ENZYMATIC STUDIES IN SORGHUM CULTIVARS UNDER PEG INDUCED WATER STRESS

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SUMMARY

The sorghum (*Sorghum bicolor*) cultivars M 35-1, Phule Maulee (RSLG-262), Phule Yashoda, Sel-3, Swati 108 and CSH-15 R were screened for drought tolerance under PEG-6000 induced water stress from –2 to –8 bars, on the basis of activities of nitrate reductase, peroxidase and polyphenol oxidase. It was noted that the cultivars CSH-15 R and RSLG-262 were drought tolerant as compared to Phule Yashoda and Swati 108. Among the cultivars studied, Sel-3 and M 35-1 were found to be susceptible to water stress at seedling stage. The study indicated that enzymes like peroxidase and polyphenol oxidase along with nitrate reductase may serve as the indicators for testing the water stress tolerance and may be useful for sorghum breeders to evolve drought tolerant cultivars.

***Key words*: Nitrate reductase, peroxidase, polyphenol oxidase, sorghum, water stress.**

INTRODUCTION

Water stress not only affects cell water potential, but also induces closure of stomata, decrease in photosynthesis, nitrate assimilation and various anabolic reactions (Sairam *et al.*1990, Sairam 1994). Water is a solvent for translocation of minerals and metabolites with in plant and is essential for cell growth and enlargement. Reduction in soil moisture affects plant metabolism, resulting in reduced growth, principally due to development of low (greater –ve values) osmotic pressure in the roots and leaves. Water stress generally triggers many metabolic changes and results in synthesis or accumulation of various stress metabolites in plants. Water stress induces generation of active oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical (Gamble and Burke 1984, Gillham and Dodge 1987, Cadenas 1989) causing lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Fridovich 1986, Liebler *et al.* 1986, Davies 1987, Imlay and Linn 1988, Arora *et al.* 2002). The present study deals with alterations in enzymatic activities due to water stress in sorghum cultivars so as to screen the cultivars for drought tolerance.

MATERIALS AND METHODS

The seeds of six sorghum (*Sorghum bicolor*) genotypes were obtained from Sorghum Improvement Project, MPKV Rahuri, Dist. Ahmednagar. Seeds were surface sterilized with 0.1 per cent $HgCl_2$ for 5 min, and after washing with distilled water, soaked in water for 12 hours. Fifty well-imbibed seeds were sown in eartern pots (20 x 30 x 20 cm.) containing garden soil and FYM (3:1). Seedlings were grown under natural condition. Fifteen days old seedlings were cut just above the soil surface and were subjected to water stress by dipping them in 50 ml PEG-6000 solutions of –2, -4, -6 and –8 bar osmotic potential for 4 hours. Seedlings dipped in 50

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ml distilled water served as control. Leaves from stressed and control seedlings were harvested immediately after stress treatment and used for enzymatic analysis. The *in vivo* anaerobic nitrate reductase assay was carried out according to the method of Sawhney *et al.* (1978).The peroxidase and polyphenol oxidase activities were assayed by the method of Vidyasekharan and Durairaj (1973).

RESULTS AND DISCUSSION

The data recorded in Table 1 shows that the increase in water stress induced the decrease in the nitrate reductase activity (NRA). The mean NRA actvity was 2.463 µmol NO_2^- g^{-1} fw hr^{-1} in control, while it was 1.193 µmol NO_2^- g^{-1} fw hr^{-1} at –8 bar. The decrease in nitrate reductase activity during water stress may be due to the inactivation of the enzyme as was reported for wheat seedlings (Plaut 1974).

There was significant variation in nitrate reductase activity in sorghum cultivars due to water stress. The mean activity ranged from 0.560 to 4.726 (µmol NO_2^- g^{-1} fw hr^{-1}) in different sorghum cultivars under water stress. The sorghum cultivar Swati-108 was found significantly superior over the other sorghum cultivars for nitrate reductase activity. The mean NRA in Swati-108 was significantly higher (4.726 µmol NO_2^- g^{-1} fw hr^{-1}) followed by Phule Yashoda (2.060 µmol NO_2^- g^{-1} fw hr^{-1}) and Sel-3 (1.814 µmol NO_2^- g^{-1} fw hr^{-1}). However, NRA was significantly low in sorghum cultivar RSLG-262 (0.560 µmol NO_2^- g^{-1} fw hr^{-1}) and M 35-1 (0.566 µmol NO_2^- g^{-1} fw hr^{-1}). These observations indicated that the sorghum cultivars RSLG-262 and M 35-1 had comparatively less reduction in nitrate reductase activity, which is an indication of drought tolerance in these cultivars.

The sorghum cultivars RSLG-262 and M 35-1 showed stability in nitrate reductase activity to various water stress treatments, which may serve as a reliable biochemical indicator for screening for drought tolerance. As proposed by Sairam and Dubey (1984), Foyer et *al.* (1998) and Bardzik *et al.* (1971) observed that in maize leaves the NR activity declined rapidly in response to drought.

The response of sorghum to water stress with respect to peroxidase activity indicated that the activity was stimulated significantly with the increase in water

Table 1. Effect of water stress on nitrate reductase activity (µmol NO_2^- g^{-1} fw h^{-1}) in sorghum leaves

Cultivars	Stress levels (bars)					
	Control	-2	-4	-6	-8	Mean
Sel-3	2.720	2.080	1.810	1.390	1.070	1.814
Phule Yashoda	3.150	2.350	2.240	1.440	1.120	2.060
CSH-15 R	1.440	1.130	0.890	0.640	0.540	0.928
Swati-108	5.870	5.120	4.750	4.160	3.730	4.726
RSLG-262	0.800	0.640	0.540	0.490	0.330	0.560
M 35-1	0.800	0.750	0.480	0.430	0.370	0.566
Mean	**2.463**	**2.012**	**1.785**	**1.425**	**1.193**	
			SEm±	CD 5%		
Cultivars			0.021	0.063		
Water Stress			0.019	0.055		
Cultivars × Water Stress			0.047	0.135		

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stress (Table 2). Peroxidase activity was significantly lower in Swati-108 followed by Sel-3 and Phule Yashoda. However it was significantly higher in CSH-15 R followed by RSLG-262 and M 35-1. These results are in conformity with the observations of Jagtap and Bhargava (1995) and Kadlag (1997) for water stressed sorghum. Similar was the observation of Asharf *et al.* (1994).

Water stress induced the stimulation in peroxidase activity was more pronounced in drought resistant cultivars. Increase in peroxidose activity has also been reported by Srivastava *et al.* (1995), Jha and Singh (1997) and Li *et al.* (2003) in tomato, rice and liquorice respectively under PEG induced water stress. Sairam *et al.* (1997) also noted increase in peroxidase activity in three wheat genotypes differing in their sensitivity to moisture and or temperature stress.

The data on polyphenol oxidase activity in sorghum cultivars as influenced by PEG induced water stress (Table 3) revealed that the activity of polyphenol oxidase was significantly low in Sel-3, but it was very high in CSH-15 R. The activity in remaining cultivars such as Phule Yashoda, Swati-108, RSLG-262 and M 35-1 did not differ significantly with each other. The increase in water stress caused significant increase in polyphenol oxidase activity. The increase was more in drought tolerant cultivars than the susceptible ones. The increase in polyphenol oxidase and peroxidase activities have also been reported in several plant species subjected to various abiotic stress conditions (Subhashini and Reddy 1990).

Venkatsan and Chellappan (1999) suggested that the increase in polyphenol oxidase activity under stress may indicate the rate of oxidation and degradation of phenolic compounds. The phenols on oxidation get converted in to quinones, which in fact induces resistance to biotic and abiotic stress. Dwivedi (1990) suggested that the increased activity of polyphenol oxidase might be due to increased synthesis of polyphenols under stress condition.

The present investigation on the impact of water stress on various enzymatic changes in cultivars of sorghum clearly revealed that there is an association between the degree of water stress and the level of the activities of NRA, POX and PPO. The response to water stress (resistance and susceptibility) by these cultivars mainly depends on adaptive physiological / biochemical mechanisms. The results of present study indicated that the level of changes in NR, POX and PPO activities may serve as the drought tolerance screening parameters.

Table 2. Effect of water stress on peroxidase activity ($\triangle A_{470}$ min^{-1} mg^{-1} protein) in sorghum leaves

Cultivars	Stress levels (bars)					
	Control	**-2**	**-4**	**-6**	**-8**	**Mean**
Sel-3	1.000	1.370	1.440	1.970	1.980	1.552
Phule Yashoda	1.230	1.380	1.460	1.860	2.130	1.612
CSH-15 R	2.600	2.940	4.227	6.730	7.710	4.841
Swati-108	0.710	0.800	0.850	0.920	0.930	0.842
RSLG-262	2.190	2.470	2.510	2.570	2.680	2.484
M 35-1	1.640	2.230	2.250	2.270	2.300	2.138
Mean	**1.562**	**1.865**	**2.123**	**2.720**	**2.955**	
			SEm±	CD 5%		
Cultivars			0.021	0.063		
Water Stress			0.019	0.055		
Cultivars × Water Stress			0.047	0.135		

Table 3. Effect of water stress on polyphenol oxidase activity ($\triangle A_{495}$ min^{-1} mg^{-1} protein) in sorghum leaves

Cultivars	Stress levels (bars)					
	Control	-2	-4	-6	-8	Mean
Sel-3	0.030	0.050	0.070	0.090	0.110	0.070
Phule Yashoda	0.061	0.090	0.110	0.120	0.140	0.104
CSH-15 R	0.073	0.120	0.130	0.161	0.180	0.133
Swati 108	0.060	0.080	0.100	0.130	0.153	0.105
RSLG-262	0.090	0.110	0.121	0.150	0.171	0.109
M 35-1	0.071	0.090	0.100	0.120	0.140	0.104
Mean	**0.064**	**0.074**	**0.105**	**0.128**	**0.149**	

	SEm±	CD 5%
Cultivars	0.003	0.009
Water Stress	0.003	0.008
Cultivars × Water Stress	0.007	0.021

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