

# Sorbitol-induced changes in various growth and biochemical parameters in maize

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## ABSTRACT

Treatment of maize seedlings with different concentrations of sorbitol decreased the rate of germination substantially. Root and shoot length was also reduced by sorbitol treatment, however, decrease in root length was lower than shoot length. Incubation of leaf segments from maize seedlings grown in continuous light with sorbitol decreased the fresh weight and increased the dry weight in a concentration-dependent manner. Sorbitol treatment also reduced the total chlorophylls, chlorophyll a as well as chlorophyll b; the decrease in chlorophyll 'b' being more prominent than chlorophyll 'a', however, carotenoid content was declined marginally. Supply of sorbitol decreased the protein and RNA content; however, proline content and *in vivo* nitrate reductase activity (NRA) were increased. The results demonstrate an inhibitory effect of sorbitol-induced stress on overall growth in maize. Amongst the biochemical parameters analysed, chlorophyll, protein and RNA contents were declined, while proline content and nitrate reductase activity were enhanced with sorbitol treatment.

**Keywords:** proline-rich glycopeptides; osmotic stress; *Zea mays*; pigments; abiotic stress; nitrate reductase activity

Influence of some abiotic factors, such as As and Cd (Jain and Gadre 1997, Jain et al. 2007), osmotic or water stress (Lei et al. 2009), and salts (Parvaiz and Satyavati 2008), on growth and metabolism of plants has been intensively studied. Drought-induced osmotic stress triggers a wide range of perturbations ranging from growth and water status disruption to the modification of ion transport and uptake systems (Bajji et al. 2000). Inhibition of leaf growth is a primary whole plant response to water stress which has been reported in maize, barley and rice seedlings (Lu and Neumann 1998). Amongst other parameters, decline in nitrate reductase activity in *Zea mays* (Abd-El Baki et al. 2000), reduction in chlorophyll level (Tomati et al. 1978) and decrease in the rate of photosynthesis, levels of PSII mRNAs and proteins, half-lives of D1 and D2 proteins in barley leaves and *Triticum aestivum* due to osmotic stress (Yuan et al. 2005) have been reported. Moreover, chloroplastic activities under water stress conditions favour the formation of reactive oxygen species, which can cause irreversible damage to the cell. In response to such oxidative stress, plants adapt themselves by some changes in their antioxidative system for scavenging active oxygen (Moran et al. 1994). Differential response during sorbitol-induced

stress in drought tolerant and resistant varieties of maize has been reported in relation to anti oxidative defence mechanism (Kolarovic et al. 2009). Additionally, plants also accumulate organic osmolytes and ions, such as proline and K<sup>+</sup>, during growth under water deficit conditions.

Sorbitol, a six carbon sugar alcohol, is one of the most frequently found polyols in plants. It is a direct product of photosynthesis in mature leaves, in parallel with sucrose, and both serve similar functions, such as translocation of carbon skeletons and energy between sources and sink organs. Increased transport of polyols, both in the xylem and phloem, occurs frequently as a result of salt or drought stress (Noiraud et al. 2001). The present study was aimed to investigate the changes in growth and biochemical parameters in maize subjected to osmotic stress by sorbitol.

## MATERIALS AND METHODS

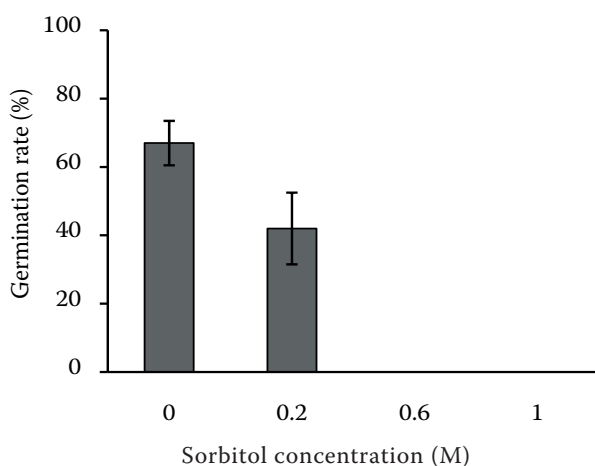
**Plant material.** Sterilized seeds of *Zea mays* L. cv. Ganga Safed-2 were raised in continuous light for 7–8 days at 26 ± 2°C. They were watered with half strength Hoagland's solution containing 5mM NH<sub>4</sub>NO<sub>3</sub>. To analyse growth parameters, sterilized

maize seeds were grown on filter paper placed in Petri plates and treated with varying concentrations of sorbitol (0.0M, 0.2M [-0.58 MPa], 0.6M [-1.78 MPa] and 1.0M [-3.09 MPa]) at  $25 \pm 3^\circ\text{C}$  in continuous light supplied with fluorescent tubes for 4–5 days. For analysis of biochemical parameters, excised primary leaves from light-grown maize seedlings were treated with different concentrations of sorbitol in continuous light supplied with fluorescent tubes for 24 h at  $25 \pm 3^\circ\text{C}$ . Leaf segments were thoroughly washed with distilled water prior to analysis.

**Estimations and assay.** For estimation of pigment content, leaf tissue was extracted with 80% acetone in cold. The extract was centrifuged and the absorbance of clear supernatant was measured at 646, 663 and 470 nm. The Chl a, Chl b and carotenoid content was calculated in  $\mu\text{g/ml}$  using equation of Linchtenthaler and Welburn (1983). Protein content was estimated with Folin Ciocalteu reagent according to the method of Lowry et al. (1951). Proline content was estimated as described by Bates et al. (1973). Total RNA was extracted and estimated by the method of Webb and Levy (1958) using orcinol reagent. In vivo NRA was assayed by estimating the amount of nitrite produced and secreted under dark anaerobic conditions (Srivastava 1974).

The data presented are the average values of at least three replicate experiments with  $\pm$  S.E. Student's *t*-test was applied to test the significance of difference obtained for various treatments.

Figure 1. Effect of different concentrations of sorbitol on germination rate in maize seedlings



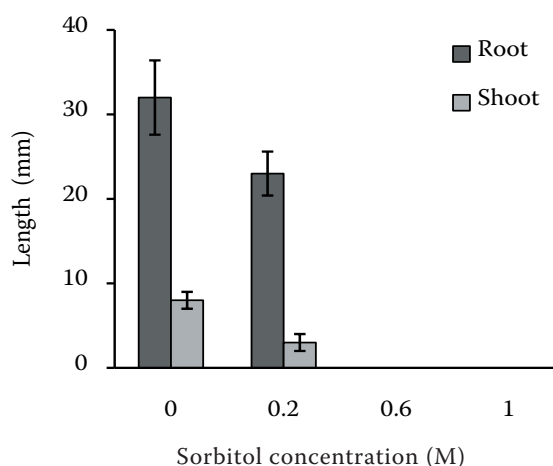
Maize seeds were grown on filter paper sheets in Petri plates treated with different concentrations of sorbitol. Distilled water was used as control. Germination rate was calculated on day 3. The plates were incubated at  $25 \pm 3^\circ\text{C}$  in continuous light for 3 days

## RESULTS AND DISCUSSION

The results of the present investigation demonstrate the decrease in growth parameters of maize seedlings due to osmotic stress imposed by sorbitol. Thus, the treatment of maize seeds with 0.6M and 1.0M sorbitol completely suppressed the germination rate; however, there was 25% reduction in the germination rate at 0.2M sorbitol (Figure 1). Similar findings involving a marked decrease in germination due to NaCl and draught treatment were reported earlier in *Sorghum bicolor* (L.) Moench seeds (Sharma et al. 2004). Other growth parameters affected by osmotic stress are root and shoot lengths with the shoot length being decreased more than the root length (Figure 2). Incubation of leaf segments with different concentrations of sorbitol decreased the fresh weight in a concentration-dependent manner, but increased the dry weight (Figure 3). An increase in dry weight of leaf segments may be due to accumulation of sorbitol. It has been found that unlike polyethelene glycol, sorbitol enters the cell wall space (Flores and Galston 1984).

Photosynthetic pigments determine the physiological status of the plants. A decrease in total chlorophyll and carotenoid contents has been shown in the seedling of Lentil genotypes with the rise in the level of salt stress (Singh et al. 2004). In the present study, pigment analysis demonstrates a concentration-dependent reduction in total chlorophylls, chlorophyll 'a' and chlorophyll 'b' in maize leaf segments subjected to osmotic

Figure 2. Effect of different concentrations of sorbitol on root and shoot length in maize seedlings



Maize seeds were grown on filter paper sheets in Petri plates treated with different concentrations of sorbitol. Distilled water was used as control. Root and shoot lengths were calculated on day 5. The plates were incubated at  $25 \pm 3^\circ\text{C}$  in continuous light for 6 days

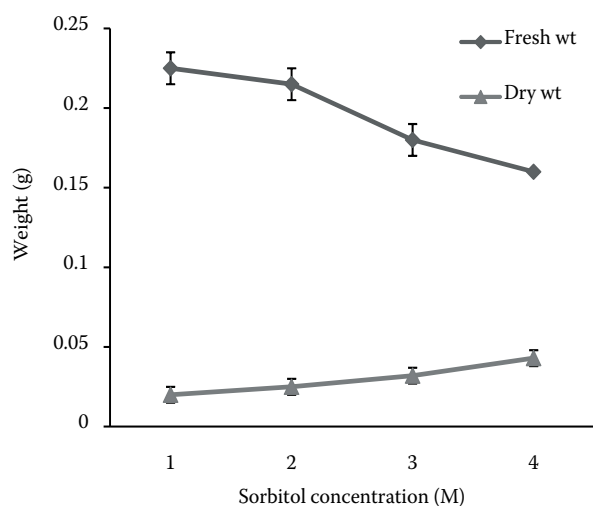


Figure 3. Effect of different concentrations of sorbitol on fresh weight and dry weight. Leaf segments were floated on distilled water and different concentrations of sorbitol for 24 h in continuous light intensity of 30 W/m<sup>2</sup> at 25 ± 3°C. Prior to the treatment, weight of the leaf segments was 0.2 g

stress (Table 1A and 1B). Due to a higher reduction in chlorophyll 'b' than in chlorophyll 'a' at all concentrations of sorbitol, chl a/b ratio was found to be higher. Hence, structural changes of the photosynthetic apparatus in leaves are likely, which may include loss of chloroplast membranes,

excessive swelling, and distortion of the lamellae vesiculation and appearance of lipid droplets (Kaiser et al. 1981).

Experimental stress could evoke compensatory metabolic changes through modification and modulation of the quantity and quality of proteins (Ramagopal 1987). A concentration-dependent decrease in protein content under osmotic stress in the present study (Table 2) could be due to enhanced proteolysis and decreased protein synthesis, respectively, which decreased the total amount of biosynthesized proteins. High protease activity of water-stressed maize plants compared to control plants was reported by Thakur and Thakur (1987). Reduction in RNA content was also observed with increasing concentration of sorbitol (Table 2). Reduced RNA synthesis with increased water stress was previously reported by He et al. (1999) in wheat leaves; they also suggested upregulation of chloroplast RNAase as one of the possible reason for the degradation of RNA. Furthermore, many researchers showed that the ribosomes and the proportion of polyribosomes decreased remarkably during water stress (Scott et al. 1979, Mason et al. 1988).

Stress imposition by sorbitol resulted in an increased proline level (Figure 4). There was more than four-fold rise in proline content at the highest concentration of sorbitol used. In general, water

Table 1A. Effect of different concentrations of sorbitol on total chlorophylls and carotenoids in maize leaf segments

Sorbitol concentration (M)	Total chlorophylls (µg/ml)	Carotenoids (µg/ml)
0.0	8.6 ± 1.42 (100)	1.12 ± 0.13 (100)
0.2	7.08 ± 1.23 (82)	1.09 ± 0.17 (97)
0.6	6.88 ± 0.96 (80)	1.08 ± 0.10 (97)
1.0	6.17 ± 0.69 (72)	1.05 ± 0.11 (94)

Leaf segments were floated on different concentrations of sorbitol for 24 h in continuous light intensity of 30 W/m<sup>2</sup> at 25 ± 3°C. Values relative to control are given in parentheses

Table 1B. Effect of different concentrations of sorbitol on chlorophyll a, chlorophyll b and chlorophyll a/b ratio in maize leaf segments

Sorbitol conc. (M)	Chl a (µg/ml)	Chl b (µg/ml)	Chl a/b ratio
0.0	5.69 ± 0.95 (100)	2.91 ± 0.47 (100)	1.95 ± 2.01 (100)
0.2	5.02 ± 0.92 (88)	2.06 ± 0.31 (71)	2.43 ± 2.96 (125)
0.6	4.86 ± 0.71 (85)	2.02 ± 0.25 (69)	2.40 ± 2.84 (123)
1.0	4.20 ± 0.47 (74)	1.97 ± 0.22 <sup>s</sup> (68)	2.13 ± 2.13 (109)

s = P < 0.05; same as in Table 1

Table 2. Effect of different concentrations of sorbitol on total protein, total RNA and NRA in maize leaf segments

Sorbitol conc. (M)	Total protein (mg/g FW)	Total RNA (mg/g FW)	NRA (nmoles NO <sub>2</sub> formed/h/g FW)
0.0	8.58 ± 0.30 (100)	10.42 ± 0.49 (100)	310.86 ± 32.64 (100)
0.2	6.21 ± 0.20 <sup>S</sup> (72)	7.53 ± 0.35 <sup>S</sup> (72)	554.82 ± 50.36 <sup>S</sup> (178)
0.6	4.64 ± 0.15 <sup>S</sup> (50)	5.26 ± 0.19 <sup>S</sup> (50)	924.92 ± 68.8 <sup>S</sup> (297)
1.0	3.22 ± 0.15 <sup>S</sup> (38)	4.47 ± 0.16 <sup>S</sup> (43)	463.91 ± 54.99 (149)

Same as in Table 1

stress has been known to increase free proline in the leaves (Stewart et al. 1966). Further, enhanced proteolysis has been shown to increase proline levels under osmotic stress (Pandey et al. 2004). Plant growing in stress conditions (for example osmotic stress) need to produce specific proteins having higher proline content (hydroxyproline-rich glycopeptide or proline-rich glycopeptide) (Battaglia et al. 2006, Ueda et al. 2007). The high content of free proline inhibits proline biosynthesis in plants growing under heavy metal contamination (Štefl and Vašáková 1982). Effect of this change is subsequently contributed to higher production of glutamic acid, which stressed plants (stress by heavy metals) need to create phytochelatins (Pavlíková et al. 2007, 2008).

Nitrate reductase, a key enzyme of nitrate assimilation, is correlated with the overall nitrogen status of the system. Decline in NRA due to water deficit was reported in several systems (Larson et al 1989, Foyer et al. 1998, Bertamini et al. 2006). However, in this study when sorbitol was supplied to the leaf segments, *in vivo* NRA was stimulated at all the concentrations with the increase being more at 0.6M sorbitol (Table 2). An increase in NO level

as well as NRA due to supply of PEG has been observed in pea and wheat roots (Kolbert et al. 2005). This suggests that higher NRA increases the NO level under osmotic stress. The results demonstrate an inhibitory effect of sorbitol-induced stress on overall growth and photosynthetic pigments in maize; to overcome the stress possible ways may involve osmolyte and/or NO accumulation.

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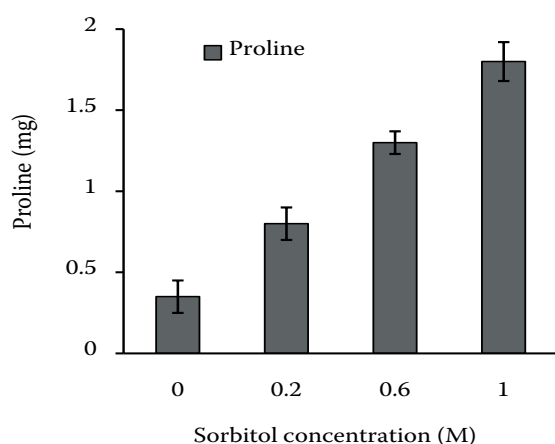


Figure 4. Effect of different concentrations of sorbitol on proline content in maize leaf segments. Leaf segments were floated on distilled water and different concentrations of sorbitol for 24 h in continuous light of intensity of 30 W/m at 25 ± 3°C

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