DEVELOPMENTAL CHANGES OF DNA, RNA AND PROTEIN IN COTYLEDONS AND EMBRYONIC AXIS OF GERMINATING SOYBEAN SEEDS[1]

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Abstract: The changes of RNA, DNA and protein in cotyledons and embryonic axis of germinating soybean seeds were studied. In the first two days, there was less low molecular weight proteins than was in subsequent stages. The small protein molecules incraesed after the 4th day of germination. During early stages of germination, the RNA content in cotyledons increased gradually from an initial amount of 30.78 mg to 39 mg on the 4th day, and then decreased. After 10th day, the RNA content decreased rapidly and on the 16th day it had decreased to 8.37 mg which is only 27.2% of its initial content. But the RNA content in embryonic axis had increased about 25-fold. The electrophoretic patterns of RNA in cotyledons changes according to its stages of growth, but in embryonic axis there were no significant differences. Concomitant with development of embryonic axis was an increase in DNA content. But in cotyledons the DNA content stayed at the same level except when chloroplasts were turning green at that time DNA increased slightly. The diversity of protein and RNA implies the complexity of their physiological reactions.

INTRODUCTION

It is well known that when a dry seed imbibes water, its metabolic activity is activated. Under the proper environmental conditions, the imbibed seed will germinate. The cells in embryonic axis begin to enlarge and proceed with cell division in the meristematic regions. In the storage organ of seed, the endorsperm or cotyledons, several newly synthesized hydrolytic enzymes such as protease, lipase, invertase, amylase, and ribonuclease, and by these the storage substances are gradually degraded. The small molecules from the degradation of reserve materials are used in embryonic axis for the growth and development of seedlings[5,9,12,19,20,22,24,25].

In legumes, the storage materials such as starch, lipids, and proteins are synthesized during seed maturation. After the cessation of cell division, there is a continuing increase of DNA and RNA contents in cotyledons. This increase of RNA may be involved in protein synthesis which is concerned with the metabolism of the maturation of the seed. Scharpe and Parijis suggested that the polyploidization of the storage organs made available extra copies of those citrons involved in the synthesis of storage proteins during seed development. But this hypothesis is still short of unequivocal evidence. However, we cannot rule out the possibility that the extra DNA acts as a reserve of deoxyribonucletides destined to help with the extensive cell division in the embryonic axis during the seed germination[4].

Compared with corn and other legume seeds, the soybean seed has a high content of RNA.

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This may be due to the presence of abundant protein bodies in the cells of the cotyledons, and the protein bodies contain ribonucleic acid[26]. If soybean seeds are allowed to germinate and grow under 25 C and in light, the cotyledons will fall from the seedling on about the 18th to 20th day after germination. This paper describes quantitative and qualitative changes of nucleic acides and protein which occur in the cotyledons and embryonic axis, and attempts to explain the eventual fate of RNA and DNA in the storage organ during the germination of soybean seeds.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* cv. Kaoshiung No. 3) were sterilized and soaked in distilled water for 2 hours, then were incubated in moist vermiculite in a growth chamber. The environmental conditions for germination were: relative humidity 75%, light intensity 10,000 lux, day/night temperature 25/20 C. During the experimental period, distilled water is the only supplied medium. Cotyledons and embryonic axis of the seedlings were harvested at 2 day intervals respectively until the 16th day after sowing.

Protein extraction and SDS-gel electrophoretic analysis

The harvested cotyledons and embryonic axis were dried, milled, and then de-lipided with *n*-hexane. The delipided samples were extracted with 1 N NaOH for 24 hr. After homogenization and centrifugation, the proteins in the supernatant were precipitated by adding trichloroacetic acid (TCA) and washed with 95% ethanol, The isolated protein was resolved in the sample buffer containing 0.0625 M tris-HCl buffer, pH 6.8, 2% SDS, 4 M urea, and 5% 2-mercaptoethanol, and kept in the waterbath at 30 C for 48 hours before electrophoresis. The concentration of acrylamide gel was 8.75%, and the SDS-gel was prepared according to Laemmli[15]. During electrophoresis, the current was kept at 2 mA per gel. The protein bands were identified by staining with coomassie blue. After destaining, the ISCO gel scanner and the UA-4 monitor were used to determine the position and the color density of the prorein bands.

Quantitative determination of RNA and DNA

The extraction of RNA and DNA from the cotyledons and the embryonic axis was accomplished by using Howell's method[13]. DNA content was determined by the diphenylamine method using calf thymus DNA as standard[7]. The content of RNA was determined by the orcinol method using yeast RNA as standard[23].

Isolation of RNA for electrophoretic analysis

Fresh harvested tissues were homogenized in buffer-phenol solution containing 0.005 M tris buffer, pH 7.4, 0.5% SDS, 1.2% bentonite 40%, phenol, 5% m-cresol, and 0.005% 8-hydroxyquinoline for 2 minutes at moderate speed with Virtis 45 homogenizer. After centrifuging at 10,000 xg for 10 minutes, the aqueous phase was re-extracted three times with an equal volume of 80% phenol solution containing 10% m-cresol and 0.01% 8-hydroxyquinoline. The following precedures were similar to the method as described by Trewavas[27], expect that the methoxyethanol was replaced by ethylene glycol monomethyl ester. A certain amount of isolated RNA was dissolved in tris buffer containing 36 mM trif-HCl buffer, pH 7.8, 30 mM NaH_2PO_4, 1 mM EDTA, 5% sucrose. Acrylamide gel separation was carried out as described by Loening[20], and the gels were scanned at 265 nm in a Gilford spectrophotometer Model 2400 S.

RESULTS AND DISCUSSION

The total extractable proteins from the cotyledons were run on the 8.75% SDS gel, the results are shown in Fig. 1 and Fig. 2. There were more than 20 protein bands on the gel,

and there at least nine distinct components present in cotyledons. The reserve proteins in the soybean seeds appears to be located in the cotyledons in subcellular entities designated as protein bodies. Tombs found that the protein, glycinin which is the major protein, could be detected in protein bodies, and it contributes about 60 to 70% of total soybean proteins[9,24]. Using the techniques of disc electrophoresis and immunoelectrophoresis, Catsimpoolas *et al.* investigated the changes of reserve protein in germinating soybean seeds (*Glycine max* var. Harosoy 63), and found at least six distinct components were present in the protein bodies of the cotyledons.[8]. These components are metabolized at different rates during germination. The major soybean protein, glycinin (11S) is found to be present even after 16 days of germination, whereas the 7 S component disappears after the ninth day. The results shown in Fig. 1 and Fig. 2 represent the total extractable proteins from the cotyledons. This fraction contains the storage proteins and other proteins or enzymes concerned with metabolism in the cells. But the storage protein is predominant in the total extractable proteins. From the

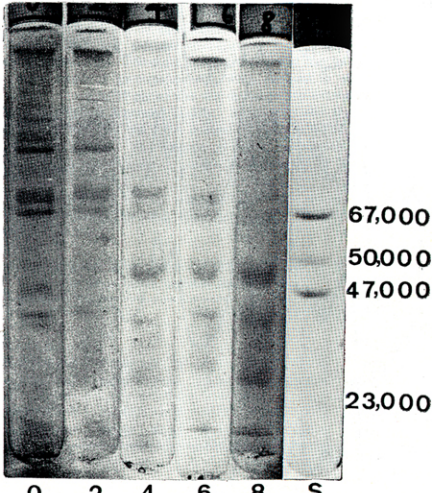

Fig. 1. The separation of total cotyledonary proteins on 8.75% SDS acrylamide gel during seed germination.
Number under each gel denotes the duration of seed germination in days, and S is the standard molecular weight of proteins, including bovine serum albumin (67,000), *r*-globumin (H-chain 50,000), alcohol dehydrogenase (36,000) and *r*-globumin (L-chain 23,000).

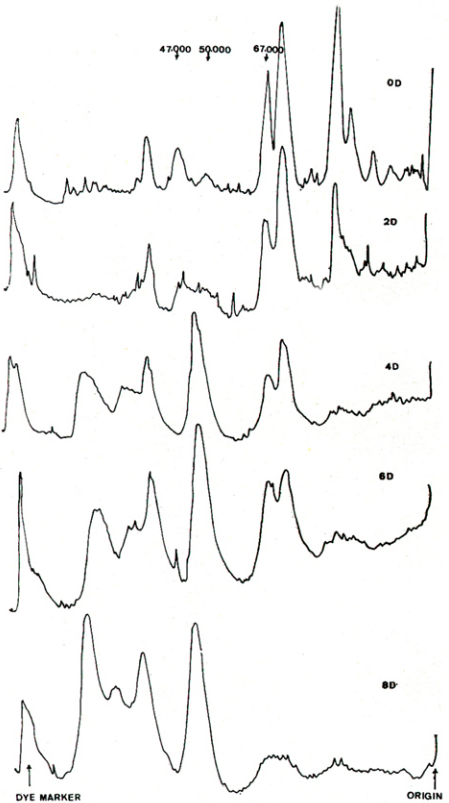

Fig. 2. Developmental changes of total cotyledonary proteins in 8.75% SDS-acrylamide gel.

electrophoretic patterns taken during the course of germination we can find the markedly different changes in the reserve protein in cotyledons. In the first two days, the amount of low molecular weight protein was less than that in subsequent stages. The small molecules increased after the 4th day of the germination. This result is in agreement with the results reported by Catsimpoolas[8]. The activity of protease in cotyledons increased from the 2nd day after seed germination, and rose to its peak on the 10th day[28]. Chrispeels *et al* reported that the endopeptidase reponsible for reserve protein breakdown is synthesized de novo and becomes associated with the protein bodies. From ultra-structural evidence they suggested that vesicles originating from the rough endoplasmic reticulum may mediate the transport of the enzyme from its site of synthesis to the protein bodies. The endopeptidase is not present in the cotyledons during the first 2 days of growth, but appeared on the third day, and subsequently increased[11]. The protein content in the cotyledons of *Glycine max* cv. Kaoshiung No. 3 was 54% of dry weight, and the protein declined rapiply from the 2nd to 10th day of germination[28].

The developmental changes of the nucleic acids in the cotyledons and in the embryonic axis are shown on Table 1. After the imbibition for one hour (0 day), the water contents of the seeds was still quite low and the concentration f RNA and DNA was very condensed. So that there are significant differences between 0 day and the following developmental stages. During the early stages of germination, the RNA contents in cotyledons increase gradually from the initial 30.78 mg to 39 mg on the 4th day and then decreases. After the 10th day, the RNA content in cotyledons decreased rapidly. Because by this time the cotyledons began to be senescence. On the 16th day, the RNA content decrease to 8.37 mg and that amount was only 27.2% of its initial content. The increase of the RNA content may be due to the rapidity of its synthesis. This phenomenon is similar to that occur in the cotyledons of peanut and cucumber[10,17]. Cherry showed that there is some RNA synthesis in the cotyledons of peanut and the storage materials are actively degraded[10]. But in the cotyledons of the starchy seeds, such as *Pisum sativum* and *Vicia faba*, there was no sign of RNA increase before eventually beclining[2,21].

Table 1. The quantitative changes of DNA and RNA in cotyledons and embryonic axis in germinating soybean seeds

		Duration of germination period in days								
		0	2	4	6	8	10	12	14	16
DNA*	Embryonic axis	676.69	222.12	257.12	367.17	312.92	320.48	243.67	212.65	236.60
	Cotyledon pair	240.67	217.89	214.88	213.59	200.31	206.41	136.62	153.12	181.03
RNA**	Embryonic axis	64.05	13.39	11.71	9.35	7.87	9.86	9.15	8.93	11.36
	Cotyledon pair	105.06	86.44	83.98	33.13	37.55	33.09	22.27	17.66	16.97

* μg DNA/g tissue
** mg RNA/g tissue

During the experimental period, the total RNA content in the embryonic axis increased gradually (Fig. 3). Generally, sufficient hydration of embryo can result in the formation of polyribosomes and the RNA synthesis may start within 30 to 60 minutes after imbibition[19,23]. The synthesis of nucleic acid and cell division may follow in the embryonic axis and the RNA content of the whole plant gradually increased. Both RNA synthesis and RNA degradation may occur in storage tissues.

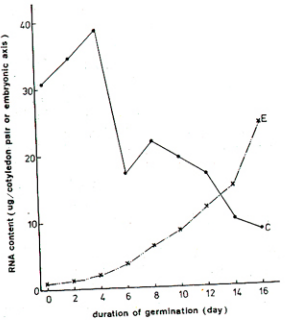

Fig. 3. Changes of RNA content in cotyledons and embryonic axis in germinating soybean seeds.

The developmental changes of RNA patterns are shown in Fig. 4 and Fig. 5. Both in the cotyledons and embryonic axis, the RNA patterns at zero time are simple. From the top we can find four major bands in the gel, named as DNA, 26S r-RNA, 18S r-RNA and 4S +5S RNA, respectively. There is no chloroplastic 16S r-RNA present before the 2nd day. The cotyledons turned green color on about the 4th day and the protoplastids developed into chloroplasts on about the 4th day. During this transition, chloroplastic r-RNA appeared, and increased, but after the 10th day, the cotyledon became senescent and chloroplastic r-RNA first declined and disappeared. After the 14th day because the reserve foods in the cotyledons had been exhausted and the cotyledons became senescent, the RNA patterns in the cotyledons reappeared as simple patterns. As shown in Fig. 4, 3 to 4 additional small peaks were observed on the 4th to 12th day in the gel between 18S r-RNA and 4-5S RNA. This may have been due to the degradation of the 23S chloroplastic ribosomal RNA. In the great majority of plant studied, the 23S chloroplastic ribosomal RNA molecules shows some degree of instability[14,16]. If chloroplastic RNA is extracted and fractionated on polyacrylamide gels in the absence of magnesium ions, the optical density peak corresponding to a molecular weight of 1.1×10^6 daltons (23S) is much smaller than expected. Further there are "extra" optical density peaks coresponding to the molecular weights of $0.6\text{-}0.7 \times 10^6$ daltons (16S) and $0.4\text{-}0.5 \times 10^6$ daltons.

The soybean seeds contains about 21% of lipids on dry weight basis. The initial total RNA increment may be due to the increment of cytoplasmic r-RNA. Using cucumber seeds, Becker *et al.* reported that the rapid increase in glyoxysomal enzyme activities between days 2 and 4 correlates well with the accumulation of cytoplasmic r-RNA and therefore presumably has the capacity for cytoplasmic protein synthesis in the cotyledons[1]. Figure 5 indicates that the chloroplastic r-RNA appears in the embryonic axis after 2nd day of germination and then is present continuously through the 16th days of the experimental period in embryonic axis.

There is no doubt that DNA synthesis occurs following the germination of seeds. This is an integral part of the growth of the embryonic axis. Fig. 6 shows the growth of the em-

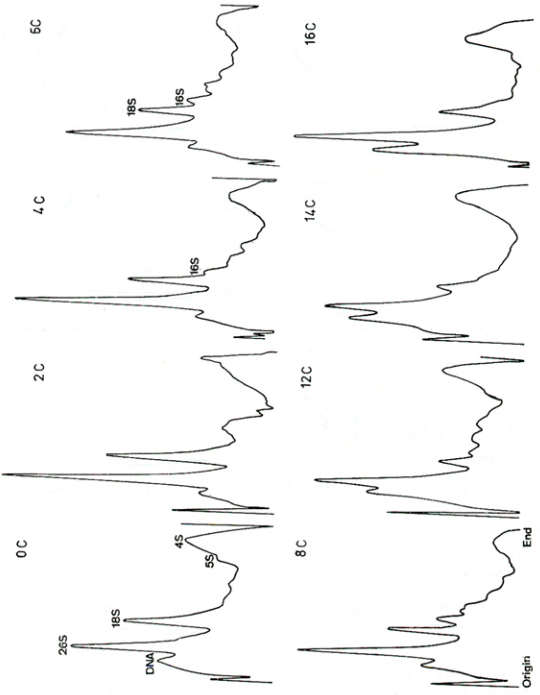

Fig. 4. Developmental changes of cotyledonary RNA in 2.4% acrylamide gel.

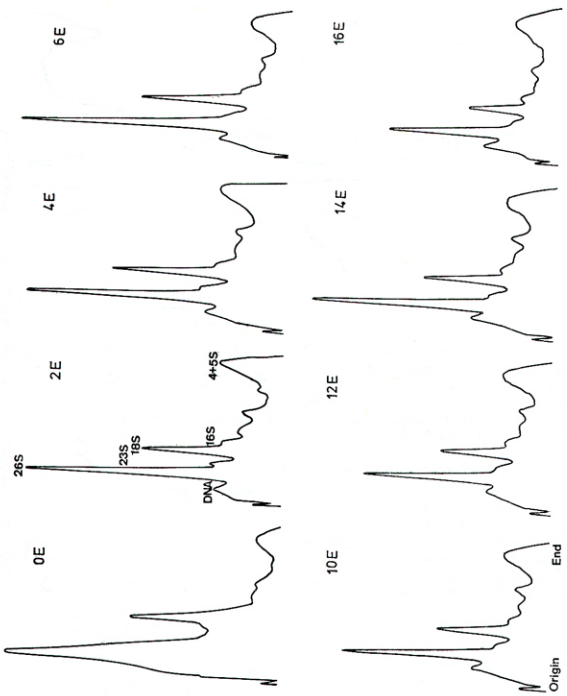

Fig. 5. Developmental changes of embryonic axis RNA in 2.4% acrylamide gel.

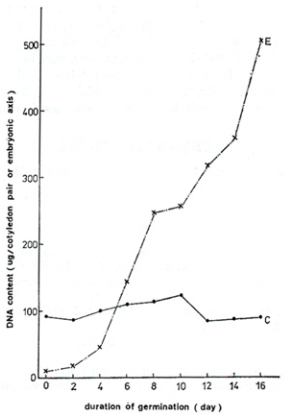

Fig. 6. Changes of DNA content in cotyledons and embryonic axis in germinating soybean seeds.

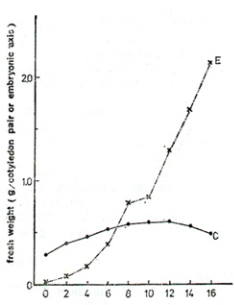

Fig. 7. Changes of fresh weight in cotyledon and embryonic axis in germinating soybean seeds.
Embryonic axis includes root, stem and leaves.

bryonic axis is accompanied with an increase of the DNA content, and the increment of the DNA almost parallels the increase of the fresh wesght (Fig. 7). In the cotyledons, the DNA content only increased from the period of the 4th to 10th day. This phenomenon may be attributable to the formation of DNA-containing organelles, such as chloroplasts and mitochondria[3]. On the 16th day, the DNA content in the cotyledons still remained at the initial level, but the RNA content was only 27.2% of the initial level. The diversity in the protein and RNA contents point up the complexity of their physiological reactions.

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