose gels containing formaldehyde, and blotted to nitrocellulose as described in Maniatis *et al.* (1982). The hybridization probe was pMON200 containing Gmg 17.1 Δ + 14 labeled with ^{32}P by nick-translation to a sp. act. of $1-3 \times 10^8$ c.p.m./μg DNA. The filters were hybridized in a solution containing 4 × SSC and 50% formamide at 42°C. After hybridization filters were washed at 65°C in 1 × SSC containing 0.1% SDS prior to exposure to X-ray film for 1–4 days.

Sucrose density gradient centrifugation

Linear sucrose gradients containing 5–20% sucrose in grinding buffer were overlaid with 5 mg of proteins extracted from petunia seeds. After centrifugation at 20°C for 14–16 h at 35 000 r.p.m. in a Beckman SW40 rotor, gradients were fractionated on an ISCO gradient fractionator and monitored at 280 nm. The collected fractions were dialyzed to remove sucrose, and proteins were precipitated by addition of five volumes of acetone. Proteins were suspended in Laemmli (1970) sample buffer and subjected to SDS-PAGE.

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