BULG. J. PLANT PHYSIOL., 2002, 28(3–4), 12–25

OSMOTIC STRESS-INDUCED CHANGES IN GERMINATION, GROWTH AND SOLUBLE SUGAR CONTENT OF *SORGHUM BICOLOR* (L.) MOENCH SEEDS

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Received November 8, 2002

Summary. The effect of osmotic stress on germination, growth and soluble sugar content in *Sorghum bicolor* (L.) Moench cv. CSH 9 seeds and seedling components (endosperm and embryos) during early germination was investigated. Under stress conditions germination decreased markedly, whereas the control at the same time reached its maximum germination (99%). A high percentage (67%) of ungerminated seeds from mannitol treatment germinated after washing with distilled water. Both water potential and tissue water content of both embryo and endosperm showed a slight recovery after re-watering. As compared to the control, fresh weight was reduced by osmotic stress both in germinated embryos and endosperm after 14 h of development. On the contrary, a substantial increase in dry weight was observed in both embryo and endosperm. Furthermore, a considerable increase in the sugar levels in both embryo and endosperm were detected under stress conditions. Total sugar and reducing sugar content increased markedly after 14 h of stress imposition. These parameters were maintained principally in endosperm after stress was released. The fructose level was always higher than glucose and sucrose in response to mannitol treatment. However, the level of glucose and sucrose was higher in the embryos and endosperm after stress treatments than in the controls. Based upon these results a possible physiological role of sugars in germination of sorghum seeds was suggested.

Keywords: Growth, sorghum, sugars, water stress, mannitol

Abbreviations: FW – fresh weight, DW – dry weight

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Introduction

Due to their sedentary mode of life, plants resort to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold, heat and excessive osmotic pressure, which ultimately affect plant growth and productivity (Epstein et al., 1980; Yancey et al., 1982). Plants adapt to stresses by different mechanisms, including changes in morphological and developmental patterns as well as physiological and biochemical processes (Bohnert et al., 1995). Adaptation to all these stresses is associated with metabolic adjustments that leads to the accumulation of organic solutes such as sugars, polyols, betaines and proline (Flowers et al., 1977; Gorham et al., 1981; Yancey et al., 1982). Among accumulating solutes, starches and lipids give rise to sugars (Amuti and Pollard, 1977; Bewley and Black, 1994; Koster and Leopold, 1988) which are synthesized during seed germination and transported to sites where they are required for growth (Mayer and Poljakoff-Mayber, 1975). Soluble sugars also seem to play an important role in osmotic regulation of cells during germination (Gorham et al., 1981; Bolarin et al., 1995). In addition to this role, sugars also regulate the expression of some genes involved in germination of seeds (Reynolds and Smith, 1995; Yu et al., 1996). Accumulation of sugars, a characteristic of mature seeds appears to be central to the development of desiccation tolerance (Hoekstra et al., 2001 for review). Sugars also facilitates vitrification (a phenomenon in which intracellular water hardens like glass with no ice crystal formation during freezing or chilling stress) and thus avoids the damage to cells caused by crystallization as water is withdrawn (Williams and Leopold, 1989). Earlier studies report on carbohydrate accumulation during various abiotic stresses in temperate grasses and cereals where long term carbohydrate storage occurs during reproductive development (Archbald, 1940; Meier and Reid, 1982). Accumulation of sugars in different parts of plants is enhanced in response to a variety of environmental stresses (Macleod and Orquodale, 1958; Gorham et al., 1981; Wang et al., 1996; Prado et al., 2000; Gill et al., 2001). In the case of salt (Gill and Singh, 1985) and water stress (Prado et al., 2000; Siddique et al., 2000), adaptation to these stresses has been attributed to the stress-induced increase in carbohydrate levels. There are few studies on carbohydrate status in germinated seeds and their early developmental stages under stress conditions, possibly, because metabolism of these compounds can be affected by a number of environmental factors such as irridance, temperature, salinity and type of ions present (Bohnert et al., 1995). Thus, the variation that occurs in carbohydrate level, during early germination is poorly understood and information on the physiological events involved in this process is scarce. Therefore, in this study, we present details on germination and status of soluble carbohydrates in germinating embryos and endosperm during early stages of germination and development of sorghum seeds under osmotic stress. Sorghum is a C_4 grass that is well adapted to semiarid tropics (Quinby, 1974). This grain crop is the fifth most important cereal grown worldwide, due in large part to its unusual tolerance to adverse environmental conditions (Doggett 1988).

Materials and methods

Plant material and chemicals

The seeds of *Sorghum bicolor* (L.) Moench cv. CSH-9 were purchased from the National Seed Corporation, Pusa, New Delhi, India. Fine chemicals and reagents used in this study were purchased from Sigma chemicals, St. Louis, USA. All the other chemicals were of analytical grade.

Seed germination and stress conditions

Washed grains of sorghum were surface sterilized with 1% (w/v) mercuric chloride followed by 70% ethanol. Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibation, 100 seeds were placed in a Petri plate (in triplicate) containing sterile filter sheets, moistened with 2 ml of distilled water or with mannitol solution (0.75 M, -1.86 MPa). Germination percentage was determined and sampling was carried out at 4, 6, 10 and 14 h when the germination in the water-irrigated seeds reached its maximum (99%) using radicle protrusion (2 mm) as a criterion for germination (Prado et al., 2000). Embryos and endosperm were separated and stored immediately in the liquid nitrogen for further analysis. The percentage of abnormal germination, i.e proportion of seeds with cotyledons without radicle protrusion. (aborted seeds), was also determined. Post stress kinetics analysis, both mannitol-irrigated and water-irrigated germinated seeds (after 14 h of germination) were rinsed with distilled water (three times) and transferred to the new petri plates containing filter paper moistened with distilled water. Biochemical analysis was carried out after 18, 22 and 26 h.

In addition, after 14 h ungerminated seeds under mannitol-irrigated stress (0.75 M, -1.86 MPa), were rinsed with distilled water for 3 min and transferred to the new petri plates containing filter paper moistened with distilled water. Biochemical analysis was carried out at 4, 6, 10 and 14 h after transferring the ungerminated seeds to the new petriplates. Embryos and endosperm were separated and stored immediately in liquid nitrogen for further analysis. Parts of these tissues were weighed to obtain the fresh weight (FW). The dry weight (DW) was obtained after drying the different tissues for 48 h at 75ºC. Tissue water content was obtained from the FW-DW/DW ratio. Water potential measurements were made with a Themocouple Dew Point Microvoltmeter (Model HR 33T, Wescor, USA).

Extraction and estimation of sugars

The different tissues (embryos and endosperm) were extracted twice with 80% ethanol at 90ºC followed by extraction (4 times) with 70% ethanol (Singh et al., 1987). The ethanol extracts were pooled separately and concentrated with a rotatory evaporator at 70ºC under vacuum (Gupta et al., 1993) and their qualitative make up was ascertain-

ed by paper chromatography (PC) on 3 MM Whatman Chromatography paper using *n*-butanol:acetic acid:water (4:1:5, v/v/v) as an irrigating solvent and $AgNO_3$ in acetone as stain (Trevelyan et al., 1950). From the extract obtained above, reducing sugars were quantitatively estimated by Nelson's method (1944), total sugars were estimated by the method of Dubois et al. (1956) and free sugars were estimated by the method of Singh and Machlachlan (1983).

Results

Effect of osmotic stress on Germination, Water potential, FW, DW, Tissue water content and Carbohydrate content of germinated embryos of sorghum seeds

Germination of sorghum seeds in distilled water reached a maximum (99%) after 14 h (Fig. 1). However, in 0.75 M mannitol germination was considerably lower (25%). Imposition of osmotic stress resulted in a concomitant lowering of water potential (more negative) from 0 h to 14 h, whereas in controls no significant change was observed (Table 1). Concordingly, a noticeable decrease in tissue water content was observed after 14 h of stress treatment. Re-watering of embryos resulted in significant increase in water potential. During early germination from 4 h to 14 h, the FW values increased considerably both in water-irrigated and mannitol-irrigated embryos, but the rate of increase was higher in the controls (Table 1). Maximum decrease in FW of embryos was observed after 14 h of mannitol treatment. Stressed embryos, however, showed considerable increase up to 26 h after re-watering.

A similar trend was observed in DW of germinated embryos (Table 1). A concomitant increase in DW was observed from 0 h to 14 h of stress treatment. Maximum increase (4-fold) was observed after 14 h. Even after relieving the stress, DW values continued to increase up to 26 h. The tissue water content (FW-DW/DW) ratio, a

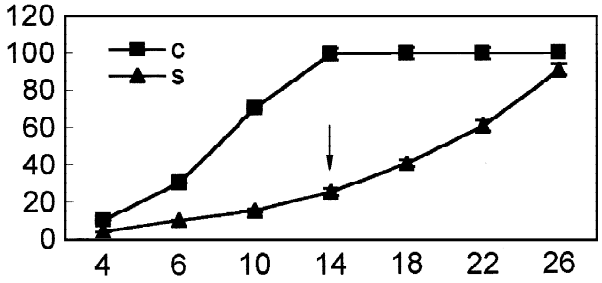

Fig. 1. Effect of osmotic stress on germination of sorghum seeds. Arrow indicates time when stress was released. Values are mean of three independent experiments ± SE C – control; S – stress

Table 1. Effect of osmotic stress(s) on water potential, fresh weight (FW), dry weight (DW) and tissue water content in germinating embryos and endosperms of sorghum. Arrow indicates time when stress was released. Values are mean of three independent experiments ±SE. C – control, S – mannitol stress (0.75 M)

Time (h)	Water potential (-MPa)		FW (mg)		DW (mg)		Tissue water content (FW-DW/DW)	
	C	S	C	S	C	S	C	S
Embryo								
0	1.8±0.07	1.7±0.03	0.82±0.01	0.81±0.02	0.21±0.01	0.23±0.02	2.90±0.05	2.52±0.01
4	1.9±0.01	1.7±0.02	0.91±0.02	0.73±0.01	0.20±0.09	0.21±0.01	3.55±0.02	2.47±0.03
6	1.8±0.01	1.6±0.04	3.21±0.71	1.23±0.15	0.54±0.11	0.39±0.04	4.94±0.11	2.15±0.11
10	1.9±0.01	1.8±0.07	7.12±1.23	3.21±0.18	1.91±0.24	1.12±0.21	2.72±0.22	1.86±0.11
14 ▬	1.8±0.01	2.0±0.01	13.23±2.11	9.91±1.23	2.1U0.54	4.12±0.98	5.27±0.55	1.40±0.11
18	1.9±0.00	1.8±0.01	14.31±3.21	12.2U2.34	2.5H0.85	5.21±0.55	4.57±0.57	1.34±0.21
22	1.8±0.01	1.8±0.01	14.92±2.11	13.31±3.33	3.12±0.54	5.51±0.98	3.78±0.55	1.41±0.22
26	1.9±0.01	1.8±0.01	16.31±2.25	14.21±2.12	3.55±0.33	5.32±0.28	3.59±0.99	1.67±0.11
Endosperm								
0	3.1±0.21	3.0±0.11	40.H2.11	39.3±2.10	25.3±3.31	24.9±3.33	0.58±0.08	0.57±0.01
4	3.2±0.08	3.1±0.11	41.2±2.23	38.3±4.33	27.2±2.21	27.3±3.33	0.51±0.07	0.40±0.02
6	3.1±0.58	3.1±0.08	41.5±3.11	36.3±2.11	28.3±3.31	28.1±4.11	0.46±0.05	0.29±0.01
10	3.0±0.25	3.2±0.11	42.0±2.25	34.2±4.13	28.3±2.82	29.5±3.33	0.48±0.02	0.15±0.05
14 ▬	3.1±0.25	3.5±0.11	42.5±4.21	33.2±1.13	29.5±3.33	30.1±1.55	0.44±0.06	0.10±0.02
18	3.2±0.31	2.7±0.21	41.9±3.33	36.3±3.33	29.1±2.22	30.3±4.10	0.43±0.03	0.19±0.02
22	3.2±0.54	2.7±0.08	42.5±3.31	39.3±2.31	29.2±3.33	31.3±2.55	0.45±0.05	0.25±0.04
26	3.1±0.20	2.8±0.11	41.9±2.32	38.9±1.10	29.1±2.55	31.5±3.25	0.43±0.03	0.23±0.04

measure of expansion growth, of embryos in distilled water showed a substantial increase up to 14 h. The water content of embryos after 14 h of stress treatment was considerably lower (about 4-fold) than the control. After re-watering, stressed germinated embryos showed slight increase in tissue water content.

The influence of 0.75 M mannitol on total soluble sugar content during germination is shown in Table 2. Under osmotic stress conditions, total soluble sugar content increased considerably between 0 h to 14 h, reaching maximum at 14 h. Similarly in the control embryos, the total soluble sugar content increased markedly after 14 h of stress treatment, but the percent increase was more under stress conditions. Relieving the osmotic stress, resulted in a substantial change in total soluble sugar content. In fact a significant decrease in total soluble sugar content under osmotic stress was observed from 14 h to 26 h. A similar trend was observed in reducing sugar content in stressed germinated embryos. Reducing sugar content continued to increase from 0 h to 14 h, showed maxima at 14 h, but rate of increase was more under stress conditions. Re-watering of germinated embryos resulted in a concomitant decrease in reducing sugar content. Among the free sugars, fructose content was always higher than glucose and sucrose (Table 2). Maximum fructose content was observed after 14 h of stress. The increase was greater in the stressed embryos than the control. Stressed embryos, however, showed significant change in glucose and sucrose content from 14 h to 26 h after re-watering.

Effect of mannitol stress on Water potential, FW, DW, Tissue water content and Carbohydrate content in endosperm

Water potential and tissue water content decreased considerably after stress treatment. Maximum decrease in water potential and tissue water content was observed after 14 h of stress imposition (Table 1). On the contrary, in the control endosperm, no significant change in water potential was observed. Stressed endosperms, however, showed a rapid recovery in water potential and tissue water content after re-watering.

During germination from 0 h to 14 h, no significant change in FW of control endosperm was observed whereas stressed endosperms showed a decrease in FW up to 14 h (Table 1). Relieving of stress resulted in considerable recovery in FW, whereas, no significant change in FW was observed in water irrigated endosperms. Under stress conditions, maximum DW was observed after 14 h. In fact rate of increases in DW was almost same both in the control and stress conditions. Even post stress recovery did not show much variation in DW values in the control and stressed endosperms.

Under stress conditions, maximum total soluble sugar content of endosperms was observed after 14 h (Table 2). In control endosperms, maxima were observed at 14 h, but accumulation of sugars was more under stress conditions. Even after stress was relieved, no significant change in total soluble sugar content was observed in the control and stressed endosperms. Similar increase in reducing sugar content was observed

Table 2. Osmotic stress-induced changes in total soluble sugar content, reducing sugar content and free soluble sugars in germinating embryos and endosperm of sorghum. Arrow indicates time when stress was released. Values are mean of three independent experiments ± SE. C – control, S – stress.

Time (h)	Sugar content (mg.g^{-1} FW)									
	Total		Reducing		Fructose		Glucose		Sucrose	
	C	S	C	S	C	S	C	S	C	S
Embryo										
0	15.5±3.3	15.1±4.3	8.7±3.3	9.0±1.0	3.1±0.89	3.2±0.08	1.0±0.08	1.1±0.01	0.51±0.02	0.48±0.02
4	14.1±5.2	15.2±4.1	8.3±3.3	9.2±1.5	3.2±0.87	3.4±0.31	1.U0.02	1.3±0.02	0.61±0.01	0.69±0.10
6	14.8±3.2	15.5±3.5	8.7±4.3	9.8±2.2	3.5±0.75	3.7±0.21	1.5±0.03	1.4±0.03	0.63±0.02	0.68±0.11
10	15.7±3.3	18.9±4.2	9.0±5.1	11.2±2.2	3.9±0.34	5.2±0.11	1.4±0.01	1.7±0.02	0.81±0.03	0.91±0.09
14	19.1±1.5	27.2±3.9	13.1±1.5	15.3±1.1	4.1±0.55	7.1±1.21	1.3±0.05	1.9±0.01	0.92±0.01	0.98±0.01
18	18.5±4.3	18.9±3.3	11.0±3.3	12.0±1.9	3.9±0.45	6.9±0.89	1.0±0.02	1.1±0.02	0.89±0.02	0.91±0.08
22	17.5±3.3	17.7±4.0	8.8±2.2	8.9±3.1	3.8±0.23	6.3±1.00	1.1±0.03	1.0±0.01	0.91±0.01	0.88±0.02
26	17.1±3.2	17.0±2.5	9.0±4.2	9.1±1.0	3.5±0.33	6.0±2.11	0.9±0.01	1.0±0.02	0.90±0.10	0.88±0.01
Endosperm										
0	20.3±1.2	21.2±31	10.2±2.1	9.9±2.3	5.2±0.89	4.8±0.89	3.2±0.85	3.0±0.55	1.1±0.08	0.9±0.08
4	20.1±2.3	20.9±3.3	10.5±2.3	10.9±3.3	5.3±0.58	5.5±1.22	3.5±0.55	3.4±0.55	1.1±0.05	1.2±0.02
6	19.9±2.1	21.5±5.1	11.2±3.1	11.1±3.3	4.8±0.84	5.1±1.10	3.7±0.55	3.9±0.33	1.2±0.03	1.4±0.01
10	25.3±3.1	31.4±4.2	10.1±1.8	12.3±3.1	5.5±0.55	8.1±1.11	4.H0.66	5.1±0.23	1.3±0.02	1.5±0.02
14	27.3±4.1	35.3±2.5	12.3±2.2	18.3±1.5	5.9±0.64	7.1±0.99	4.2±0.54	5.9±0.41	1.8±0.05	2.0±0.03
18	27.9±3.3	37.3±5.6	12.5±3.1	18.5±4.1	6.1±0.66	6.9±1.22	4.3±0.45	5.1±0.32	1.4±0.02	1.9±0.02
22	27.0±3.3	37.1±6.1	13.2±4.1	18.0±3.1	5.9±0.66	7.0±2.11	4.1±0.35	5.2±0.25	1.6±0.01	1.8±0.01
26	26.5±2.2	36.9±4.3	13.5±1.1	18.3±2.1	6.0±0.75	7.U1.31	4.0±0.65	4.2±0.51	1.5±0.01	2.1±0.03

from 0 h to 14 h of stress imposition, with maxima at 14 h. In the controls no considerable change in reducing sugar content was observed. Relieving of stress, showed no significant change in reducing sugar content. Differences were observed in fructose, glucose and sucrose contents. Fructose content was always higher than glucose and sucrose. Under stress conditions an increase in fructose content was observed up to 10 h. Re-watering, resulted in a significant decrease in glucose content. Glucose and sucrose content was higher than their respective controls, reaching maximum levels at 14 h. Relieving of stress did not result in significant change in fructose content from 14 h to 26 h.

Profile of Water potential, FW, DW, Tissue water content and Carbohydrate changes in the ungerminated seeds

Changes in Water potential, FW, DW, Tissue water content and Carbohydrate content of dissected embryos

After 14 h, (67%) of ungerminated seeds from the mannitol treatment germinated after washing with distilled water. No significant variation in water potential was observed from 4 h to 14 h of germination whereas embryo water content increased considerably from 4 h to 14 h. (Table 3). This increase in water content was concordent with an increase in FW up to 14 h. Similarly an increase in embryo DW was observed (Table 3). Furthermore, only a slight increase in total sugar content and reducing sugar content was observed from 4 h to 14 h of germination (Table 4). Among the free sugars, fructose level was markedly higher than glucose and sucrose. During germination from 4 h to 14 h, significant change in fructose content was observed after 14 h of germination.

Table 3. Changes in water potential (-MPa), fresh weight (FW), dry weight (DW) and tissue water content in the ungerminated seeds of sorghum at various time points of germination. Values are mean of three independent experiments ±SE.

Time (h)	Water potential (-MPa)	FW (mg)	DW (mg)	Tissue water content (FW-DW/DW)
Embryo				
4	1.9±0.02	0.89±0.02	0.19±0.02	3.68±0.85
6	1.9±0.01	2.93±0.89	0.56±0.03	4.23±0.99
10	1.8±0.01	6.71±1.23	1.00±0.11	5.71±1.22
14	1.9±0.03	12.91±3.31	1.99±0.019	5.48±1.11
Endosperm				
4	2.8±0.02	39.5±2.21	34.3±3.33	0.15±0.02
6	2.7±0.01	39.1±3.31	35.5±4.55	0.10±0.01
10	2.8±0.01	38.9±5.21	34.5±5.51	0.12±0.01
14	2.9±0.08	40.1±1.32	35.0±1.21	0.14±0.09

Changes in Water potential, FW, DW, Tissue water content and Carbohydrate content in endosperms

During early germination, from 4 h to 14 h, the water potential and tissue water content of endosperm did not changed (Table 3). Furthermore, a little variation in FW and DW was observed from 4 h to 14 h. A considerable increase in total soluble sugar content was observed at 14 h of germination (Table 4). In contrast, no significant variation was observed in reducing sugar content. Among the free sugars, fructose content was always higher than glucose and sucrose.

Discussion

The present investigation monitored changes caused by mannitol stress in germination rate and carbohydrate level of *S. bicolor* (L.) Moench cv. CSH-9 seed embryos and endosperm. Fourteen hours of osmotic stress caused a considerable decrease in germination of control seeds. Upon relieving stress with distilled water, a high percentage of ungerminated seeds under mannitol treatment germinated. Similar declines and recoveries in seed germination have been reported in the literature (Khan and Ungar, 1984; Woodell, 1985; Gupta et al., 1993; Singh et al., 1996; Ungar, 1996). Reduced germination under water stress conditions may be attributed to the effect that seeds seemingly develop an osmotically enforced "dormancy" under water stress conditions, which may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Singh et al., 1996, Prado et al., 2000). Furthermore, under osmotic stress a significant reduction in water potential and tissue water content of germinated embryos and endosperm was obser-

Table 4. Changes in total soluble sugar content, reducing sugar content and free sugars in the ungerminated seeds of sorghum at various time points of germination. Values are mean of three independent experiments ±SE.

Time (h)	Sugar content (mg.g^{-1} FW)				
	Total	Reducing	Fructose	Glucose	Sucrose
Embryo					
4	17.1±2.3	10.3±3.3	2.9±1.20	0.9±0.02	0.31±0.02
6	17.5±3.3	10.5±2.2	2.8±0.99	0.8±0.01	0.33±0.02
10	17.9±4.2	11.5±3.2	3.3±0.85	1.1±0.02	0.31±0.01
14	18.U1.5	11.0±1.3	3.5±0.15	0,9±0.01	0.34±0.02
Endosperm					
4	21.3±1.3	10.9±2.3	4.9±1.1	3.1±0.88	1.0±0.05
6	22.3±5.5	10.3±3.3	4.7±0.88	2.9±0.58	0.9±0.02
10	23.4±6.1	10.8±4.1	5.2±0.94	3.3±0.66	1.1±0.05
14	25.4±1.3	11.1±2.3	5.4±0.88	3.9±0.65	1.5±0.02

ed, indicating that these tissues were under stress. Similar observations of decreases in water level under stress conditions were made by Gill et al. (2001) in sorghum, Siddique et al. (2000) in wheat (*Triticum durum*), Prado et al. (2000) in Chenopodium (*Chenopodium quinoa*), Pennypacker et al. (1990) in alfalfa (*Medicago sativa*) and Gupta et al. (1993) in chickpea (*Cicer arietinum*).

In relation to growth, both germinated embryos and endosperm were suppressed by mannitol treatment. They were smaller than in distilled water because of reduced FW, resulting from reduced water absorption (Prado et al., 1995). The increase of embryos FW in distilled water was mainly due to an increase of tissue water content which was reflected from FW-DW/DW values (Table 1). This increase was not significant in endosperm where the (FW-DW/DW) ratio showed no significant variation. Osmotic adjustment has been shown to reduce growth sensitivity to water stress (Cutler et al., 1980) or to allow growth to proceed at a slower rate under water stress (Meyer and Boyer 1981) by maintaining turgor. Thus, it can be concluded that growth at lower water potential was a result of turgor maintenance, whereas the inhibition of growth was not entirely dependent on turgor (BassiriRad and Coldwell 1992). In contrast to a significant decrease in FW, stress imposition resulted in a significantly higher gain in biomass in germinating embryos and endosperm as is shown from an increase in DW values after osmotic stress treatment. This osmotic stress-induced DW increase, principally in germinated embryos, might be attributed to the increased synthetic activity associated with cell division and new material synthesis (Sunderland 1960). In contrast, DW values of endosperm did not show much difference in water-irrigated and stressed endosperms, probably because cell expansion was not accompanied by cell division. Thus, the FW increase is largely attributable to cell enlargement by water absorption, cell vacuolation and turgor-driven wall expansion (Prado et al., 2000).

In earlier research, Gill and Singh (1985) have reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stresses. Changes in anyone of these processes can affect other metabolic activities, particularly carbohydrate metabolism that plays an important role in germination and seed development. In earlier research Schubert et al., (1995) and Kameli and Losel (1996) reported a linear correlation between water stress and dry matter accumulation in *Medicago sativa* and *Triticum durum* respectively. Our findings are similar to results of *M. sativa* and *T. durum*. A significant increase in DW from 0 h to 14 h of stress imposition coincided with an increase in total soluble sugar content and reducing content both in germinated embryos and endosperm. These results can be compared with the earlier observations where imposition of water stress was related to an elevated level of sugars in corn (Barlow et al., 1976), soybean seedlings (Meyer and Boyer, 1981), in the leaves and seeds of wheat (Kameli and Losel, 1996), cotton (Timpa et al., 1986) and chickpea (Gupta et al., 1993). This increase in the sugar levels of stressed tissues might also help in effective osmoregulation under

water stress conditions (Spyropoulos, 1982). Inspite of a significant increase in DW, stressed embryos, did not show rapid recovery in total soluble sugar content and reducing sugar content after re-watering. Among the free sugars, fructose was accumulated to a greater extent than glucose and sucrose. Maximum fructose level was observed after 14 h of stress treatment in germinating embryos and after 10 h of stress imposition in endosperm. Kameli (1990) and Spyropoulos (1982) had reported a similar increase in fructose levels after stress treatments. Soluble organic compounds may act as osmoprotectants of proteins under salt and drought stress in addition to their role in osmoregulation (Kameli and Losel, 1995). The overall low levels of glucose and sucrose under control and stress conditions may be due to a general decrease in total metabolic activity caused by stress. It is also possible that more rapid hexose metabolism, supplying compounds involved in water balance is involved (Prado et al., 2000). This assertion was further supported by studies with a variety of plants that demonstrate a salt- or drought-induced conversion of hexoses and other carbohydrates, such as sucrose and starch, into sugar alcohols (polyols) and proline (Perez-Alfocea and Larher 1995, Wang et al., 1996).

Further studies with ungerminated seeds revealed that a high percentage (67%) of ungerminated seeds under osmotic stress germinated after washing with distilled water. However, significant variations in water potential and tissue water content of embryos were observed. A similar trend was observed in total soluble and reducing sugar contents. Among the free sugars, fructose accumulation was higher than glucose and sucrose. However, endosperm showed a substantial increase in DW and carbohydrate contents from 4 h to 14 h of germination, suggesting that a high degree of osmotic adjustment occurred in the seeds since seeds represents the synthetic site where a series of metabolic changes takes place that are necessary for the further development in plants. Consequently, our study indicates that changes observed under stress conditions are associated with adaptation of plants to stresses that leads to a gain in synthetic activity, carbohydrate content and other changes associated with them. Furthermore, investigations are needed to enhance our understanding of how different abiotic stresses effect early seed development.

Acknowledgement. Financial assistance for this work was provided by the Department of Biotechnology, Government of India.

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