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Dynamics of seed protein biosynthesis in two soybean genotypes differing in drought susceptibility

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Abstract

The dynamics of seed storage protein biosynthesis was studied under field conditions during two vegetative seasons. Two soybean (*Glycine max* L. Merr.) genotypes were examined: BOSA (drought tolerant) and L 121 (drought susceptible). Seed samples were taken from plants at three stages of seed maturation (50 and 70 d after flowering, and at full maturity). The earlier synthesis of the β-subunit of the 7S protein occurred in the drought susceptible cultivar. We have not found such differences in the synthesis of the α- and α'-subunits of the 7S protein. Our results did not confirm significant genotypic differences in protein composition of the mature seeds between the cultivars studied, but have pointed out to the differences in the dynamics of protein biosynthesis during seed maturation and desiccation.

Additional key words: drought resistance, *Glycine max*.

Introduction

During soybean (*Glycine max* L. Merr.) seed development four major phases can be recognized (Meinke *et al.* 1981): morphogenesis and cell division, cell enlargement, seed maturation, and desiccation and dormancy. The synthesis of storage proteins, sugars and lipids occurs during cell expansion (Bewley and Marcus 1990). The two major proteins in soybean seeds were identified (Hill and Breidenbach 1974): 11S protein (consisting of at least four acidic and four basic polypeptides) (Nielsen 1985) and 7S (β-conglycinin) storage protein (composed of three subunits α, α' and β) (Thanh and Shibasaki 1978). The sum of 7S and 11S proteins constitute approximately 70 % of the total seed proteins at maturity (Meinke *et al.* 1981).

Developmental changes in the synthesis of seed storage proteins have been described previously for a number of legumes including *Glycine max* (Hill and Breidenbach 1974, Meinke *et al.* 1981, Chrispeels *et al.* 1979, Pernollet 1985). According to the findings of Meinke *et al.* (1981) α- and α'-subunits of 7S storage protein begin to accumulate shortly after the cessation of cell division, followed by the accumulation of 11S basic, and subsequently 11S acidic subunits. The β-subunit of the 7S protein does not begin to accumulate until the maturation phase of seed development.

Protein biosynthesis can be investigated not only from the point of view of normal seed development (Rosenberg and Rinne 1988), but also from the point of plant's resistance to different stress factors. It is well known that the specific proteins are accumulated during drought stress in vegetative tissues (Mundy and Chua 1988), *e.g.* "late embryogenesis abundant" proteins - LEAs (Galau *et al.* 1986, Baker *et al.* 1988, Hsing *et al.* 1995), "water stress proteins" - WSPs (Dure *et al.* 1989) and dehydrins (Close 1996, 1997). There are numerous data describing the dynamics of storage protein biosynthesis in *Glycine max* seeds, but the information regarding the genotypic differences in protein biosynthesis is scarce. Therefore, the aim of this study was to examine the dynamics of protein biosynthesis in soybean genotypes differing in drought sensitivity.

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Abbreviations: DAF - days after flowering; SDS-PAGE – sodium dodecyl sulphate - polyacrylamide gel electrophoresis.

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Materials and methods

Plants: Two soybean (*Glycine max* L. Merr.) cultivars, classified as a drought tolerant cv. BOSA and drought susceptible cv. L 121 - according to the visual estimation under field conditions and yield (unpublished data), were used for this investigation. Plants were grown at the Maize Research Institute (Belgrade - Zemun, Yugoslavia) in a calcareous chernozem soil. Plants were grown in 2 blocks: one block under rain-fed conditions and one block irrigated at regular intervals to maintain soil near field capacity. On each elementary block soybean plants were planted in 4 rows, 9 m long, 0.5 m apart. Plant density was 50 plants m^{-2}. At two stages of seed maturation (1/3 of the filled grain and fully filled grain, *i.e.* approximately 50 and 70 d after flowering - DAF, respectively) and at full maturity seeds were harvested, frozen at -20 °C and stored for subsequent biochemical analyses. Seeds were sampled from the pods on the eleventh node of ten plants per genotype in each block. At these stages, measurements of leaf water potential (ψ_L) were performed with a pressure chamber according to Scholander *et al.* (1965). It was measured between 09:00 and 12:00 prior to seed sampling on the middle leaflet of the trifoliate leaf on the eleventh node.

Protein assays and SDS-PAGE: The proteins of soybean seeds were extracted with 0.03 M Tris-HCl buffer containing 0.01 M 2-mercaptoethanol (pH 8.0), according to Thanh and Shibasaki (1976). Protein extractions were carried out by homogenization in a mortar for the seeds at 50 and 70 DAF, and using a shaker for the mature seeds ground to fine flour (buffer:sample ratio 20:1). The homogenate was centrifuged at 13 500 *g* for 15 min, and the supernatant was used for further analyses. The amount of protein in the supernatant was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. The protein concentration was monitored spectrophotometrically at 750 nm (*DB-GT, Beckman*, Palo Alto, USA). The change of content and composition of soluble polypeptides was determined by densitometric analysis of SDS-polyacrylamide gels. Electrophoresis was performed according to Fling and Gregerson (1986). Slab gel of 14.5× 15.5 × 0.15 cm was prepared. Stacking gel was 5 % and running gel was 12.5 % acrylamide. Prior to electrophoresis protein extracts were diluted to 2 mg cm^{-3} using sample buffer (pH 6.8). Vertical electrophoresis unit *LKB-2001-100* was used in conjunction with power supply *LKB-Macrodrive 5* and *LKB-Multitemp* as a cooling unit (*LKB*, Bromma, Sweden). Samples (0.025 cm^3) were run at 30 mA per gel for 6 h. The gel was stained with 0.23 % solution of Coomassie blue *R-250* for 90 min, and destained in 7 % methanol-acetic acid. Destained gels were scanned using *Scanexpress 12000SP* (*Mustek*, Neuss, Germany). Densitometric analysis of the scanned gels was performed using *SigmaGel for Windows* (*Gel Analysis Software, Jandel Corporation*, Erkhart, Germany). Protein subunits were identified by the use of low molecular mass calibration kit (*Pharmacia*, Uppsala, Sweden). Molecular mass markers included: phosphorylase *b* (94 kDa), albumin (64 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

Results and discussion

Leaf water potential: Values of leaf water potential were expressed in absolute terms (ψ_L), and also as water potential difference ($\Delta\psi_L$) between the rain-fed and irrigated plants. Drought declined ψ_L. In 1996, at the latter date of measurement, $\Delta\psi_L$ was as high as 0.72 and 0.74 MPa for cultivars BOSA and L 121, respectively (Table 1). In 1997, relatively low ψ_L values were recorded in both genotypes. Although the same irrigation doses were applied in both experimental years, measurements in the irrigated field in1997 showed lower ψ_L values. Low ψ_L was reported by Zur *et al.* (1981) and Reicosky *et al.* (1985) in soybean plants in the phase of active seed growth.

Table 1. Leaf water potential [-MPa] of genotypes BOSA and L 121 in two experimental years at two stages of seed maturity. Means ± SE, $n = 10$.

Cultivar		1996			1997		
		rain-fed	irrigated	$\Delta\psi_L$	rain-fed	irrigated	$\Delta\psi_L$
BOSA	50 DAF	1.70 ± 0.02	1.19 ± 0.05	0.51	1.96 ± 0.02	1.85 ± 0.05	0.11
	70 DAF	2.26 ± 0.04	1.54 ± 0.02	0.72	2.14 ± 0.03	1.98 ± 0.07	0.16
L 121	50 DAF	1.34 ± 0.05	0.89 ± 0.05	0.45	1.85 ± 0.04	1.79 ± 0.04	0.06
	70 DAF	2.04 ± 0.03	1.30 ± 0.06	0.74	2.07 ± 0.04	1.95 ± 0.04	0.12

Developmental changes in the accumulation of soybean seed proteins: The seed protein composition of both examined cultivars was the same in the stage of the mature seed, in both experimental years. However, differences in the dynamics of protein biosynthesis during seed maturation and desiccation are evident in both years of the experiment (Figs. 1, 2). In 1996, at the stage of 50 DAF, α- and α'-subunits of 7S storage protein have already been considerably accumulated. Accumulation of β-subunit of the 7S protein and basic polypeptide of the 11S protein was especially notable in the seeds of drought susceptible cv. L 121 under water stress.

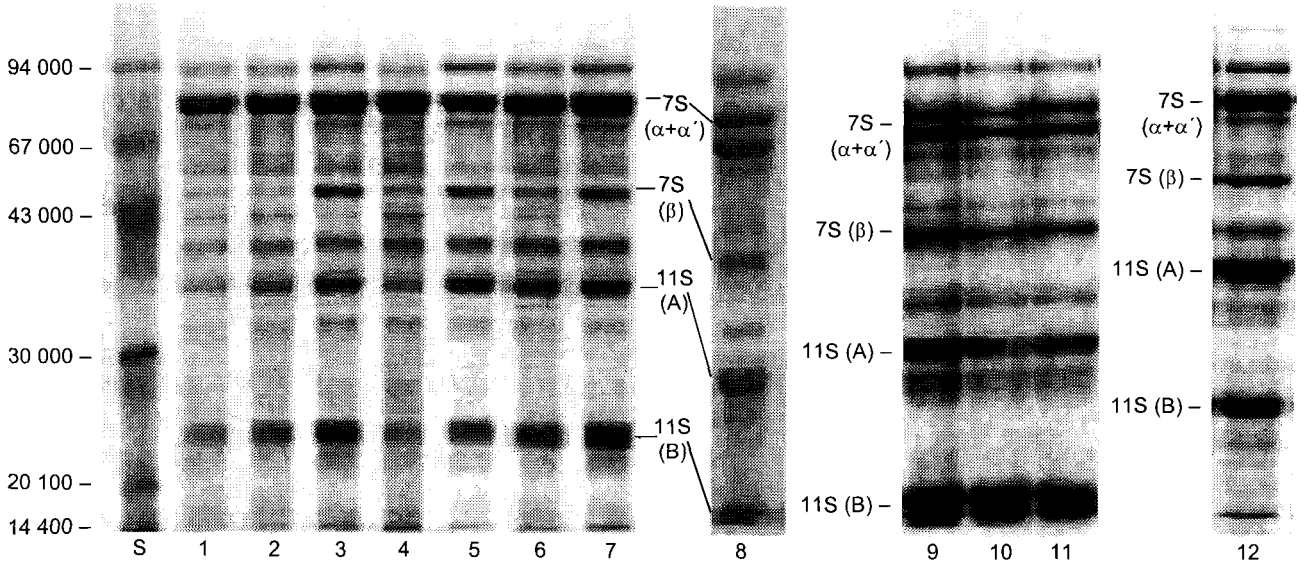

Fig. 1. Seed storage proteins (determined by SDS-PAGE) of soybean seeds at different stages of maturation in 1996. Comparison of control and droughted plants of two cultivars (*S* - standard, *lane 1* - 50 DAF BOSA droughted, *lane 2* - 50 DAF BOSA irrigated, *lane 3* - 50 DAF L 121 droughted, *lane 4* - 50 DAF L 121 irrigated, *lane 5* - 70 DAF BOSA droughted, *lane 6* - 70 DAF BOSA irrigated, *lane 7* - 70 DAF L 121 droughted, *lane 8* - 70 DAF L 121 irrigated, *lane 9* - mature seed BOSA droughted, *lane 10* - mature seed BOSA irrigated, *lane 11* - mature seed L 121 droughted, lane 12 - mature seed L 121 irrigated).

Bray and Beachy (1985) have investigated the effect of abscisic acid (ABA) on protein expression in soybean seeds. The pattern of ABA-induced protein synthesis in their experiment was similar to the pattern induced by drought in our experiment. They showed that application of ABA to cultured soybean cotyledons increases accumulation of β-subunit of the 7S protein, while the amounts of α- and α'-subunits of 7S storage protein remain the same. Bradford and Chandler (1992) found in maize that ABA was capable of inducing synthesis of a protein with similar electrophoretic mobility and molecular mass to the one induced by dehydration and water stress. However, as the patterns of temporal and spatial expression of these proteins vary in seeds, suggesting that factors other than ABA primarily control their expression, it is questionable whether these proteins play role in the plant's response to osmotic stress.

In 1997, when $\Delta\psi_L$ was low, at 50 DAF the following was noticed: β-subunit of the 7S protein was synthesized earlier in the drought susceptible genotype L 121 in comparison with drought tolerant genotype BOSA. At the same time, α- and α'-subunits of the 7S protein have been mostly accumulated by this time in both genotypes.

At 70 DAF, in both experimental years, no differences were noticeable between the genotypes or fields in the synthesis of α- and α'-subunits of the 7S protein. However, faster synthesis of β-subunit of the 7S protein was occurring in both genotypes under drought in 1996, while in 1997 at 70 DAF, more intensive synthesis of the β-subunit was noticed in the drought susceptible genotype L 121 in both experimental fields.

Regarding the order of appearance of seed storage proteins our results are in accordance with those of Kondo *et al.* (1986) and Meinke *et al.* (1981) who have found that among the first proteins to accumulate in soybean seed were α- and α'-subunits of 7S storage protein, followed by acidic and basic polypeptide of 11S protein, while the β-subunit of 7S protein began to accumulate later, which was also the case in our experiment.

In conclusion, the obtained results confirmed the existence of differences in the biosynthesis of β-subunit of the 7S seed storage protein between the two investigated genotypes. The earlier synthesis of the

β-subunit of the 7S protein in both experimental years was detected in drought susceptible genotype L 121 compared to drought tolerant BOSA. Since similar values of $\Delta\psi_L$ for the genotypes were detected in both investigated years, the differences in protein biosynthesis could not be attributed to the differences in water regime of the investigated genotypes. Further investigation of the relevance of these data for adaptation and resistance of soybean to drought would be very worthwhile.

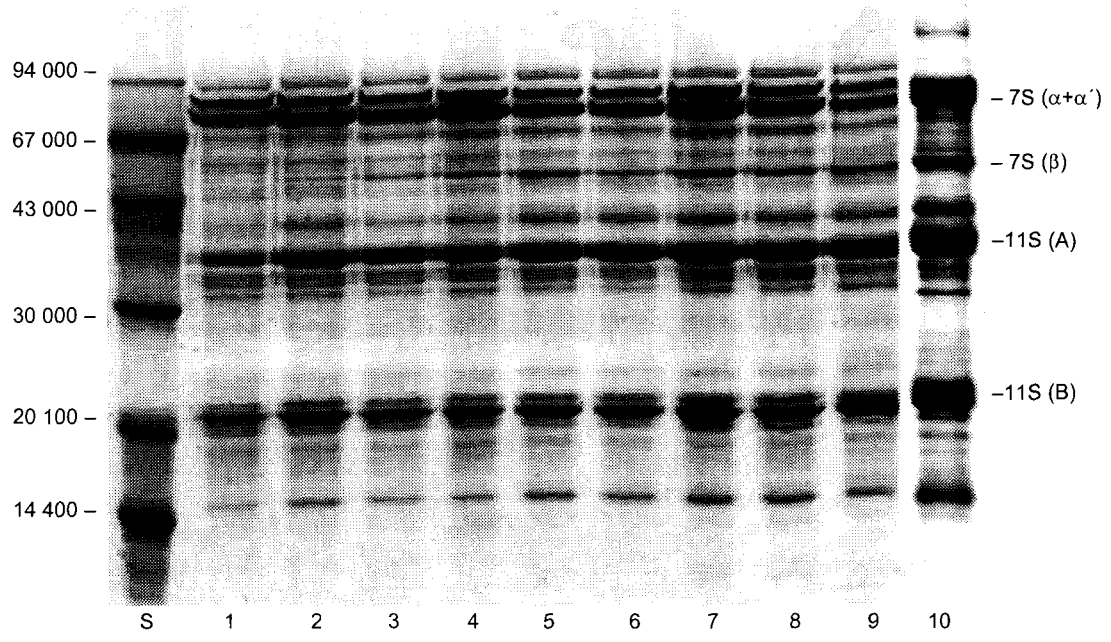

Fig. 2. Seed storage proteins (determined by SDS-PAGE) of soybean seeds at different stages of maturation in 1997. Comparison of control and droughted plants of two cultivars (*S* - standard, *lane 1* - 50 DAF BOSA droughted, *lane 2* - 50 DAF BOSA irrigated, *lane 3* - 50 DAF L 121 droughted, *lane 4* - 50 DAF L 121 irrigated, *lane 5* - 70 DAF BOSA droughted, *lane 6* - 70 DAF BOSA irrigated, *lane 7* - 70 DAF L 121 droughted, *lane 8* - 70 DAF L 121 irrigated, *lane 9* - mature seed BOSA droughted, *lane 10* - mature seed BOSA irrigated).

References

Baker, J., Steele, C., Dure, L.: Sequence and characterization of 6 Lea proteins and their genes from cotton. - Plant mol. Biol. **11**: 277-291, 1988.

Bewley, J.D., Marcus, A.: Gene expression in seed development and germination. - Progr. nucl. Acid Res. mol. Biol. **38**: 165-193, 1990.

Bradford, K.J., Chandler, P.M.: Expression of "dehydrin-like" proteins in embryos and seedlings of *Zizania palustris* and *Oryza sativa* during dehydration. - Plant Physiol. **99**: 488-494, 1992.

Bray, E.A., Beachy, R.N.: Regulation by ABA of β-conglycinin expression in cultured developing soybean cotyledons. - Plant Physiol. **79**: 746-750, 1985.

Close, T.J.: Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. - Physiol. Plant. **97**: 795-803, 1996.

Close, T.J.: Dehydrins: A commonality in the response of plants to dehydration and low temperature. - Physiol. Plant. **100**: 291-296, 1997.

Chrispeeles, J.M., Bollini, R., Harris, N.: Biosynthesis, accumulation and catabolism of reserve proteins in legume seeds. - Ber. deut. bot. Ges. **92**: 535-551, 1979.

Dure, L., Crouch, M., Harada, J., Ho, T.-H.D., Mundy, J., Quatrano, R., Thomas, T., Sung, Z.R.: Common amino acid sequence domains among the LEA proteins of higher plants. - Plant mol. Biol. **12**: 475-486, 1989.

Fling, S.P., Gregerson, D.C.: Peptide and protein molecular weight determination by electrophoresis using a high molarity Tris buffer system without urea. - Anal. Biochem. **155**: 562-567, 1986.

Galau, G.A., Hughes, D.W., Dure, L.: Abscisic acid induction of cloned cotton late embryogenesis-abundant (Lea) mRNAs. - Plant mol. Biol. **7**: 155-170, 1986.

Hill, J.E., Breidenbach, R.W.: Proteins of soybean seeds I: Accumulation of the major protein components during seed development and maturation. - Plant Physiol. **53**: 747-751, 1974.

Hsing, Y.C., Chen, Z.Y., Shih, M.D., Hsieh, J.S., Chow, T.Y.: Unusual sequences of group 3 LEA mRNA inducible by maturation or drying in soybean seeds. - Plant mol. Biol. **29**: 863-868, 1995.

Kondo, K., Sugimoto, T., Saio, K.: Changes in storage protein components of developing soybean seeds (*Glycine max* var. Enrei). - Agr. biol. Chem. **50**: 581-587, 1986.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Meinke, D.W., Chen, J., Beachy, R.N.: Expression of storage protein genes during soybean seed development. - Planta **153**: 130-139, 1981.

Mundy, J., Chua, N.H.: Abscisic acid and water-stress induce the expression of a novel rice gene. - EMBO J. **7**: 2279-2286, 1988.

Nielsen, N.C.: The structure and complexity of the 11S polypeptides in soybeans. - J. amer. Oil Chem. Soc. **62**: 1680-1686, 1985.

Pernollet, J.C.: Biosynthesis and accumulation of storage proteins in seeds. - Physiol. vég. **23**: 45-59, 1985.

Reicosky, D.C., Warnes, D.D., Evans, S.D.: Soybean evapotranspiration, leaf water potential and folliage temperature as affected by row spacing and irrigation. - Field Crop Res. **10**: 37-48, 1985.

Rosenberg, L.A., Rinne, R.W.: Protein synthesis during natural and precocious soybean seed (*Glycine max* L. Merr.) maturation. - Plant Physiol. **87**: 474-478, 1988.

Scholander, P.F., Hammel, H.T., Bradsteel, E.P., Hemmingsen, E.A.: Sap pressure in vascular plants. - Science **148**: 339-346, 1965.

Thanh, V.H., Shibasaki, K.: Major proteins of soybean seeds. A straightforward fractionation and their characterization. - J. agr. Food Chem. **24**: 1117-1121, 1976.

Thanh, V.H., Shibasaki, K.: Major proteins of soybean seeds: subunit structure of β-conglycinin. - J. agr. Food Chem. **26**: 692-695, 1978.

Zur, B., Boote, K.J., Jones, J.W.: Changes in internal water relations and osmotic properties of leaves in maturing soybean plants. - J. exp. Bot. **32**: 1181-1191, 1981.