2006

Dehydrin-Like Proteins in Soybean Seeds in Response to Drought Stress during Seed Filling

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Published online September 8, 2006

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ABSTRACT

There is no information on accumulation of dehydrin proteins during seed development and maturation of soybean [*Glycine max* (L.) Merr.] in response to drought stress. Our objective was to study accumulation of dehydrin-like proteins in developing soybean seeds in response to drought stress. A greenhouse experiment and a field experiment were conducted. In the greenhouse experiment, three treatments were imposed on soybean plants after beginning of linear seed filling (R_5): well-watered (WW), gradual stress (GS) imposed before severe stress, and sudden severe stress (SS). In the field treatments were irrigation (I) and nonirrigation (NI) (rainfed) conditions imposed from R_5 to R_8 (mature seeds). Greenhouse results indicated dehydrin-like proteins (28 and 32 kDa) were detected 18 d after R_5 ($R_{5.8}$) in developing seeds from drought-stressed plants but not in seeds from the well-watered plants. In the mature seeds, dehydrin-like proteins (28, 32, and 34 kDa) were detected in seeds from drought-stressed plants as well as the well-watered plants. In the field, dehydrin-like proteins accumulated similarly under irrigation and nonirrigation conditions, with the first detection for dehydrins (28 and 32 kDa) at 22 d after R_5 (R_6). Accumulation of dehydrin-like proteins was maximal in seeds harvested at 43 d after R_5 (seed physiological maturity).

Changes in protein expression, accumulation, and synthesis have been observed in many plant species resulting from plant exposure to drought stress during growth (Ramagopal, 1987; Chen and Tabaeizadeh, 1992; Cheng et al., 1993). In maize (*Zea mays* L.), it has been observed that drought stress increased expression of 50 proteins, decreased that of 23, and induced synthesis of 10 proteins as detected by two-dimensional gel electrophoresis (Riccardi et al., 1998). The newly synthesized proteins in response to drought stress were known to be involved in plant response to water stress such as RAB17 [Response to ABA] protein and enzymes involved in metabolic pathways such as glycolysis, Krebs cycle, and lignin synthesis (Riccardi et al., 1998).

Newly synthesized proteins in response to drought stress are called dehydrin (dehydration-induced) and belong to the Group II LEA proteins (late embryogenesis abundant) (Close and Chandler, 1990; Leprince et al., 1992). Dehydrin proteins are also produced in response to various environmental stresses such as salt and cold stress (Close, 1996) and have been characterized as hydrophilic, heat-stable, free of cysteine and tryptophan, responsive to ABA, and rich in lysine (Close et al., 1989; Mundy and Chua, 1988; Vilardell et al., 1990; Close, 1996; Godoy et al., 1996). Dehydrin proteins accumulate along with other LEA proteins in response to a particular stress and have been proposed to play an important role in membrane and protein stability, osmotic adjustment (Carpenter and Crowe, 1988; Close 1996; Dure et al., 1989; Godoy et al., 1996), and in acquisition of desiccation tolerance in seeds (Close et al., 1989). Han et al. (1997) reported that a dehydrin of 25 kDa MW was detected in castor bean seeds (*Ricinus communis* L.) during early- to mid-seed development. In mature dry seeds of castor bean, other dehydrin polypeptides of 28 to 30 and 41 kDa were also detected (Han et al., 1997). These observations also suggested that dehydrins as well as other LEA proteins might play a role in acquisition of desiccation tolerance in seeds (Close et al., 1989; Dure et al., 1989; Dure, 1993a).

Soybeans are planted over a wide range of conditions; however, information related to protein accumulation and synthesis under drought stress is limited. In 1988, Bensen et al. reported that drought stress in soybean resulted in an overall decline in protein synthesis, although synthesis of several other proteins detected by two-dimensional gel electrophoresis increased in the seedling tissue. There is no information available on the accumulation of dehydrin proteins during seed development and maturation of soybean plants in response to drought stress. The objective of the experiment was to study accumulation of dehydrin-like proteins during seed development and maturation of soybean and whether drought stress and nonirrigation treatments induce expression of these proteins earlier than that of well-watered plants.

MATERIALS AND METHODS

Experiments

Two experiments, one in the greenhouse and one in the field, were conducted to determine accumulation of dehydrin-like proteins in developing soybean seeds in response to drought stress and nonirrigation conditions. The greenhouse experiment was initiated in 10 Oct. 1998 and ended 10 Mar. 1999 at Iowa State University, Ames, IA. The medium used in the greenhouse experiment was a mixture of soil: silica sand: peat: perlite in a volume ratio of 2:2:1:1, later referred to as "soil." Sixty 3.8-L pots containing an equal weight of soil mixture were prepared. Ten pots of soil were randomly picked and allowed to saturate overnight in a water bath. Pots were drained the next day and weighed to determine field capacity. Four soybean seeds of the semideterminant early maturing cultivar Harosoy were planted in each pot. Two weeks after planting, seedlings were thinned to two plants per pot. Plants were watered to 80% field capacity (FC) until the beginning seed fill growth stage (R_5) (Fehr and Caviness, 1977). At R_5, one of three drought-stress treatments was imposed on the plants (Fig. 1). Treatments were (i) well-watered (WW) (control), (ii) gradual

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Published in Crop Sci. 46:2141–2150 (2006).
Seed Physiology, Production & Technology
doi:10.2135/cropsci2006.02.0066

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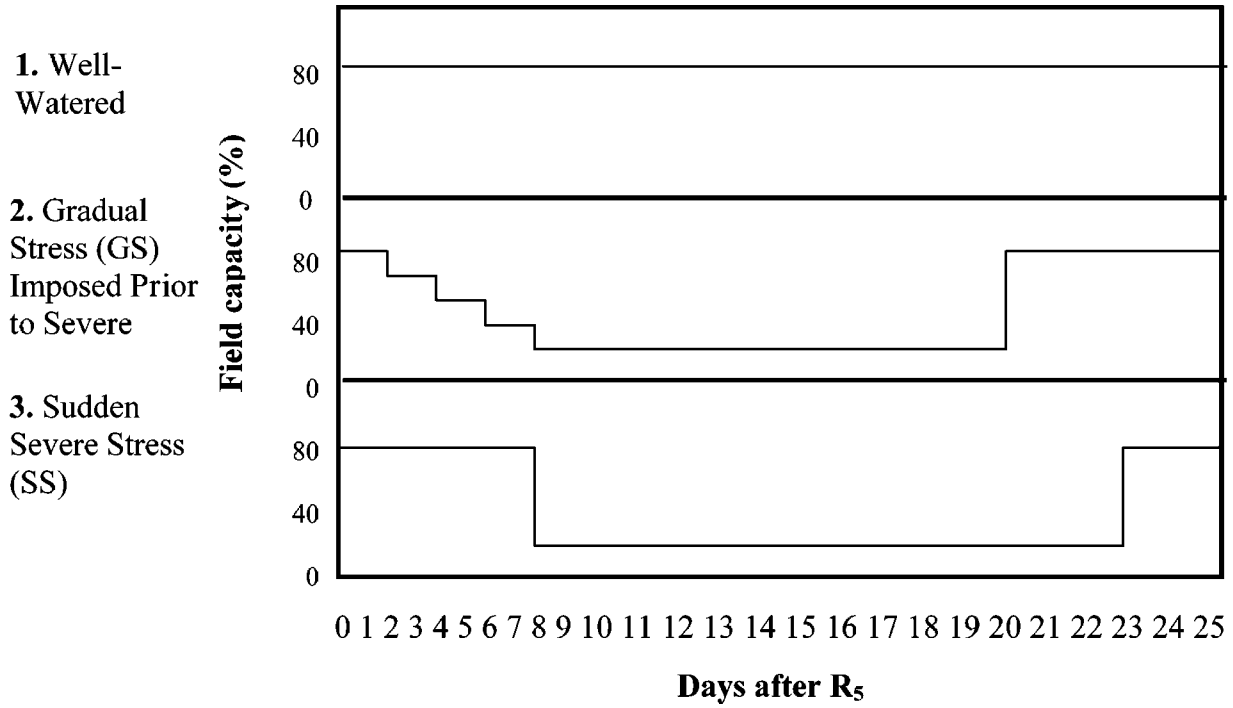

Fig. 1. Three drought stress treatments imposed on soybean plants at beginning seed fill (R_5) in the greenhouse experiment. WW: plants were maintained at 80% field capacity (FC) after R_5. GS: the soil moisture content was dropped gradually in 7 d after R_5 from 80% to 20% FC; then, maintained at 20% FC for 13 d. SS: plants were maintained at 80% FC for 7 d from R_5; then, soil moisture content was allowed to drop suddenly in 1 d to 20% FC and maintained for 16 d.

stress (GS) (drought preconditioned by exposing the plants to gradual reductions in soil moisture for 7 d) imposed before severe water stress, and (iii) sudden severe water stress (SS) (suddenly stressed without drought preconditioning). In the WW treatment, plants were maintained at 80% FC from R_5 to maturity (R_8). In the GS treatment, plants were exposed to a gradual reduction in soil moisture content from 80 to 20% FC applied during 7 d after R_5. At Day 8, plants in GS treatment were exposed to severe drought for 13 d. In the SS treatment, plants were maintained at 80% FC for 7 d after R_5, and then, were suddenly exposed to severe water stress (20% FC) at Day 8 after R_5. The severe stress period in SS treatment was imposed for 16 d. The difference between the SS and GS treatments in the duration of the severe drought (16 versus 13, respectively) was designed to equalize the total water consumption of plants between the two treatments during the first 7 d after R_5 and was based on water consumption patterns of treated plants. Average midday air temperature, light intensity, and air relative humidity at the top of the canopy during the seed-filling stage was 27°C, 998 μmol of photon m^{-2} s^{-1}, and 39%, respectively.

The experimental design was a randomized complete block with four replications. Five pots of two debranched plants each were used for the experimental treatment unit using a total of 120 plants (4 replications × 3 treatments × 5 pots × 2 plants per pot).

The field experiment was conducted in Puerto Rico from 25 Oct. 1999 to 20 Jan. 2000. Seeds of semideterminant early maturing cultivar Harosoy were planted in 3-m rows with 13 seeds per meter. Five 3-m rows were planted per replication (200 seeds per replication). All plots were irrigated with 38 mm of water per week until R_5. At R_5, plants from WW treatment were irrigated until maturity once a week, irrespective if natural rain occurred or not. Plants from the nonirrigated treatment were left to grow under the natural rainfed conditions. Average maximum and minimum temperature, percentage relative humidity in air, and rainfall during treatment period (from R_5 to seed physiological maturity) were 27 and 18°C, 84%, and 100 mm, respectively (Fig. 2a,b,c).

During development, seeds were sampled from plants exposed to drought-stress treatments in the greenhouse experiment and nonirrigation treatment in the field experiment to determine the effect of these conditions on the accumulation of heat-stable proteins and dehydrin-like proteins in soybean seeds. In the greenhouse experiment, seeds were sampled at 2, 4, 10, and 18 d after R_5 (Table 1). In the field experiment, seeds were sampled at 0, 5, 15, 22, and 43 d after R_5 (Table 1). Seed dry weight and moisture content of the seed at each sampling date were measured.

In both experiments, remaining seeds were harvested at maturity (R_8) at a moisture content of approximately 130 g kg^{-1} based on wet weight. Seeds were screened into different seed size categories to allow comparisons of proteins accumulation for seeds of uniform size and weight. In the greenhouse experiment, seeds retained above a 3.97 × 19.05 mm (width × length) oblong screen and a 6.35-mm diameter round screen were classified as large seeds. Seeds that passed through a 6.35-mm diameter round screen, and were retained above a 5.56-mm diameter round screen were classified as medium seeds. The seeds that passed through a 5.56-mm diameter round screen and a 3.97 × 19.05 mm (width × length) oblong screen were combined and classified as small seeds. In the field experiment, full rounded seeds retained above a 3.97 × 19.05 mm (width × length) oblong screen and a 5.56-mm diameter round screen were used to study protein accumulation in seeds. In both experiments, seed number, seed weight, and yield were measured to quantify drought stress.

Protein Extraction

Harvested seeds were immediately frozen in liquid nitrogen and stored at −80°C until used. Seeds were ground to a powder and equal weights of seed powder were extracted in a Tris

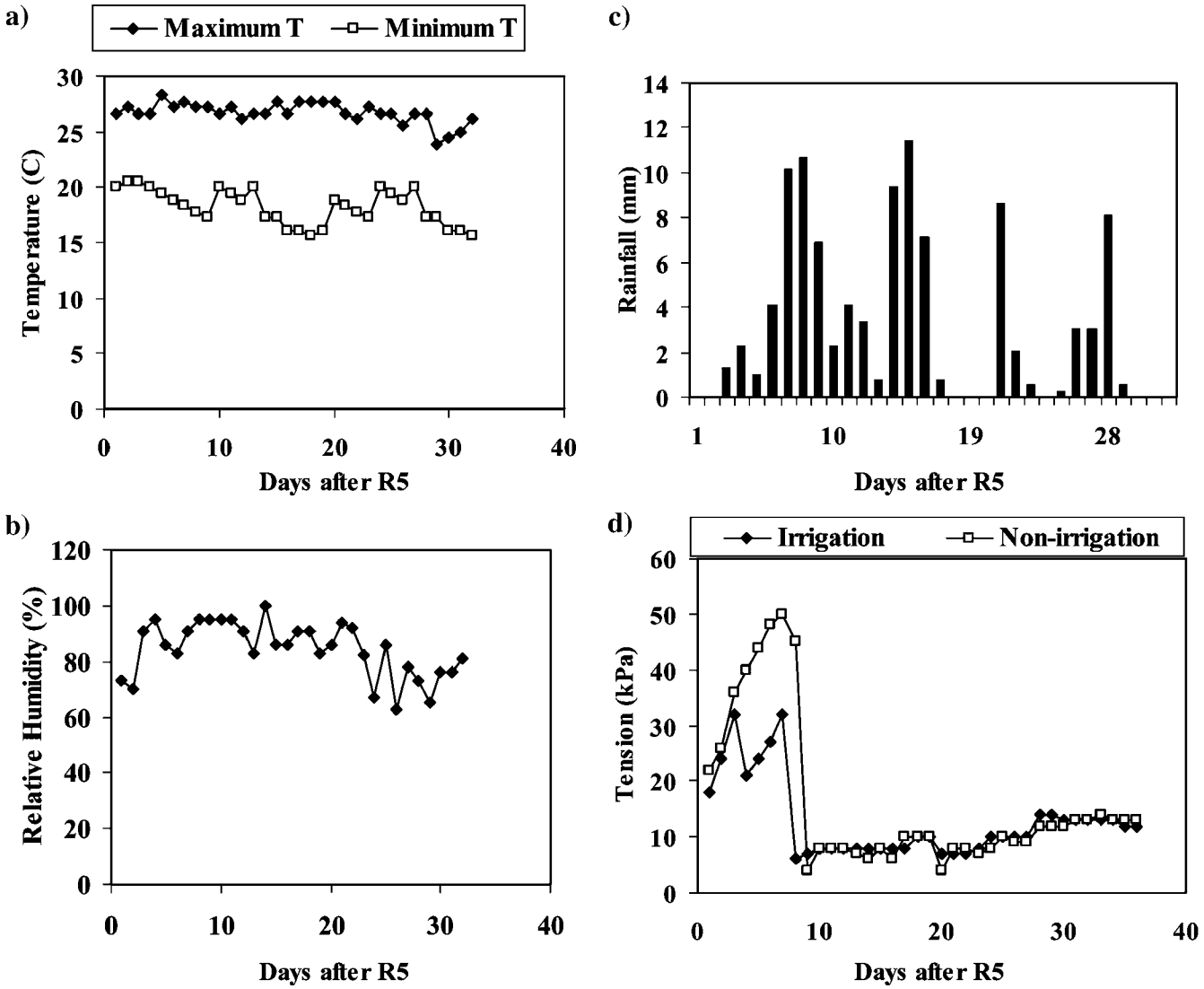

Fig. 2. Average air temperature (a), relative humidity (b), rainfall (c), and soil tensiometer readings at a 45-cm depth (d) for irrigated and nonirrigated conditions recorded after treatments were imposed on soybean plants at beginning of seed fill (R_5) in the field experiment.

buffer (20 m*M* Tris, 0.5 *M* NaCl, pH 7.5) and centrifuged at 20000 *g* for 20 min. For electrophoresis of heat-stable proteins, supernatant was heated in boiling water for 15 min, transferred to ice for 4 min, centrifuged at 20000 *g* for 10 min, and then extracted. Protein concentration was determined by the method of Bradford (1976), with bovine serum albumin as a standard.

Table 1. Days after R_5 and reproductive stages for sampling of developing soybean seeds in the greenhouse and field experiments.

Experiment	Days after R_5	Reproductive stage† Code	Description
Greenhouse	2	$R_{5.2}$	5-mm-long seeds
	4	$R_{5.4}$	7-mm-long seeds
	10	$R_{5.6}$	8-mm-long seeds
	18	$R_{5.8}$	11-mm-long seeds (seeds did not fill pod cavity)
Field	0	R_5	3-mm-long seeds (beginning seed)
	5	$R_{5.4}$	7-mm-long seeds
	15	$R_{5.7}$	10-mm-long seeds
	22	R_6	full pods (seeds filled pod cavity)
	43	PM‡	yellow pods (seeds reached maximum dw)

† Reproductive stages described according to Fehr and Caviness (1977).
‡ PM, physiological maturity.

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

Equal weights of proteins from supernatants were fractionated by discontinuous SDS-PAGE (12.5%, w/v) using Mini-Protean II Electrophoresis Cells (Bio-Rad Laboratories, Hercules, California). The gels were stained with Coomassie blue.

Western Blotting

Heat-stable proteins fractionated by SDS PAGE were blotted onto Immobilon PVDF Transfer Membranes (Millipore, MA, Bio-Rad Laboratories, Hercules, CA) using Hoefer Transfer Electrophoresis Unit (Amersham Pharmacia Biotech, CA). The blotted proteins on the membranes were probed with a 1:2000 dilution of a polyclonal antibody (kindly provided by Dr. Timothy Close) raised against a synthetic peptide of 15 amino acids representing a consensus sequence (TGEKKGIMDKIKEKLPGQH) of dehydrin proteins. The membranes were then incubated with a secondary antibody

(horseradish peroxidase conjugate) (Bio-Rad Laboratories). The secondary antibody was visualized using horseradish peroxidase color developer (4-chloro-1-napthol) (Bio-Rad Laboratories) and hydrogen peroxide.

Statistical Analysis

Analysis of variance was performed on the variables of seed number, weight, and yield using GLM procedures (SAS Institute, Inc., 1996). When F values were significant ($P < 0.05$), Fisher's least significant differences were calculated.

RESULTS AND DISCUSSION

Seed Yield

Seed yield was measured in both experiments to determine the occurrence of the drought stress (Table 2). The drought stress treatments in the greenhouse and nonirrigated condition in the field reduced seed yield by 42 to 54% compared with the well-watered plants, indicating that drought stress had effectively occurred in both experiments. In soybean, a reduction in seed yield > 40% has been used as an indication for the occurrence of severe drought stress condition and has been associated with many physiological changes in plants such as a reduction in leaf photosynthesis, stomata conductance, and transpiration rate (Dornbos et al., 1989; Vieira et al., 1991).

Seed Dry Weight and Moisture Content in Developing Seeds

Seed dry weight and moisture content at each sampling date were measured in both experiments to further describe the stage at which seeds were harvested during development (Fig. 3a,b). In the greenhouse experiment, seed dry weight and moisture content changed from 7.5 mg seed^{-1} and 81% at 2 d after R_5 to 86 mg seed^{-1} and 67% at 18 d after R_5. Seed dry weight was less for drought-stressed plants at 4 and 10 d after R_5 than for well-watered plants, although seed moisture content was not significantly changed under drought stress at any sampling date. In the field experiment, seeds were sampled during the entire seed-filling period from R_5 to R_7 (seed physiological maturity). Irrigation treatments did not change seed dry weight and moisture content at any sampling date. For both treatments, seed dry weight and moisture content changed from 0.68 mg seed^{-1} and 83% at R_5 to 174 mg seed^{-1} and 53% at 43 d after R_5 (at R_7), respectively.

Table 2. Yield and yield components of soybean plants exposed to drought stress treatments at beginning of seed fill (R_5) in the greenhouse experiment and irrigation treatments in the field experiment.

Treatment†	Seed number	Seed weight	Yield
	no. plant^{-1}	mg seed^{-1}	g plant^{-1}
Greenhouse experiment			
WW	128	202	25.9
GS	94	157	14.8
SS	97	142	13.8
LSD	14	25	3.6
Field experiment‡			
	no. plot^{-1}		g plot^{-1}
I	8550	198	1693
NI	4146	190	787
LSD§	490	ns	129

† WW, well-watered treatment. SS: sudden severe stress treatment. GS, gradual stress before severe water stress treatment (See Fig. 1 for details). I, irrigated conditions throughout the growing season. NI, nonirrigated (rainfed) conditions imposed on the soybean plants at beginning of seed fill (R_5).

‡ Mean seed number and yield of plants harvested per plot (200 seeds were planted per plot).

§ LSD, Fisher's least significantly difference at probability level ($p \leq 0.05$) to compare treatment means.

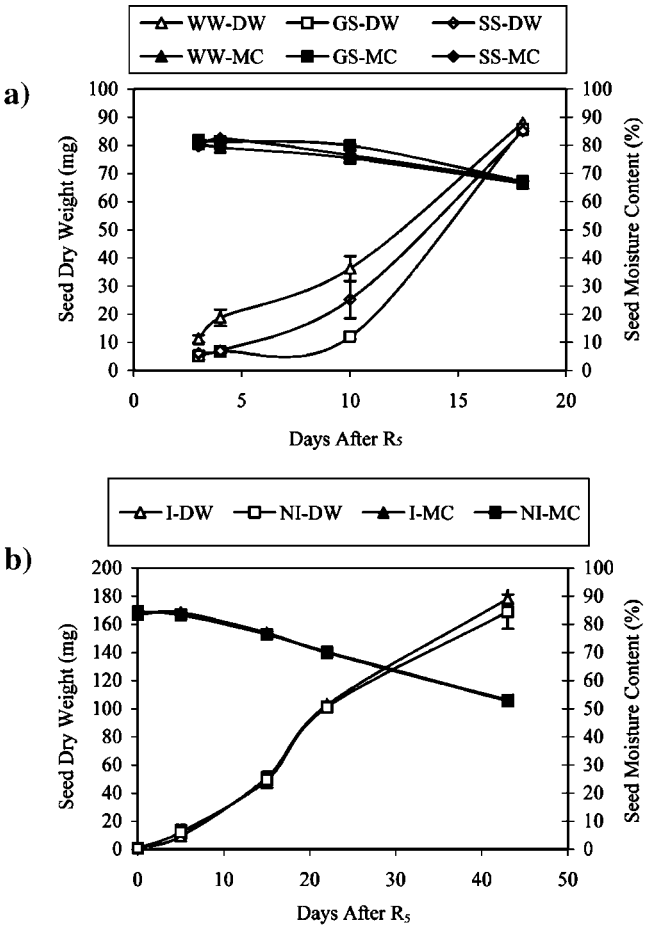

Fig. 3. Dry weight (DW) and moisture content (MC) of developing soybean seeds harvested from plants exposed to drought stress treatments at beginning seed fill (R_5) in the greenhouse and the field experiments. a) Seed dry weight (mg) and moisture content (percentage based on wet weight) of developing soybean seeds from plants exposed to well-watered (WW), gradual stress (GS), and sudden stress (SS) treatments in the greenhouse experiment. b) Seed dry weight (mg) and moisture content (percentage based on wet weight) of developing seeds from plants grown under irrigated (I) and nonirrigated (NI) conditions in the field. Means ± standard error (n = 3–4).

Proteins in Developing and Mature Seeds

In the greenhouse experiment, the heat-stable and dehydrin-like proteins were detected by SDS PAGE and Western blots for the developing seeds sampled at 2, 4, 10, and 18 d after R_5, respectively (Fig. 4a,b, 5a,b). In general, accumulation of heat-stable proteins as detected by SDS PAGE increased in developing seeds of plants in all treatments, except for the protein of 28 kDa MW which was expressed at a lower level at 18 d than at

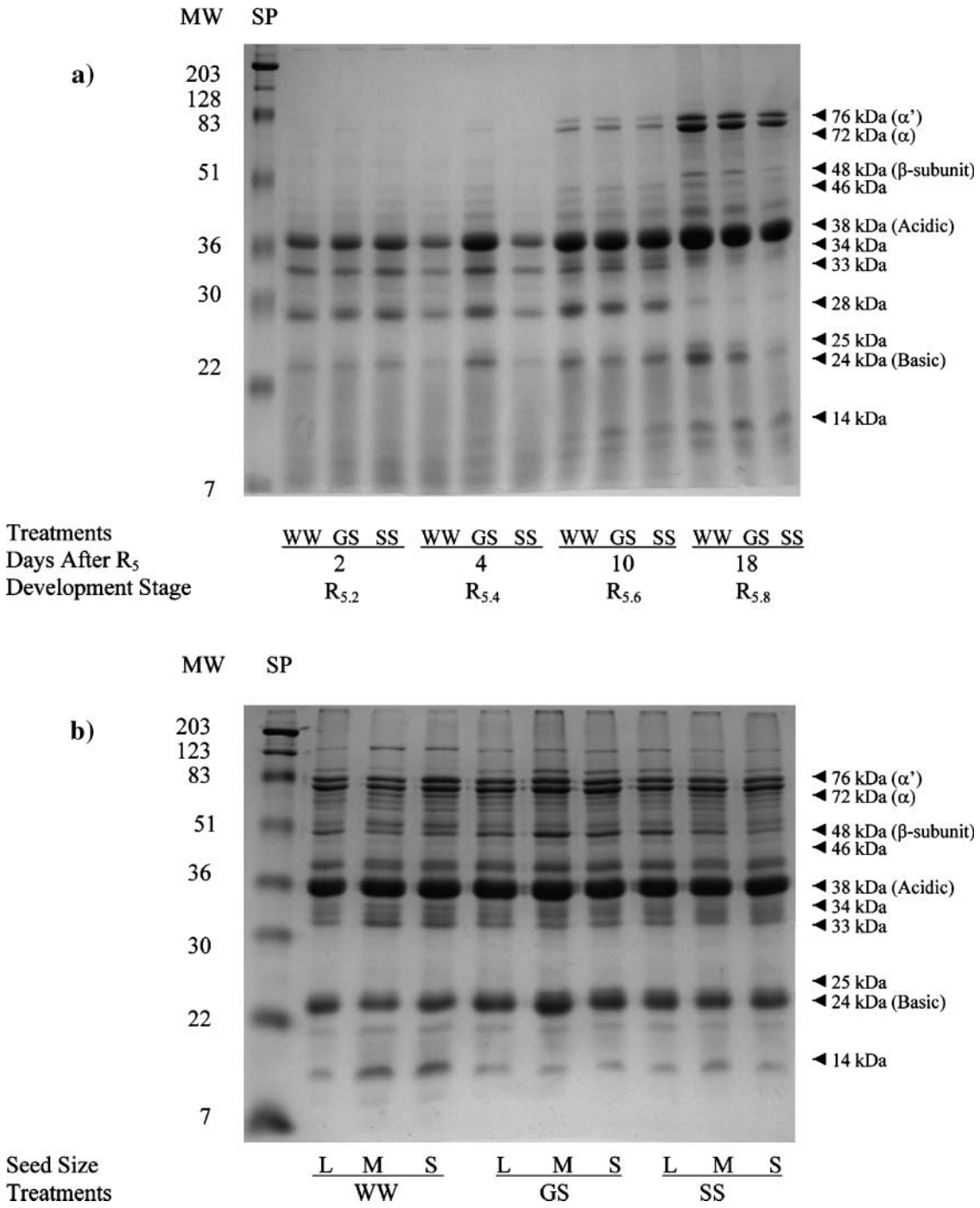

Fig. 4. SDS PAGE of heat-stable proteins for a) developing and b) mature soybean seeds from plants exposed to three drought stress treatments at beginning seed fill (R_5) in the greenhouse experiment. WW: well-watered; GS: gradual stress; SS: sudden severe stress treatments. L: large; M: medium; S: small seeds. Equal weight of protein (7 μg) was loaded per lane. Developing seeds were sampled at 2 ($R_{5.2}$), 4 ($R_{5.4}$), 10 ($R_{5.6}$) and 18 d ($R_{5.8}$) after the stress treatments were imposed at beginning seed fill (R_5). The lane on the left side indicates the molecular weights (MW) of standard proteins (SP). The lane on the right is used to specify MW of proteins indicated by arrows.

2, 4, and 10 d after R_5 (Fig. 4a). These proteins correspond to soybean storage proteins and other unidentified proteins.

The major seed storage proteins in soybeans are glycinin (11S globulin) and β-conglycinin (7S globulin), accounting for approximately 70% of the total storage proteins (Derbyshire et al., 1976). The glycinin protein is composed of acidic subunits, with approximate molecular weight of 45 and 38 kDa, and basic subunit with 22 kDa; the β-conglycinin is composed of major subunits of α′,α, and β-subunits with 76, 72, and 48 kDa, respectively (Shuttuck-Edidens and Beachy, 1985). In our study, the acidic subunit of the glycinin proteins appeared earlier in WW treatment (at 2 d after R_5) than the α′ and α subunits (at 10 d after R_5) and the β-subunit (at 18 d after R_5) of the β-conglycinin (Fig. 4a). The

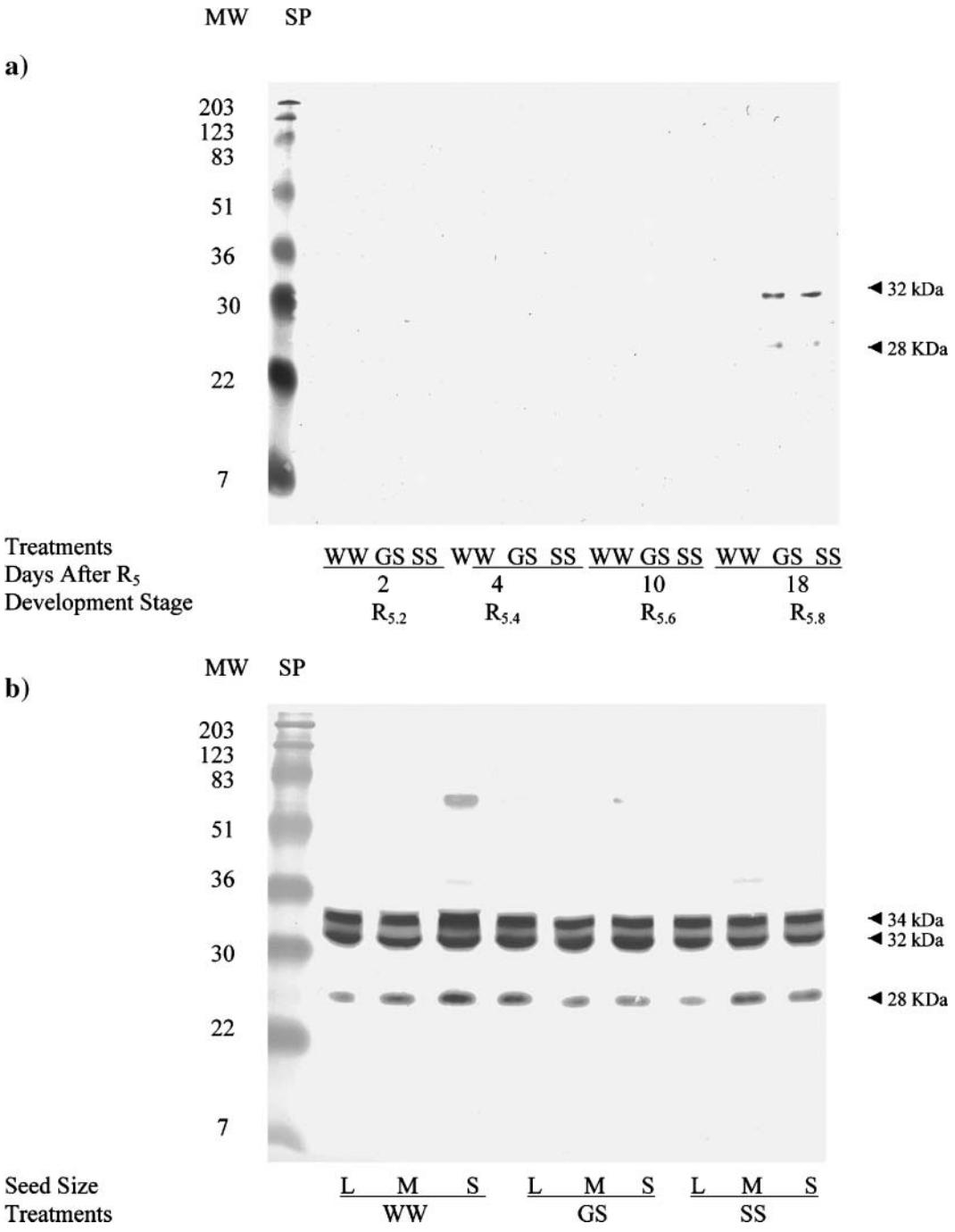

Fig. 5. Western blot of the heat-stable proteins immunologically reacting with dehydrin antibody for a) developing and b) mature soybean seeds from plants exposed to three drought stress treatments at beginning seed fill (R_5) in the greenhouse experiment. WW: well-watered; GS: gradual stress; SS: sudden severe stress treatments. Equal weight of protein (7 μg) was loaded per lane. L: large; M: medium; S: small seeds. The developing seeds were sampled at 2 ($R_{5.2}$), 4 ($R_{5.4}$), 10 ($R_{5.6}$) and 18 d ($R_{5.8}$) after the stress treatments were imposed at beginning seed fill (R_5). The lane on the left side indicates molecular weights (MW) of standard proteins (SP). The lane on the right is used to specify MW of proteins indicated by arrows.

observed accumulation of the α′ and α subunits during seed development earlier than the β-subunit is consistent with the onset of these subunits at the 6.5 to 8.5 mm and 8.5 to 11.5 mm cotyledon stage as reported by Honeycutt et al. (1989), respectively.

The severe drought stress treatment in the greenhouse environment did not effect the accumulation of the α′ and α subunits but delayed the appearance of the β-subunit of the β-conglycinin and the basic subunit of the glycinin storage proteins compared with the WW treatment (Fig. 4a). The data suggest that accumulation of the α′ and α subunits of the β-conglycinin proteins was insensitive to drought stress. Nevertheless, drought stress might have accounted for the delayed onset of the β-subunit relative to the onset of the α′ and α subunits as observed in the SS treatment. These results were con-

sistent with Samarah and Mullen (2006), who reported that shriveled soybean seeds produced under drought stress had a variation in β-subunit of the β-conglycinin, probably because of degradation of proteins in the shriveled seeds produced under drought stress. Similarly, Honeycutt et al. (1989) observed a reduced level of the β-subunit of the β-conglycinin in shriveled seeds produced from a mutant soybean line. The level of β-subunit of β-conglycinin has been shown to be affected by abscisic acid (Bray and Beachy, 1985), methionine (Holowach et al., 1984), and low temperature (Honeycutt et al., 1989).

At Day 4 after R_5, seeds from the gradually stressed plants had an increase in abundance of the acidic subunit of glycinin as compared with the seeds from WW and SS plants (Fig. 4a). When developing seeds from GS and SS plants were sampled 10 d after R_5, a 14 kDa MW proteins were more abundant in the drought-stressed seeds compared with the seeds from WW plants. At Day 18 after R_5, unidentified proteins with 46, 34, 33, and 25 kDa MW were expressed at lower levels in the seeds from SS plants than in seeds from GS and WW plants. The increase and decrease in expression of proteins under drought stress was consistent with the findings of Riccardi et al. (1998) in maize and Bensen et al. (1988) in soybean. These authors reported that drought stress resulted in an increase of some proteins and decrease of others. The increase in the accumulation of heat stable protein with seed maturation in our study was consistent with the findings of Blackman et al. (1991). In the mature dried seeds, heat-stable proteins as detected by SDS-PAGE were not different among different sizes of seeds from the drought-stress treatments (Fig. 4b).

Western blot analysis did not detect dehydrin-like proteins at 2, 4, and 10 d after R_5 (early during seed development) in any treatment (Fig. 5a). Expression of dehydrin-like proteins of 28 and 32 kDa MW was detected in the developing seeds from GS and SS plants but not in the developing seeds from WW plants at 18 d after R_5 (mid-seed development). In the mature dried seeds, dehydrin-like proteins (28, 32, and 34) were detected in different sizes of seeds harvested from drought-stressed and well-watered plants (Fig. 5b). Results indicate that dehydrin-like proteins are present in the mature seeds of soybean and that drought stress induced earlier expression of the proteins in developing seeds.

In the field experiment, heat-stable and dehydrin-like proteins were determined at 0, 5, 15, 22, and 43 d after R_5 (during the entire seed-fill period) and for the mature dried seeds (Fig. 6a,b, 7a,b). Seeds from irrigated and nonirrigated plants were sampled to determine the time of dehydrin appearance in developing seeds from the WW plants and the accumulation pattern of dehydrin later during seed development. The accumulation of heat-stable proteins increased in the developing seeds in both irrigated and nonirrigated plants (Fig. 6a). Developing seeds from both treatments did not differ in the accumulation of heat-stable proteins at any sampling date. Similar results were observed in the mature seeds (Fig. 6b). Western blotting indicated that dehydrin-like proteins were not detected at 0, 5, or 15 d after R_5 (early during seed development) (Fig. 7a). Dehydrin-like proteins of 28- and 32 kDa MW were slightly expressed at 22 d after R_5 (full-seed growth stage [R6]) in the developing seeds from both irrigated and nonirrigated plants. The accumulation of dehydrin-like proteins of 28, 32, and 34 kDa MW increased at 43 d after R_5 (yellow-pod stage [physiological maturity]) before seed desiccation (Fig. 7a) and in the mature dried seeds (Fig. 7b). The treatments did not change dehydrin-like proteins seeds harvested at 22 and 43 d after R_5, neither did they in the mature dried seeds.

The expression of heat stable and dehydrin-like proteins was changed in response to drought stress treatments in the greenhouse experiment but was not affected by the nonirrigated (rainfed) condition in the field experiment. In the greenhouse experiment, there was an increase and decrease in the expression of heat-stable proteins under drought stress treatments. In the field experiment, the expression of heat stable proteins was similar for developing seeds from both irrigated and nonirrigated (rainfed) condition. In both experiments, dehydrin-like proteins were not detected at early sampling dates (0–15 d after R_5) for any of the treatments. Dehydrin-like proteins of 28 and 32 kDa were detected under irrigated and nonirrigated conditions in the field at 22 d after R_5 (R_6). The accumulation of dehydrin-like proteins of 28, 32, and 34 kDa was increased as the seed matured, achieving the maximum accumulation at physiological maturity (defined as the maximum accumulation of seed dry weight). These results were consistent with findings of Galau et al. (1986) and Dure et al. (1989), who observed that dehydrins were synthesized before the dehydration of the maturing seeds. In the greenhouse experiment, drought stress treatments induced expression of dehydrin-like proteins of 28 and 32 kDa at 18 d after R_5, slightly earlier than that observed in the field experiment. In both experiments, dehydrin-like proteins of 28, 32, and 34 kDa were detected in the mature dried seeds of soybean.

The differences in the expression of heat-stable proteins and dehydrin-like proteins in developing seeds between the greenhouse and field experiment might be due to differences in stress severity, developmental stage at which seed samples were taken, and/or the time of stress occurrence between the two experiments. In the greenhouse experiment, the severe stress period was imposed on the plants from Day 8 to Day 23 after R_5. In the field experiment, plants were grown under natural rainfed condition during the entire seed-filling period. The reduction in seed yield in greenhouse experiment was mainly due to the reduction in seed number and weight per seed, while reduction in seed yield in the field was mainly due to the reduction in seed number rather that the weight per seed (Table 2). Reduction in seed yield but not the weight per seed in the field experiment indicates that drought stress occurred early in the seed-filling period, resulting in reduction in seed number and yield, although rainfall occurrence during the remaining filling period resulted in filling the remaining seed to a larger size (Fig. 2c). The tensiometer readings at 45-cm soil depth were significantly higher for the nonirrigated condition than the irrigated condition at the beginning

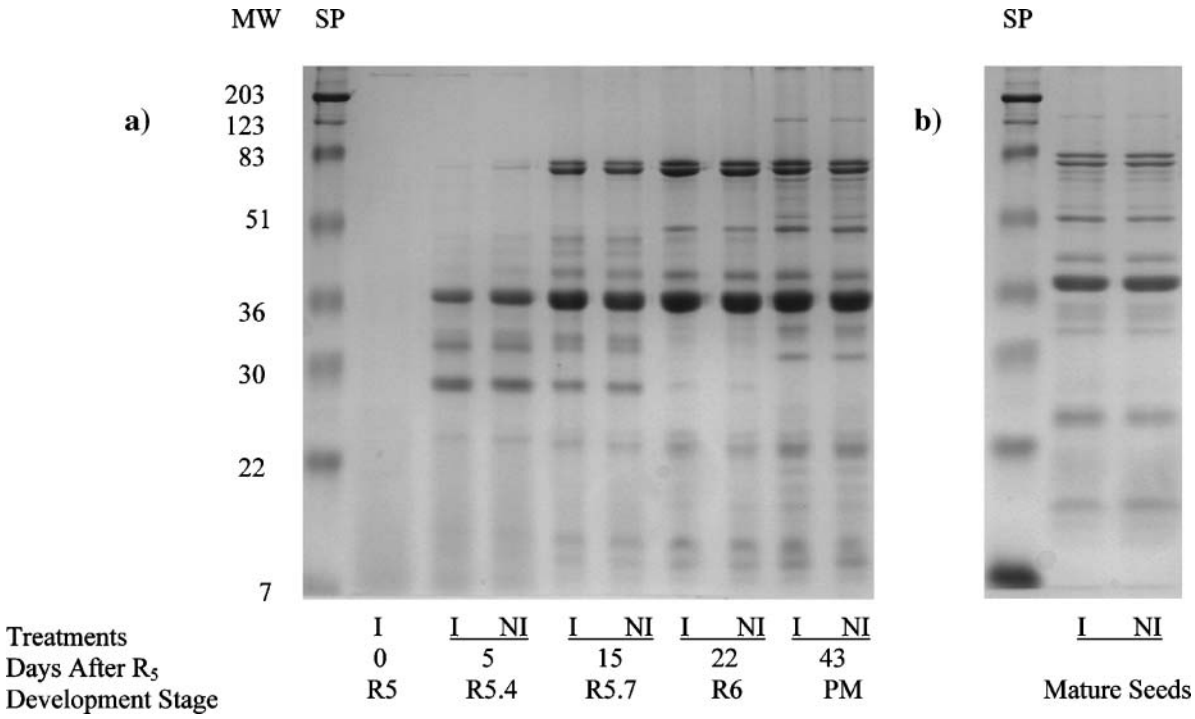

Fig. 6. SDS PAGE of heat-stable proteins for a) developing and b) mature soybean seeds from plants exposed to irrigated (I) and nonirrigated (NI) (rainfed) conditions at beginning seed fill (R_5) in the field experiment. Equal weight of protein (7 μg) was loaded per lane. The developing seeds were sampled at 0 (R_5), 5 ($R_{5.4}$), 15 ($R_{5.7}$), 22 (R_6), 43 d (physiological maturity [PM]) after the stress treatments were imposed at beginning seed fill (R_5). Mature seeds were sampled at harvest maturity when seeds dried in the field. The lane on the left side indicates the molecular weights (MW) of standard proteins (SP).

of seed fill, which supports the conclusion that drought stress occurred early after beginning seed fill in the field experiment (Fig. 2d).

In pea (*Pisum sativum* L.), drought stress imposed before linear seed-fill period, reduced seed yield because of reduction in seed number but not in weight per seed (Ney et al., 1994). When the stress was imposed after the linear filling phase, seed number was maintained by remobilization of reserves, but the remaining seeds were smaller because of shortening of seed-filling period (Ney et al., 1994). In our experiment, drought stress treatments (SS and GS) reduced the duration of seed-filling period by 4 d in the greenhouse experiment although the nonirrigation treatment did not reduce duration of seed filling in the field experiment (data not shown). These results suggest that the difference between the two experiments in the accumulation of proteins might be due to differences in the stress time and severity between the two experiments.

Several hypotheses may explain the mechanism by which drought stress might induce dehydrin-like protein in the developing soybean seeds at 18 d after R_5 (around midseed development) in the greenhouse experiment. The first hypothesis was that drought might have accelerated seed development, resulting in earlier expression of dehydrin-like proteins. On the basis of this hypothesis, dehydrin-like proteins are expressed because of development rather than environmental stress. In our experiment, drought stress significantly shortened the seed-filling period (period from R_5 to R_8) by 4 d (data not shown). However, we selected seeds with the same developmental stage from WW, GS, and SS plants at each sampling date for dehydrin measurements. The developing seeds from WW, GS, and SS plants did not differ in seed dry weight or moisture 18 d after R_5 (Fig. 3a) when dehydrin proteins were first detected in the developing seeds from the drought-stress treatments (GS and SS) compared with the seeds from WW (Fig. 5a). Thus, the induction of dehydrin-like proteins by drought-stress treatments was unlikely to be due to the acceleration of seed development.

The second hypothesis was that the change in water potential in developing seeds under drought stress might have resulted in the expression of dehydrin-like proteins. Water contents of seeds from GS and SS plants were the same as the water content of the seeds from the WW plants at 18 d after R_5 (Fig. 3a). Thus, attributing the dehydrin response observed in drought-stressed seeds to early seed desiccation appeared unlikely.

A third hypothesis was that drought stress increased the biosynthesis of abscisic acid (ABA), which might induce the expression of dehydrin-like proteins. Abscisic acid has been reported to mediate plant response to drought stress (Davies and Mansfield, 1983). The expression of numerous genes was induced in response to ABA (Bray et al., 1996; Espelund et al., 1995). Quatrano (1986) reported that ABA might be involved in regulating the expression of maturation proteins. The regulation of gene expression by ABA reported in the literature suggests that ABA might have a role in mediating drought tolerance in plants. The relationship between the ABA level in the seeds under drought stress

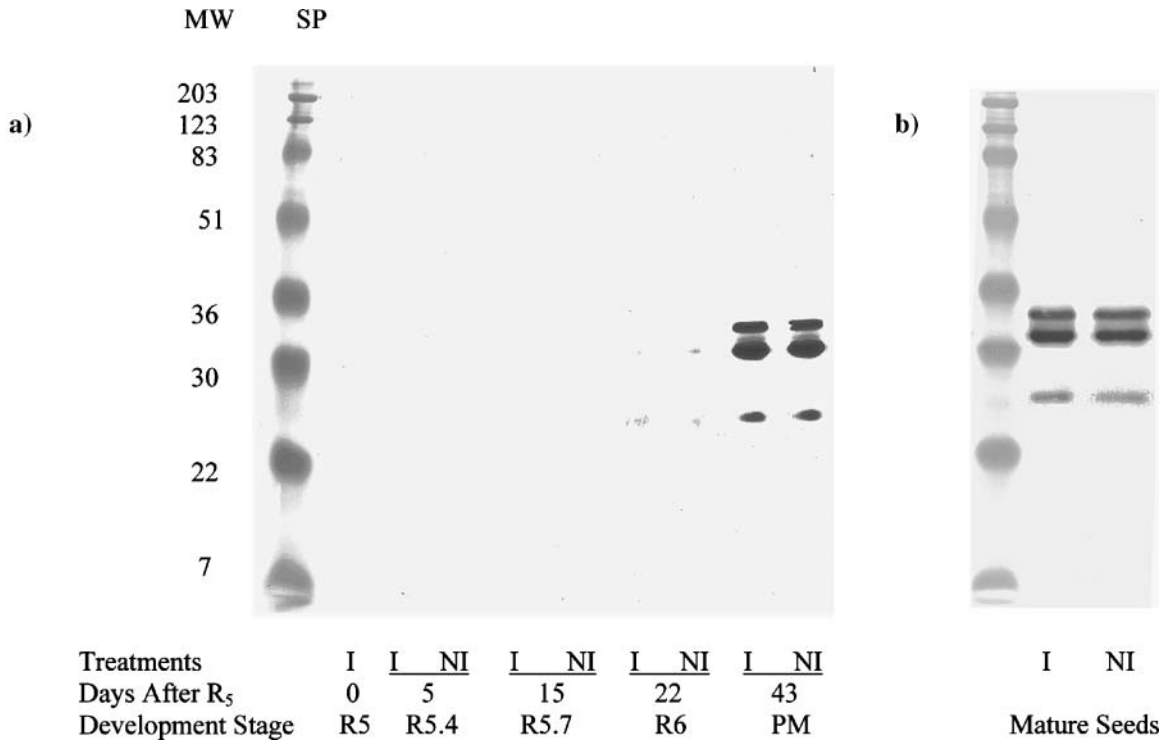

Fig. 7. Western blot of heat-stable proteins immunologically reacting with dehydrin antibody for a) developing and b) mature soybean seeds from plants exposed to irrigated (I) and nonirrigated (NI) (rainfed) conditions at beginning seed fill (R_5) in the field experiment. Equal weight of protein (7 μg) was loaded per lane. The developing seeds were sampled at 0 (R_5), 5 ($R_{5.4}$), 15 ($R_{5.7}$), 22 (R_6), 43 d (physiological maturity [PM]) after the stress treatments were imposed at beginning seed fill (R_5). Mature seeds were sampled at harvest maturity when seeds dried in the field. The lane on the left side indicates the molecular weights (MW) of standard proteins (SP). The lane on the right is used to specify MW of proteins indicated by arrows.

and the expression of dehydrin proteins was not measured in this study and may be an important factor in the development of dehydrin expression in response to drought stress.

A proposed role of dehydrins under drought stress has been in protecting cells from dehydration stress (Close and Chandler, 1990; Dure et al., 1989). The highly conserved lysine-rich sequence (K segment) within dehydrin proteins forms a secondary structure (an amphiphilic α helix), which suggests that the K segment is an essential part for dehydrin function under dehydration stress (Close et al., 1989; Dure et al., 1989). The hypothesized role for the K segment of dehydrin is to form a hydrophobic interaction with DNA (Godoy et al., 1996), partially denatured proteins, and damaged membranes, thus acting as a chaperone to stabilize protein folding under dehydration (Close, 1996; Godoy et al., 1996). Dehydrin may also have a role similar to compatible solutes (such as proline, sucrose, and glycine betaine) in osmotic adjustment. Another possible role of dehydrins is to bind with the accumulated ions (ion sequestering) under water stress and to control solute concentration in the cytoplasm (Dure, 1993b). Dehydrin may act as a cryoprotective role in macromolecular stabilization by binding water molecules on their hydrophilic surfaces, which reverses or prevents further denaturation of cellular proteins (Close, 1996). Maturation proteins, which were induced in response to ABA or dehydration, might protect the plant under stress by stabilizing cell membranes (Dure et al., 1989). The results in the literature and our results on developing soybean seeds suggest that dehydrin might have a role in response to drought stress.

CONCLUSIONS

Drought-stress treatments, regardless of stress rate, changed the accumulation of heat-stable and dehydrin-like proteins in developing soybean seeds in the greenhouse experiment but not in the nonirrigated (rainfed) conditions. Dehydrin-like proteins of 28 and 32 kDa were detected in developing seeds at 22 d after R_5 (full-size seeds in pods [R_6]) in both irrigated and nonirrigated (rainfed) plants in the field. The accumulation of dehydrin-like proteins increased as seeds matured, achieving a maximum accumulation at seed physiological maturity. In the greenhouse experiment, the drought stress treatments induced earlier expression of dehydrin-like proteins in the developing seeds (at 18 d after R_5) (4 d earlier than its appearance in the field experiment). The earlier detection of dehydrin-like proteins under drought stress in the greenhouse experiment indicated that dehydrin-like proteins might have a role in drought tolerance in developing seeds. The results in both experiments suggest that maximum accumulation of dehydrin was associated with maximum accumulation of seed dry weight (the physiological maturity). The accumulation of heat stable and dehydrin like proteins in developing soybean seeds can be used as an indicator for determining seed maturity in physiological and biochemical studies.

ACKNOWLEDGMENTS

We sincerely thank Dr. Timothy J. Close, Department of Botany and Plant Science, University of California, for providing dehydrin antibody. Special thanks are also extended to Dr. Jack Hartwigsen, Dr. Joan Peterson, and Dr. Leobigildo Cordova, Department of Agronomy, Iowa State University for their assistance on protein analysis.

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