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Cloning and characterization of cDNA clones encoding soybean seed maturation polypeptides

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Abstract. To study soybean seed maturation polypeptides, we have selected clones from a pod-dried seed cDNA library by differential screening. The clones, pGmPM 1 through 9, whose mRNAs are abundant in the dry seed, were characterized. These clones hybridize to RNA classes of approximately 900 to 2400 nucleotides whose accumulation are detected in pod-dried as well as mature soybean seeds. The molecular mass of their corresponding proteins were determined by hybrid select translation. These cDNA clones correspond to soybean seed maturation polypetides of 18, 19, 20, 22, 31, 32, 41, 48, 50, 52, 60, and 70 kD. It is also demonstrated in this study that the cotyledon tissue is a better material than whole seed to select for maturation polypeptide cDNA clones.

Key words: Late embryogenesis abundant *(Lea)* proteins; cDNAs; Precocious mature.

Introduction

Studies have indicated that water loss or dessication is an important part of the maturation process in many monocot and dicot seeds, such as corn (Spraque, 1936), wheat (Armstrong *et al.*, 1982), French bean (Kermode and Bewley, 1985), and soybean (Rosenberg and Rinne, 1986). In many species, premature drying of immature seeds and the subsequent rehydration of these seeds results in a transition from embryonic development to germination and seedling growth. Thus, when drying treatments are imposed, the seeds are said to be precociously matured, as compared to naturally matured (Rosenberg and Rinne, 1988). In the present study, immatured soybean seeds as well as precociously mature soybean seeds are used for cDNA cloning work. The developmental stage between these two is only 4 days apart, which corresponding to the drying treatment period.

During seed maturation, there are dynamic changes in the nature of proteins being synthesized (e.g. Bewley and Marcus, 1990; Galau *et al.*, 1987). Synthesis and accumulation of these polypeptides is suggested to be correlated with desiccation tolerance (Dure *et al.*, 1989; Blackman *et al.*, 1991), seed dormancy (Ried and Walker-Simmons, 1990), ABA content (Hughes and Galau, 1991), and transition to seedling growth (Rosenberg and Rinne, 1986). These proteins are termed maturation polypeptides (Rosenberg and Rinne, 1986) or late embryogenesis abundant, *Lea*, proteins (Galau *et al.*, 1986). Their hydrophilic characteristics has been noted (Dure *et al.*, 1989). In addition, the property of these maturation polypeptides has been shown to be associated with desiccation, ABA, and salt treatment since there are high homology between the amino acid sequences of proteins induced by these factors (e.g. Dure *et al.*, 1989). We are interested in studying the relationship between seed maturation, drying, ABA, and transition to seedling growth. As a first step, as many as seed maturation polypeptide cDNA clones have to be pulled out.

In this study, we have isolated by differential screening and have characterized several clones from a precociously matured soybean seed cDNA library The method of isolation,subsequent identification, and char-

acterizations, including message sizes and corresponding protein weight, are presented here.

Materials and Methods

Plant Materials

Soybean (*Glycine max* (L.) Merrill cv. Shi-shi) seeds were kindly provided by Kaoshung Agricultural Experimental Station. The plants were grown to maturity in a field environment. Pods were harvested at mid-development (about 35 days after flowering, DAF), and seeds were precociously matured by air-drying the intact pods (pod-dried, PD) for 4 days (Hsing *et al.*, 1990).Whole seed, embryonic axes and cotyledons of fresh or pod-dried seeds were immediately frozen in liquid N_2 after the harvesting or the drying treatments and stored at -70°C prior to extraction.

In vivo Labeling of Protein

The embryonic axis or cotyledon was dissected and placed into a 6 mm dia. Petri dish. A 50 μl volume containing 25 μCi ^{35}S-methionine (specific activity > 1000 Ci/mmol, Amersham) in 50 mM Tricine buffer (pH 7.2) was applied to the tissue. The incubation proceeded for 4 h within the closed Petri dish and was terminated by thoroughly washing the tissue with pre-chilled, unlabeled, 1 mM methionine. The tissue was ground to slurry with 500 μl of grinding buffer: 63 mM Tris-HCl (pH 7.8)/20 mM $MgCl_2$/10 mM 2-mercaptoethanol/4 mM phenylmethylsulfonyl fluoride (PMSF). Homogenization was followed by the addition of 500 μl of sample loading buffer and then incubated at 100°C for 10 min.

RNA Extraction

RNA was extracted from frozen tissue using the guanidine-HCl extraction procedure of Chirgwin *et al.* (1979). Poly (A) RNA was isolated by an oligo (dT)-cellulose chromatography procedure, in which the binding buffer was 0.5 M LiCl, 10 mM Tris-HCl (pH 7.4), 0.5% (w/v) sodium dodecylsulfate (SDS), and the elution buffer was 10 mM Tris-HCl (pH 7.4), 0.05% (w/v) SDS. The poly (A) RNA was stored at -70°C in sterile H_2O.

In vitro Translation

The poly (A) RNA were translated *in vitro* using a rabbit reticulocyte lysate system (Promega Biotec.). For 25 μl translation assays, saturating amounts of poly(A) RNA (0.5-1 μg) were incubated for 60 min at 37°C, using 5 μCi of ^{35}S-methionine (specific activity > 1000 Ci/mmol, Amersham).

Separation of Protein

Proteins were separated by 12.5% SDS-polyacrylamide gels according to Laemmli (1970). Equal amounts of radioactive TCA-precipitable ^{35}S were loaded onto each slot. The labeled polypeptides were visualized by fluorography as described previously (Hsing *et al.*, 1990).

cDNA Library Construction

A cDNA library was prepared from poly(A) RNA isolated from the cotyledons of 4 days PD, 35 DAF seeds, and cloned in lambda ZapII (Strategene) as described previously (Hsing *et al.*, 1990) with some modification. The procedures of Huynh *et al.* (1985) and Gubler and Hoffman (1983) were used to synthesize double stranded cDNA. The EcoRI methylase reaction was not carried out because the EcoRI adaptors (Promega) were used, instead of the more commonly used EcoRI linkers. The cDNA was then size-selected by running through a NACS PREPAC column (Bethesda Research Lab.). The fraction containing cDNA greater than about 800 bp in length was collected, ligated with lambda ZAPII arms, and packed into phage using GigaPack packaging extracts (Strategene) according to the manufacturer's instructions. All plating was done on *E. coli* XL1-blue.

Differential Screening

For plus/minus differential screening, about 250 pfu were plated onto 90 mm diameter plate. Duplicate replicas were lifted from each of the plates and were treated according to standard methods. The Magnagraph Nylon membrane (Micron Separations Inc.) was used for lifting.

Radioactive probes were generated by randomly primed reverse transcription of poly(A) RNA isolated from the cotyledon of 4 day PD, 35 DAF seeds, or fresh 35 DAF seeds (the plus and minus RNAs, respectively). The reaction mixtures consisted of 50 mM Tris-HCl (pH 7.5), 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, 1 mM each of dGTP, dATP, dTTP, 0.5 μg $p(dN)_6$, 100 μCi ^{32}P-dCTP, 2 μg poly(A) RNA, and 500 units M-MLV reverse transcriptase (Bethesda Research Lab.) in 50 μl. Reactions were incubated at 37°C for 15 min, after

which 1 μl of 50 μM cold dCTP was added, and the incubations continued for one hour. Labeling was terminated by adding EDTA to a final concentration of 10 mM and precipitating the nucleic acids with ethanol.

In preparation for hybridization, the RNA-cDNA molecules were treated with alkaline, and the RNA hydrolyzed. In brief, the nucleic acid was dissolved in 200 μl of water, followed by the addition of 60 μl of 1.0 N NaOH. After incubation for 10 min at room temperature, the solution was neutralized by the addition of 60 μl of 1.0 N HCl and 60 μl of 20 X SSPE (1 X SSPE: 0.18 M NaCl, 10 mM NaH_2PO_4, pH 7.5, 1 mM EDTA). Filters were prehybridized overnight at 65°C in 4 X SSPE, 5X Denharddt's solution (1 X Denhardt's solution: 0.02% each Ficoll, bovine serum albumin, and polyvinylpyrrolidone), and 0.1% SDS. The solution was replaced with a fresh mix containing 2 X 10^7 cpm labeled cDNA and incubated at 65°C for 24 h. Filters were washed twice for 20 min at room temperature in 2 X SSPE, 0.1% SDS, and twice for 20 min at 50°C in 0.1 X SSPE, and 0.1% SDS. Plaque replicas were detected by autoradiography using Kodak X-Omat AR film at -70°C with an intensifying screen. Differentially hybridizing phage were plaque purified and used to prepare plasmid using *in vivo* subcloning according to manufacture's instruction.

Hybrid-Selected Translation of cDNA Clones

Characterization of selected cDNA clones was performed by hybrid-selected translation. Fifty μg of plasmid DNA were denatured and spotted onto nitrocellulose filters. The filters were air-dried, baked at 80°C *in vacuo* for 30 min, and hybridized with poly (A) RNA prepared from the cotyledons of 4 day PD, 35 DAF soybean seeds. The hybridization was carried out at 45°C for 3 h in 50 μl of 50% formamide, 20 mM Pipes buffer [1,4-piperazinediethanesulfonic acid] /NaOH (pH 6.4), 0.2% SDS, 0.4 M NaCl,100 μg yeast tRNA, and 5 μg poly (A) RNA . The reaction mixture was then removed, and the filters were washed 10 times with 1 X SSPE and 0.1% SDS at 65°C and two times with 1X SSPE at 65°C . To these filters, 1.2 ml sterile water and 10 μg yeast tRNA were added; the mixture was incubated at 100°C for 1 min. and immediately frozen in liquid N_2. After thawing on ice, the RNA was ethanol -precipitated and resuspended in 5 μl of sterile water. The entire RNA sample was translated *in vitro*.

RNA Blot Analysis

Poly (A) RNA were size-fractionated in formaldehyde-containing gels (Bartels *et al.*, 1986) and blotted to nylon filters (Magnagraph, MSI) which were reused several times according to the manufacturers' instructions.

The RNA samples for slot blotting were denatured in TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] by heating at 65°C for 10 min, cooled on ice, and diluted with two volumes of 15 X SSPE. Samples were applied to nitrocellulose filters using a slot blot manifold, and immobilized by baking as described above. Both slot blot and northern blot filters were prehybridized and hybridized as described above for plaque hybridizations. Cloned DNAs were labeled by the random oligonucleotide priming method of Feinberg and Vogelstein (1983).

Results

Newly Synthesized Polypeptides in Different Tissue

To investigate which part of the soybean seeds would be an ideal material for cDNA library construction and screening, the cotyledon and embryonic axis were dissected from fresh or 4 day PD, 35 DAF seeds. Newly synthesized polypeptides in these tissues were then analyzed using *in vivo* labeling techniques as described above, and the results are given in Figure 1.

Seed maturation in most plant species is initiated by a natural decline in seed water content. During the course of moisture loss in soybean seeds, several new polypeptides are synthesized *in vivo*, and are designated as mature polypeptides (Rosenberg and Rinne, 1986). These mature polypeptides are not present or are present in very low amounts in cotyledons and embryonic axis of fresh seeds. However they are present with high amounts in 4 days PD seeds, as indicated in Figure 1. The fresh immature seeds are grouped into two parts: average fresh weight per seed 200 mg (Fa seeds) and 250 mg (Fb seeds). Since there is no difference between lane 1 and 2 in Figure 1, the materials used in this study included fresh 35 DAF soybean seeds with fresh weight ranging from 200 to 270 mg. Also, there are more differences between lanes 2 and 3 than between lanes 5 and 6; and the protein bands in lanes 4, 5, and 6 are much more complicated than in lanes 1, 2, and 3. Thus, the differential appearance of soybean mature polypeptides are more obvious in cotyledon tissue than in embryonic

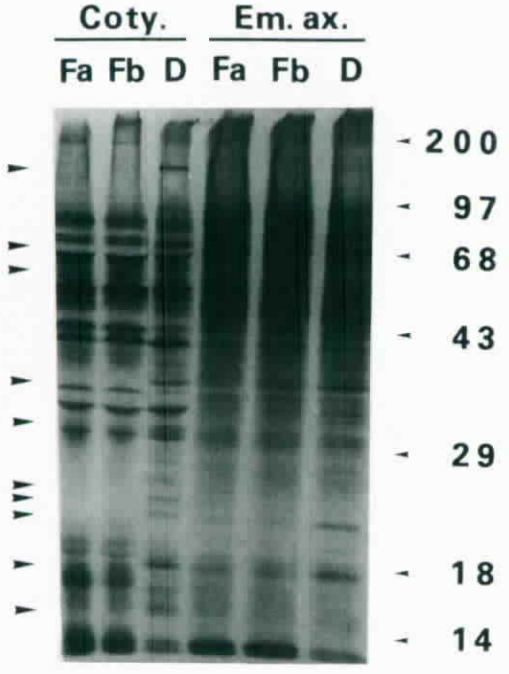

Fig. 1. Fluorogram of *in vivo* labeled proteins extracted from different parts of soybean seeds. Newly synthesized polypeptides in cotyledons of fresh 35 DAF seeds with 200 mg (Fa) average fresh weight (Lane 1), cotyledons of fresh 35 DAF seeds with 250 mg (Fb) average fresh wight (Lane 2), cotyledons of 4 days pod-dried (D) seeds with 250 mg average fesh weight (Lane 3), embryonic axis of fresh 35 DAF seeds with 200 mg average fresh weight (Lane 4), embryonic axia of fresh 35 DAF seeds with 250 mg average fresh weight (Lane 5) and embryonic axis of 4 days PD seeds with 250 mg average fresh weight (Lane 6) were seperated. Arrows on the right indicate the molecular weight markers in kD. Arrows on the left indicate the soybean seed maturation polypeptides, their apparent molecular mass are: 16, 18, 22, 23, 24, 31, 32, 41, 52, 70, and 130 kD, respectively.

axis tissue. The material for cDNA library construction and first stranded cDNA probes were therefore poly (A) RNA of cotyledon tissues, but not whole seeds or embryonic axis. According to the newly synthesized polypeptides profiles in lane 3, the apparent molecular mass of these maturation polypeptides were 16, 18, 22, 23, 24, 31, 32, 41, 52, 70, and 130 kD. Their total amount was about 2 to 5% of all polypeptides synthesized at cotyledon tissues of 4 day PD seeds.

Construction of cDNA Library

Cotyledons were dissected from 4 day PD, 35 DAF soybean seeds. Poly (A) RNA was extracted from these tissues and cDNA library was made from the RNA into the bacteriaphage vector lambda ZAP II. The size of the cDNA library was 2 X 10^6. The numbers of clones obtained in the library was large enough to represent rare messengers. The library, therefore, was more than sufficient for our screening experiments.

Identification of Clones with Preferential Expression in Pod-Dried Seeds

The cDNA library was amplified before screening work. cDNA clones from the library were plated at low density, and duplicated nylon lifts were made from each plate (see "Methods"). One filter was hybridized with ^{32}P-labeled single-stranded cDNA synthesized from the RNA that had been used to construct the library. The second filter was hybridized with a similar probe made from RNA from cotyledon of fresh seeds. The plaques showing substantially stronger hybridization to the pod-dried seed cDNA probes than to the fresh seed cDNA probes were picked up. The second screening was performed again using phage DNA. Duplicate DNA blots were hybridized with the same probes used in the original screening steps. Of approximately 8 X 10^3 phage screened, 456 contained cDNA inserts that gave strong (greater than 5-fold) preferential expression in pod-dried seeds relative to fresh seeds. Thus, the differential screening resulted in cDNA clones representing nearly 5% of the total cDNA clones screened, consistent with that expected from *in vivo* labeling data. Many clones were then subcloned into plasmid forms and characterized further.

Characterization of Soybean Maturation Polypeptide cDNA Clones

To determine which of the maturation polypeptides are encoded by these cDNA clones, hybrid select translation and northern blot hybridization experiments were performed. The 456 cDNA phages were then plated, and three duplicate lifts were made from each plate. Each set of filters was probed with known cDNA inserts. The relatioship between each clone was thus determined by DNA hybridization homology and classified into several gene families: pGmPM 1 through 9. GmPM stands for *Glycine max* physiological maturation.

Poly (A) RNA from 4 day PD seeds was hybridized with the immobilized pGmPM cDNA. The selected mRNAs were eluted in water at 100°C and translated

in vitro in a rabbit reticulocyte lysate. The *in vitro* translation products were analyzed by gel electrophoresis (Fig. 2) and compared with those obtained using total poly (A) RNA from 4 days PD seeds. In addition to the residual products from the lysate (lane I, J and K, the control), the polypeptides found in the profile correspond to the one encoded by the specific gene. During the whole studies, three lots of lysate were used. There is only one band as residual product for one lot (lane J) while many smeared bands for another (lane I and J). Lane A, B, D, F, H, and J belong to the same lot, lane C, E, G, and I belong to another, and lane K and L belong to another one. Several of these clones have 2 corresponding protein bands instead of one. For instance, clones pGmPM1 as well as pGmPM9 correspond to 20 and 22 kD maturation polypeptides and clone pGmPM2 correspond to 52 and 60 kD maturation polypeptides. The 20 kD band selected by pGmPM1 and 9 have a similar molecular mass with the lysate control, as illustrated in lane C and I. However, according to the sample applying amount and exposure time, it is certain that the 20 kD band showing in lane C being selected by pGmPM 1 but not the background. Clone pGmPM7 have also two corresponding protein bands, a heavy 32 kD band and a faint 31 kD band. This is the reverse of pGmPM6, which has a heavy 31 kD band and a faint 32 kD band. In summary, these cDNA clones correspond to soybean seed maturation polypeptides of 18, 19, 20, 22, 31, 32, 41, 48, 50, 52, 60, and 70 kD, respectively. The results for the hybrid select translation are illustrated in Table 1. The pGmPM1 clone corresponds to the maturation polypeptide of 22 kD, pGmPM2 to 52 as well as 60 kD, pGmPM3 to 18 as well as 19 kD,

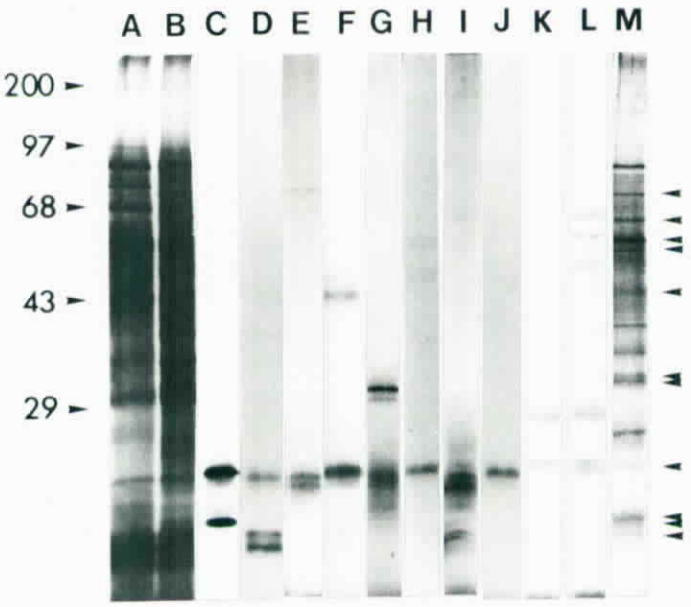

Fig. 2. Hybrid selection and translation of selected pGmPM-specific mRNAs. The figure shows the fluorographs obtained after separation on a SDS-PAGE. Lane A, poly (A) RNA of fresh 35 DAF soybean seeds; Lane B and M, poly (A RNA of 4 days PD 35 DAF seeds; Lane C, HST (hybrid select translation) of pGmPM 1; Lane D, HST of pGmPM 3; Lane E, HST of pGmPM 4; Lane F, HST of pGmPM 5; Lane G, HST of pGmPM 7; Lane H, HST of pGmPM 8; Lane I, J and K, endogenous mRNAs of cell-free systems, different batches; and Lane L, HST of pGmPM2. Sample loading amount and exposure time of each lane are: Lane A, 0.5 μl, 2 days; B, 0.5 μl, 2 days; C, 2 μl, 2 days; D, 5 μl, 4 days; E, 7μl, 5 days; F, 7 μl, 5 days; G, 7 μl, 5 days; H, 7 μI, 5 days; I,10 μl, 5 days; J, 10 μl, 5 days; K, 7 μl, 5 days; L, 7 μl, 5 days; and M, 0.5 μl, 2 days.

Table 1. *Characterization of pGmPM cDNA clones*

Clone	cDNA insert size	Estimated size of RNA	HRT*-selected protein weight	# of clones showing homology
pGmPM1	0.9 kb	0.9 kb	22 kD (20kD)	46/456
pGmPM2	1.8 kb	1.8 kb	52 kD, 60 kD	12
pGmPM3	1.0 kb	0.9 kb	18 kD, 19 kD	23
pGmPM4	2.2 kb	2.2 kb	70 kD	8
pGmPM5	1.3 kb	1.3 kb	41 kD	15
pGmPM6	1.1 kb	1.1 kb	31 kD (32 kD)	93
pGmPM7	1.3 kb	1.2 kb	32kD (31 kD)	102
pGmPM8	1.6 kb	1.6 kb	48 kD, 50 kD	19
pGmPM9	0.8 kb	0.8 kb	20 kD (22 kD)	46

*HRT: hybridization

pGmPM4 to 70 kD, pGmPM5 to 41 kD, pGmPM6 to 31 kD, pGmPM7 to 32 kD, pGmPM8 to 48 as well as 50 kD, and pGmPM9 to 20 kD.

Northern blot hybridizations were performed using poly (A) RNA from 4 day PD seeds. Several clones, labeled by random primer methods , were used as probes and illustrated in Figure 3. For these clones that encode 2 polypeptides shown by hybrid select translation, i.e. pGmPM 1, 2, 6, and 7, there are two, or one very broad, hybridization bands in the Northern blots, as illustrated in short time as well as long time exposure. For the clone that corresponds to only one polypeptide, e.g. pGmPM 4, only one sharp band is present in the blot. Cross hybridization of all of the 456 clones were also performed to find out the relationship between each clone. All of these results, including cDNA insert sizes, estimated sizes of RNA, hybrid selected protein mass, and number of plaques showing hybridization homology to each cDNA clone are summerized in Table 1.

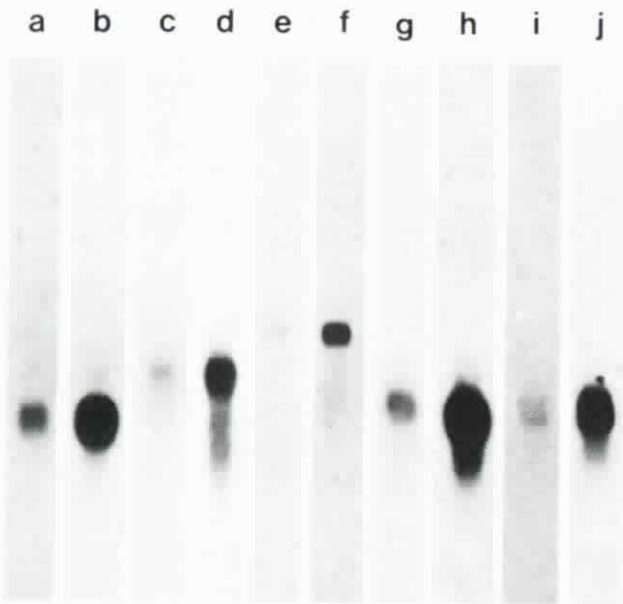

Fig. 3. RNA Northern hybridization with selected pGmPM cDNA clones. Polyadenylated RNAs were isolated from 4 days PD seeds, and equal amounts of RNA (1 μg) were fractionated by electrophoresis on a formaldehyde agarose denaturing gel, transferred to nylon membrane and hybridized with ^{32}P-labelled inserts of the clones as indicated. Lane a and b, clone pGmPM 1; Lane C and d, clone pGmPM 2; Lane e and f, clone pGmPM 4; Lane g and h, clone pGmPM 6; Lane i and j, clone pGmPM 7. The filters in Lane a, c, e, g, and i were exposed for one days while Lane b, d, f, h, and j for 3 days.

From the results of hybrid select translation, it is indicated that there is a close relationship between pGmPM 6 and 7. This is confirmed by plasmid DNA cross hybridization (data not shown). Thus, many in these 456 clones will hybridize to both pGmPM 6 and 7, i.e. there is overlap in the 93 and 102 clones showing homology to both. For clones pGmPM 1 and 9, the results of hybrid select translation indicated that both 20 and 22 kD polypeptides are the corresponding proteins. Plasmid DNA cross hybridization also indicated the close relationship between them. According to the sequencing work (unpublished data), there were 22 amino acid deletions between the two clones. Of the nine soybean seed maturation polypeptide cDNA clones selected in the present study, seven of them correspond to two protein molecules. Four of these seven had been assigned specific clone numbers because sequencing data or hybrid select translation pattern indicated their differences, they are pGmPM 1, 6, 7, and 9.

In summary, these nine different cDNA clones, each correspond to one or two different soybean maturation polypeptides, ranging from 18 kD to 70 kD, and all of these protein bands were also found in the preferentially expressed newly synthesized polypeptides in pod-dried seeds, as compared between Figure 1 and Figure 2. Most of the maturation polypeptide cDNA clones have been selected in the present work except the 130 kD one. We already purified the antibody against the 130 kD maturation polypeptide, and we are still working on selecting their cDNA clones.

Drying Regulation of Maturation Polypeptide Messages

Drying treatment of the 35 DAF immature soybean seeds had a marked effect on the one-dimensional electrophoretic pattern of newly synthesized proteins, as illustrated in Figrue 1. Total RNA was isolated from fresh 35 DAF seeds, 4 day PD seeds, and naturally matured seeds, and performed RNA slot blot analysis (Fig. 4). Transcripts hybridizing to pGmPM 1 or pGmPM4 were very rapidly induced by drying treatment; therefore, the cDNA clone pGmPM 1 and pGmPM4, corresponding to 18, 20, and 70 kD soybean seed maturation polypeptide, showed enhanced gene expression during drying treatment . All the other cDNA clones showed similar gene expression patterns.

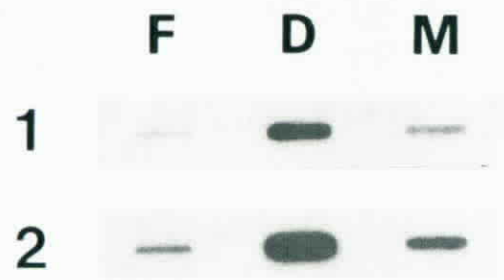

Fig. 4. RNA slot blot analysis with selected pGmPM cDNA clones. RNA was isolated from fresh (F) 35 DAF soybean seeds, 4 day PD (D) 35 DAF seeds, or mature (M) seeds. Five micrograms of total RNA were subjected to each slot. The membrane in Panel 1 was hybridized with pGmPM 1 probe and Panel 2 with pGmPM 4.

Discussion

It has been shown that seed maturation in many plant species is initiated by natural decline in seed water content. Artificially dried immature seeds mimic the naturally mature seeds in germination mode and synthesizing protein profile (Rosenberg and Rinne, 1986). The maturation polypeptides are products of mRNAs that accumulate during seed drying, either naturally drying or artifitially drying, and range in apparent molecular weight from 14 kD to 130 kD (Rosenberg and Rinne, 1988). The differential appearance of mRNAs encoding the maturation polypeptides suggested that poly (A) RNA fractions prepared from desiccating soybean seeds could be used to construct and select cDNA clones encoding seed maturation polypeptides (Hsing *et al.*, 1990). In the present study, it was indicated that the poly (A) RNA of the cotyledon is a better material than that of whole seeds. The chance to pull out the soybean seed maturation polypeptide cDNA clones was $35/4 \times 10^3$ when using whole seed poly (A) RNA (Hsing *et al.*, 1990) versus $456/8 \times 10^3$ in this study. Thus it was confirmed that poly (A) RNA of cotyledon was indeed an ideal material for seed maturation polypeptide cDNA cloning work. Furthermore, only four out of the 35 differentially expressed clones were characterized in the previous work (Hsing *et al.*, 1990), and all of these were similar to pGmPM 6. In our present study, these clones represent 22% of all the differentially expressed clones pulled out. Thus, it is obvious that the high abundancy of 31 kD maturation polypeptide message in the artificially dried soybean seeds leads to the fact that only 31 kD cDNA clones were pulled out at the previous work (Hsing *et al.*, 1990). In the present study, we used poly (A) RNA of cotyledon as the materials and we screened a larger size of the polulation. As a result, 13 fold more differentially expressed clones were pulled out, and 12 instead of 1 maturation polypeptide cDNA clones were characterized.

In the 9 soybean seed maturation polypeptide cDNA clones selected, pGmPM 1, 2, 3, 6, 7, 8 and 9 are each corresponded to two protein bands, as indicated by hybrid select translation and Northern blot. According to the sequence data of the cDNA clones and genomic clones we already finished, pGmPM 1 and 9 belong to two different genes of the same gene family, and there are high homology. One of them has a 22 amino acid deletion compared with the other (unpublished data). Therefore, we should be able to define some more pGmPM clones after further sequence data are available. At present, pGmPM 2, 3, and 8 each represent two groups of cDNAs.

There are 9 soybean seed maturation polypeptide cDNA clones, corresponding to 12 maturation polypeptides, pulled out in the present study. This situation is closely analogous to the recent finding by Galau *et al.* (1987) of late embryogenesis abundant *(Lea)* proteins and mRNAs in cotton (*Gossypium hirsutum*) seeds. In their system, they have found that cotton seeds contain about twenty *Lea* proteins which are much more abundant in late maturation stage seeds than in younger seeds or young seedlings. There are also about twenty *Lea* cDNA clones pulled out and sequenced. According to the sequence data we already finished, the soybean seed maturation polypeptide cDNAs exhibit convincing similarity to many other *Lea* proteins, including those of cotton, wheat and barley seeds (unpulished data).

In the present study, we pulled out several cDNA clones corresponding to the soybean seed maturation polypeptides. Using these as the probe, it will be possible for us to determine how these genes respond to drying treatments and developmental stages. The exact biological functions of these proteins remains to be determined. It has been suggested that they may play a role in equipping the seed for dessication survival (Blackman *et al.*, 1991). Alternatively, they might play a role during imbibition by controlling water uptake.

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大豆種子成熟多胜肽互補 DNA 系的單殖與定性

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爲了進一步研究大豆種子成熟多胜肽的基因表現及生物特性，我們進行它們的互補 DNA 系的單殖與定性工作。利用正反雜交的方法，我們找出了 pGmPM 1, 2, 3, 4, 5, 6, 7, 8, 9 等 9 個互補 DNA 系。這些訊息在人工乾燥或自然成熟的大豆種子中會大量堆積。這些互補 DNA 系的訊息大小自 800 到 2400 nucleotides，其所對應的多胜肽大小爲 18, 19, 20, 22, 31, 32, 41, 48, 50, 52, 60 以及 70 kD，這些恰與先前實驗的大豆成熟多胜肽相似。此外，本實驗亦指出利用莢乾燥處理的大豆種子的子葉(而非全粒種子)做爲材料，可以得到較完整的選拔、單殖工作。