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Enhanced tolerance of transgenic sorghum expressing *mtlD* gene to water-deficit stress

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Transgenic sorghum (T_4) lines with mannitol-1-phosphate dehydrogenase (*mtlD*) gene derived from *Escherichia coli* were evaluated for water-deficit stress tolerance in pot culture under greenhouse conditions. Leaf water relations, accumulation of total soluble sugars, membrane stability, antioxidative enzyme activity, yield and yield related parameters were analyzed in the plants subjected to well-watered and water-deficit stress conditions. Water-deficit stress caused a reduction in the soil moisture content resulting in visual symptoms of leaf rolling. Transgenics maintained relatively higher relative water content (RWC) than the untransformed control 15 d after withholding water. The transgenics accumulated more total soluble sugars when compared to untransformed control. Also the transgenics showed lower malondialdehyde (MDA) content and increased superoxide dismutase (SOD) activity. All these traits together contributed to the better performance of *mtlD* transgenics compared to untransformed control. Further, the percent decrease in total biomass and grain yield under water-deficit stress was lower in transgenics compared to control. Most importantly the root biomass was found to be significantly higher in the transgenics when compared to untransformed control. Among the transgenics, L2, L5 and L75 were found to be superior in terms of various physiological traits, such as, RWC, accumulation of total soluble sugars and SOD activity related to drought tolerance compared to control and other *mtlD* transgenic lines, L3, L4 and L72.

Keywords: *mtlD*, physiological evaluation, sorghum, water-deficit stress, yield

Introduction

Water-deficit is one of the major abiotic stresses affecting plant growth and crop productivity world over. Consequently, the need to develop varieties with enhanced tolerance is of great importance. It is well known that water-deficit disrupts cellular structures and impairs key physiological functions. In the past few decades genetic engineering has emerged as one potential approach to conventional crop breeding for enhancing abiotic stress tolerance[1].

Metabolic engineering of compatible solute synthesis pathways could be one of the strategies for enhancing the abiotic stress tolerance of plants. Expression/ overexpression of genes for sugar alcohols, such as mannitol, can improve tolerance to water-deficits through osmoregulation, free radical scavenging and stabilization of macromolecular structures[2,3]. Expression of bacterial mannitol-1-phosphate dehydrogenase (*mtlD*) gene for accumulation of mannitol has been one of the successful transgenic approaches for enhanced tolerance to water-deficit and salt stress in tobacco[4,5], Arabidopsis[6], rice[7], egg-plant[8], wheat[9] and sorghum[10].

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The transgenic crop plants overexpressing the genes of interest were, in most cases, tested under controlled conditions in the laboratory. Moreover, most of these studies revealed stress tolerance and/or survival, only very few were on effects of different stress conditions on plant productivity[11].

In the present study, physiological evaluation of transgenic sorghum lines with the *mtlD* gene derived from *Escherichia coli* [10] was carried out. The lines selected in this communication had previously been demonstrated to be tolerant to water-deficit and salinity. These transgenic lines have been evaluated for water-deficit stress tolerance in terms of leaf water relations, accumulation of metabolites, membrane stability and antioxidative enzyme activity under transgenic glasshouse conditions.

Materials and Methods

Plant Material

Six transgenic events of sorghum with *mtlD* gene, L2, L3, L4, L5, L72 and L75, which were positive as indicated by all the molecular tests and segregated in 3:1 ratio were selfed and advanced upto T_4 generation. Three seeds of each transgenic line as well as untransformed control were sown in 30× 30 cm^2 plastic pots, filled with 16 kg of sandy loamy soil

(soil, sand & farm yard manure in a ratio of 3:1:1) in a transgenic glasshouse (Category II-2 containment). A basal dose of 0.54 g per pot, both N and P were supplied before sowing. There were three replications and two treatments, well-watered and water-deficit stress. The plants were irrigated with normal tap water and grown under natural illumination (~1500 µmol m^{-2} s^{-1}) with a 14 h photoperiod. The mean air temperature during the experiment was 30°C and the relative humidity ranged between 45-55% at 14.00 h. Water-deficit was imposed at 40^{th} d after sowing by withholding irrigation for a period of 15 d. The following physiological measurements were assessed in the control and water-deficit stress plants.

Physiological Measurements

All the physiological measurements were carried out in the fully expanded third leaf. Relative water content (RWC) was determined according to Barrs and Weatherly[12].

$$RWC(\%) = [(FW - DW)/(TW - DW)]x100$$

FW is the fresh wt, TW is the turgid wt after rehydration and DW is the dry wt after oven drying. Leaf water potential (ψL) was measured using a pressure bomb apparatus according to Scholander *et al*[13] immediately after excision of the leaf from the plant. Excised leaf water retention capacity (ELWRC) curves were drawn to record the moisture percent actually retained by the leaf after it was excised from the plant according to Rao *et al*[14]. Accumulation of total soluble sugars was estimated using the method of Dubois *et al*[15] in the leaf samples fixed in 80% ethanol. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content according to Heath and Packer[16]. The absorbance of MDA content was recorded at 532 nm and the nonspecific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 mM/cm and expressed as µmol/g dry wt. Superoxide dismutase (SOD) activity was assayed by homogenizing the leaf tissue in 100 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for the *in vitro* assay of enzyme activity according to Dhindsa *et al*[17]. The activity was expressed as units/mg protein/min. One unit of SOD activity is defined as quantity of enzyme required to cause 50% inhibition.

Total biomass and grain yield of all the genotypes under well-watered and water-deficit stress treatments were recorded at harvest and expressed as g/plant. The root growth of both the transgenics and untransformed control were recorded. Standard statistical procedures were followed according to Snedecor and Cochran[18].

Results

Water-deficit stress for 2 wk caused leaf rolling in the water-deficit stress pots. At this point, the soil moisture content in the well-watered pots ranged between 19-20%, while in the water-deficit stress pots it ranged from 7.4-9.4% (Table 1).

Under water-deficit stress, although the soil moisture content was reduced by >50%, the transgenics maintained significantly higher relative water content when compared to untransformed control. Among the transgenics L75, L5 and L2 maintained relatively higher RWC content (>80%) when compared to untransformed control (62%). Under well-watered conditions the RWC varied between 90.1-94.6%. All the transgenics maintained relatively higher *ψ*L (−1.8, −2.0 MPa) when compared to untransformed control (−2.2 MPa). Significant genotype (G) × treatment (T) interaction was observed (Table 1). Significant differences in water holding capacity of excised leaves among the trangenics and untransformed control (Fig. 1) were revealed by ELWRC. All the transgenics retained higher water content (> 80%) when compared to untransformed control (78%) after excision from the plant.

Table 1 — Soil moisture, relative water content and leaf water potential of the sorghum *mtlD* transgenics and untransformed control (UC) subjected to water-deficit stress

Genotype	Soil moisture (%)		Relative water content (%)		Leaf Water potential (MPa)	
	WW	WDS	WW	WDS	WW	WDS
UC	19.7	8.1	91.3	62.0	-1.4	-2.2
L2	19.5	7.4	92.5	81.3	-1.4	-2.0
L3	20.3	7.8	91.2	71.0	-1.5	-2.0
L4	19.5	8.7	90.8	72.5	-1.6	-2.0
L5	20.7	7.4	90.5	88.9	-1.5	-1.8
L72	20.3	7.4	90.1	68.0	-1.4	-2.1
L75	20.6	9.4	94.6	88.4	-1.5	-1.9
CD at 0.05% Genotype (G)		NS		2.327		0.074
Treatment(T)		0.486		1.244		0.040
G × T		NS		3.291		0.105

WW: Well-watered; WDS: Water-deficit stress

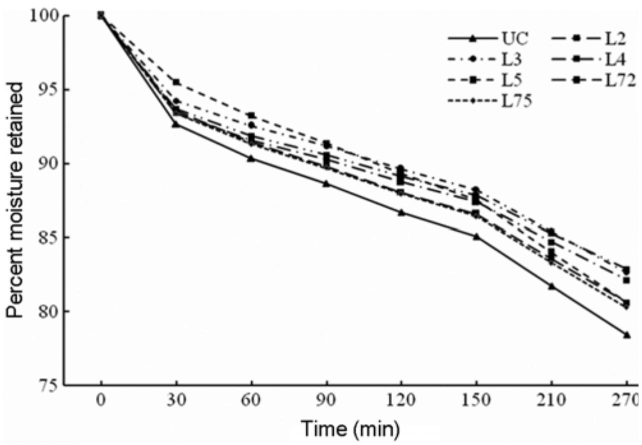

Fig. 1 — Excised leaf water retention capacity of the sorghum *mtlD* transgenic lines and untransformed control (UC).

Under water-deficit stress, the accumulation of total soluble sugars in all the transgenics was significantly increased when compared to untransformed control (Fig. 2A). Among the transgenics, L75 and L5 recorded 50% more total soluble sugars as compared to untransformed control. The transgenics maintained relatively better membrane stability than the untransformed control, which is indicated by their significantly lower MDA content (Fig. 2B). Among the transgenics, L75, L5 and L2 showed relatively better membrane stability than the other transgenics. The SOD enzyme activity was found to be significantly increased in transgenic plants as compared to untransformed control under water-deficit stress. The relative increase in SOD activity under stress ranged between 15-26% in transgenics as compared to 7.82% in untransformed control plants (Fig. 2C).

Water-deficit stress resulted in reduction of total biomass and grain yield in both untransformed control and transgenics compared to that under well-watered conditions (Table 2). Among the transgenics, L2, L5 and L75, the percent reduction in total biomass (16.26-19.27%) and grain yield (32.73-37.34%) were significantly lower compared to the reduction in total biomass (25.78%) and grain yield (45.95%) in untransformed control. Also, the root biomass was significantly increased in the transgenics in comparison to untransformed control (Fig. 3).

Discussion

The present paper describes the evaluation of whole plant transgenic *mtlD* sorghum lines to water-deficit stress in transgenic glasshouse facility. Development of *mtlD* transgenics in sorghum and

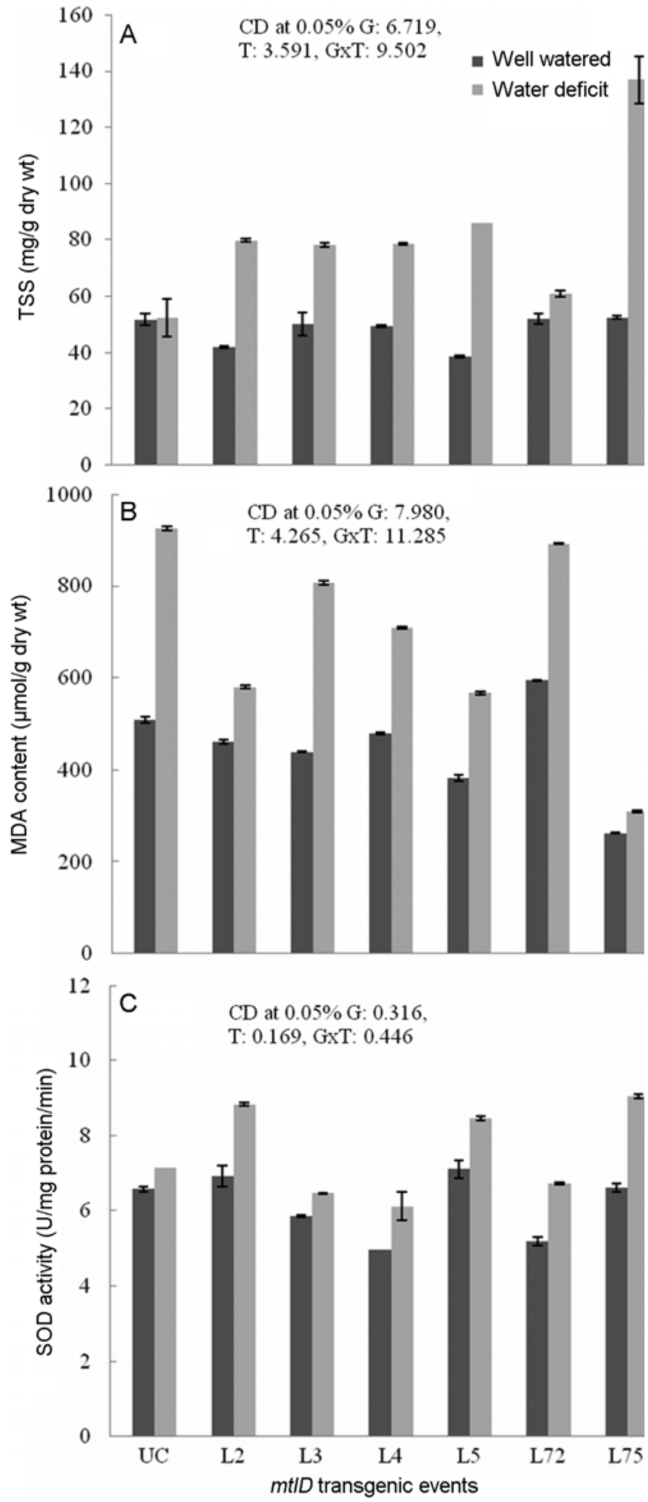

Fig. 2 (A-C) — Effect of water-deficit stress on: (A) Accumulation of total soluble sugars (TSS); (B) Malondialdehyde (MDA) content; & (C) Superoxide dismutase (SOD) activity, of the sorghum *mtlD* transgenics and untransformed control (UC).

their relative tolerance to water-deficit and salinity based on seed germination and leaf discs assay have

Table 2 — Effect of water-deficit stress on total biomass and grain yield of the sorghum *mtlD* transgenics and untransformed control (UC)

Genotype	Total biomass (g)		No. of grains		Grain wt (g)	
	WW	WDS	WW	WDS	WW	WDS
UC	83.4	62.0	1529	867	44.4	24.0
L2	91.4	72.5	1849	1298	47.8	30.5
L3	88.7	64.7	1655	1087	50.3	31.5
L4	92.9	77.8	1677	1024	48.8	28.0
L5	94.0	74.5	1957	1430	54.2	35.4
L72	91.9	75.0	1650	968	51.2	31.7
L75	85.9	69.4	1665	1243	49.9	33.6
CD at 0.05% Genotype (G)		1.719		15.031		3.967
Treatment (T)		0.919		8.034		2.121
G × T		2.430		21.257		NS

WW: Well-watered; WDS: Water-deficit stress

Fig. 3 — Root system at harvest of sorghum *mtlD* transgenics and untransformed control (UC).

been described previously[10]. Water was withheld such that the soil moisture content under stressed treatment was about 60% of the control pots. In general, the transgenics maintained higher relative water content than the untransformed control. The ability to withstand water-deficit stress depends on maintaining plant water status, accumulation of metabolites and membrane stability. Among the transgenics, L75, L5 and L2 maintainted higher relative water content compared to other transgenics. However, differences in leaf water potential were of much lower magnitude probably because the measurement was made of bulk leaf water potential. Similar to relative water content, leaf water potential was also higher in case of L5 and L75. The ELWRC of all the transgenics was higher when compared to untransformed control. A positive association of ELWRC with osmotic adjustment has been described in several rainfed crops, such as, sorghum[19], castor[20] and pearl millet[21]. Maintenance of higher osmotic adjustment could have contributed to the maintenance of higher moisture content even after excision. The ELWRC could also be attributed to the quick stomatal response to excision[14]. Increased root growth might have contributed to the better extraction of water from the soil. Also, in the controlled experiments on the validation of transgenic events, increased root growth has been observed[10]. In other crops, such as, tobacco, eggplant and wheat, *mtlD* transgenics have shown increased root growth[4,5,8,9].

In this context, the higher accumulation of total soluble sugars in all the transgenics under water-deficit stress is of significance as significant correlations are known to exist between accumulation of total soluble sugars and water-deficit stress tolerance[22]. Among the transgenics, L75 and L5 had higher total soluble sugars accumulation and better water-deficit stress tolerance in terms of leaf water relations.

The increase in MDA content, which is an indicator of extent of membrane lipid peroxidation, was relatively lower in transgenics compared to untransformed control. This supports the hypothesis that enhanced tolerance to water-deficit due to metabolic engineering with *mtlD* gene can be attributed more to the role of mannitol as a free radical scavenger[23] than to its contribution in osmotic adjustment[4,5]. Further, enhanced tolerance to chilling and salinity stresses was also reported to be attributed to maintenance of membrane stability in the *mtlD* transgenics of Petunia[24] and Populus[25], respectively. SOD enzyme activity of transgenic plants L75, L5 and L2 was found to increase as compared to untransformed control. The enhancement of SOD activity in the transgenics under water-deficit stress also suggested better free radical scavenging activity of *mtlD* transgenics.

It is interesting to find that the better performance of the transgenics in terms of various physiological parameters as described above was also reflected in terms of biomass and grain yield. Reduction in both grain number and grain wt was much lower in transgenics as compared to untransformed control under water-deficit stress. The transgenic lines exhibited variations in terms of the relative performance, which may be attributed to the differential expression of the transgene[8].

In summary, the reduction in grain yield under water-deficit was much lower in *mtlD* transgenics as compared to untransformed control, which seemed to be due to maintenance of leaf water relations, membrane stability, root growth and enhanced antioxidant activity.

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