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SN Jadhav
Department of Biochemistry, M.P.K.V, Rahuri Dist. Ahmednagar, Maharashtra, India

RM Naik
Department of Biochemistry, M.P.K.V, Rahuri Dist. Ahmednagar, Maharashtra, India

US Dalvi
Department of Biochemistry, M.P.K.V, Rahuri Dist. Ahmednagar, Maharashtra, India

Correspondence
US Dalvi
Department of Biochemistry, M.P.K.V, Rahuri Dist. Ahmednagar, Maharashtra, India

Evaluation of parents and segregating population of rabi sorghum for drought tolerance using biochemical markers

SN Jadhav, RM Naik and US Dalvi

Abstract
Laboratory and pot culture experiments were conducted to evaluate the levels of osmoprotectant such as proline, glycine betaine, activity of P_5CS and soluble proteins in the leaves of five drought tolerant and five drought susceptible cultivars with segregating crosses of rabi sorghum by the imposed stress created by withholding irrigation as well as by osmotic stress created by PEG-6000. Imposition of osmotic stress was found to increase the mean proline accumulation in leaves from 42.24 to 156.04 µmoles g^{-1} fr. wt., an increase of 3.73 folds in tolerant types. Under similar conditions, the mean proline accumulation was found to range from 61.21 to 128.26µmoles g^{-1} fr. wt. an increase of 2.15 folds in susceptible types. The crosses involving drought tolerant parents *viz* CSV 216(DS)x RSV 458(DR) and SPV1502(DS) x RSV 458 (DT) led to increased proline accumulation as evident from higher fold increase. It thus appears that the exploitation of drought tolerant male parent in breeding for drought tolerance can further improve higher proline accumulation and thus endow the plant with improved drought tolerance performance. The mean P_5CS activity was found to increase from 1.55 to 4.01 µmoles of γ -glutamyl hydroxamate formed g^{-1} tissue hr-1, an increase of 2.66 fold in tolerant type while it was found to increase from 1.67 to3.01 µ moles of γ- glutamyl hydroxamate formed g^{-1} tissue hr^{-1},an increase of 1.80 -fold in susceptible cultivars. Similarly, other osmolyte glycine betaine accumulation was found to be an increase of 3.50 -fold in tolerant cultivars and an increase of 1.84-fold in susceptible cultivars of sorghum under stress created by withholding irrigation.

Keywords: Stress, proline, glycine betaine, osmolyte, tolerant

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a major cereal during *kharif* and *rabi* seasons in India. It is a dual purpose crop providing staple food for human consumption and fodder for livestock (Salunkhe *et al*., 1984) [1]. Sorghum in Maharashtra occupies an area of about 4618 thousand ha with the annual production of 3772 thousand tones and the average productivity of 816 kg/ha (Anonymous, 2007-08) [2]. Drought is perhaps the most important abiotic factor limiting crop productivity around the world and has significance in semi-arid tropics, where rainfall is generally scanty and its distribution is erratic.

Plants have evolved an array of strategies to cope with various abiotic stresses. One common mechanism is the accumulation of compatible solutes of low molecular weight, highly soluble compounds that are nontoxic at high concentrations. The accumulation of compatible solutes differs among plant species can include betaines and related compounds such as polyols and trehalose and an amino acid such as proline (Rhodes and Hanson, 1993 and McNeil *et al.*, 1999) [3, 4]. Two pairs of near isogenic lines with contrasting glycine betaine levels within pairs were developed from the population and stable inheritance of the glycine betaine phenotype and NIL isogenecity were confirmed with progeny test (Mickelbart *et al.*, 2003) [5]. Natural osmoprotectant concentrations in cytoplasmic compartments are osmotically significant as they have pivotal roles in maintaining cell turgor and the driving gradient for water uptake under stress (Balibrea *et al.*, 2000 and Rontein *et al.*, 2002) [6, 7]. Higher accumulation of proline has been reported in seedlings of forage sorghum at various levels of osmotic stress induced by polyethylene glycol (PEG) (Jafar *et al.*, 2004) [8]. Yamada *et al.* (2005) [9] studied the effect of free proline accumulation in Petunias under stress. Petunia plants were transformed by P_5CS gene and this transgenic plant showed higher accumulation of proline in stress condition than plants grown under normal condition. Drought stress caused an increase in the GB content, paclobutrazol and ABA acts as stress ameliorating agents by further enhancing this parameter in water stressed *Cajanas cajan* plants (Jaleel *et al*., 2008) [10].

Activities of several enzymes have been shown to be affected by water stress in various plants (Mali and Mehta, 1977) [11] and the degree of stress tolerance has been positively correlated with the levels of certain organic solutes like proline and glycine betaine in a number of crop plants (Barnett and Naylor, 1966) [12]. High salinity of -15 dS m-1 and low water availability (-2 MPa) were increased the value of total soluble sugars by 48 and 19.3%, and the amount of free proline also by 60 and 15.1% respectively (Alirega Saberi, 2013) [13]. Genetic transformations have allowed the introduction of new pathways for the biosynthesis of various compatible solutes in plants, resulting in the production of transgenic plants with improved tolerance to stress. The identified molecular and biochemical markers could introduce a great benefit for breeding programmes to select salt tolerant individuals (Khalil Rasha, 2013) [14].

Materials and Methods

The seeds of five drought tolerant and five drought susceptible cultivars and segregating population of crosses were obtained from the Senior Sorghum Breeder, Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The clean and sound seeds of each cultivar and crosses were then germinated at 27°c in an incubator on previously sterilized 0.8% agar medium containing various levels of PEG 6000 and standardized. The seeds of these cultivars were initially allowed to germinate under controlled condition (without PEG 6000) and stress (with PEG 6000) condition in petridishes. The seeds were allowed to germinate for 7 days. The shoot portions of each cultivar were separated and the proline was extracted as per method of Bates *et al*., (1973) [15].

In a separate experiment, the seeds were imbibed in water for 24 hrs and sown in earthen pots in two sets. After germination, uniform plant population in both the sets was maintained and plants were allowed to germinate in natural daylight under glass house conditions. Water stress in one set was created by withholding seedlings for 30 days old. Leaf samples from both the unstressed and stressed seedlings were analyzed for the contents of free proline, P5CS activity, glycine betaine and soluble proteins.

The activity of D'-pyrroline-5-carboxylate synthetase was assayed according to the method of Hayzer and Leisinger (1980) [16]. Glycine betaine content in leaves of both the control and stressed seedlings was determined by using the Dragendorff reagent as per the method described by Stumpf (1984) [17]. Soluble proteins in the leaves were determined by the colorimetric method of Lowry *et al.* (1951) [18] using bovine serum albumin as a standard protein.

Results and Discussion

In the present study, attempts have been made to estimate the levels of proline, glycine betaine and the activity of P_5CS, a rate limiting enzyme in proline biosynthesis. First the effects of various levels of PEG 6000 on proline accumulation were studied. From this -0.5 Mpa level gives good proline accumulation and germination (Table 1) which is used for further osmotic stress study.

The proline content in the germinating seedlings of cultivars and crosses was determined after imposing osmotic stress of -0.5 MPa using PEG-6000. The proline content under control condition increased from 36.17 in *to* 51.32 µmol g^{-1}fr.wt in tolerant sorghum cultivars. The proline content under stressed condition increased from 121.63 in to 178.89 in tolerant sorghum cultivars. Mean fold increase under osmotic stress in drought tolerant sorghum genotypes was 3.73 against drought susceptible cultivars with 2.15 (Table 2). In a separate experiment, five each of drought tolerant, drought susceptible and segregating lines of three crosses were grown in earthen pots in two sets. After 30 days water stress was created by withholding irrigation for 6 days when the RWC values of stressed leaves were in the range of 57-65 percent as against 90-95 percent in turgid leaves.

The data in Table 3 show that the proline content in control (unstressed) plants varied from 28.60 to 32.94 with a mean value of 37.62 µmol g^{-1} fr.wt. in tolerant cultivars under water stress induced by withholding irrigation. The free proline content increased significantly under stressed condition, ranging from 87.22 to 127.86 with a mean value of 112.36 µmol g-1fr.wt. in tolerant cultivars with highest fold increase in SPV 1090 (4.22), while in drought susceptible cultivars free proline increased from 60.36 to 117.39 with a mean 91.10 µmol g-1fr.wt. under stressed conditions. The activity of P_5CS from unstressed seedlings ranged from 1.13 to 1.88 in tolerant cultivars which further increased under PEG-6000 induced stress from 3.45 to 4.75 µmol of g-glutamyl hydroxamate formed g^{-1} tissue hr^{-1} (Table 4). The drought tolerant cultivars of sorghum recorded comparatively higher fold increase in P_5CS activity than susceptible cultivars. The segregating crosses were also evaluated for P_5CS activity. The segregating cross CSV-216 x RSV-458 recorded the highest fold increase of 4.12 in P_5CS activity as against a 2.13 fold increase observed in CSV-216. Even, a 3.02 fold increase P_5CS activity was recorded in a cross SPV-1502 x RSV-458 as against 1.63 fold increase observed in a drought susceptible cultivar SPV-1502 (Table 4).

The glycine betaine content of unstressed plants in tolerant cultivars varied from 21.01 in RSV-658 to 41.68 in RSV- 458 with a mean value of 29.63 µg g^{-1} fr.wt. under water stress created by withholding irrigation. The glycine betaine content increased significantly under stressed condition ranging from 83.65 to 112.99 µg g^{-1} fr.wt. (Table 5). The highest fold increase of 4.11 was recorded in cultivar SPV-1090. The susceptible cultivars also accumulated glycine betaine under stress condition but the fold increase was from 1.15 to 2.71.The higher fold increase in crosses was again because of the influence of the higher glycine betaine accumulating male parent, RSV-458. In cross SPV-1502 x RSV 458, glycine betaine was increased from 29.03 (control) to 98.23 µg g^{-1} fr.wt. in stressed conditions with 3.77 fold increase. The soluble protein content of unstressed plants of tolerant cultivars varied from 10.20 to 20.94 with a mean value of 14.33 mg g-1 fr.wt. However, the soluble protein content in the leaves of tolerant cultivars increased significantly under stressed condition ranging from 51.70 to 75.52 mg g-^{1}fr.wt. (Table 6).

The correlation coefficients were calculated for proline x P_5CS activity, proline x glycine betaine, proline x soluble protein and P_5CS activity x soluble protein. All the correlations under stressed condition were found to be positively significant. These results thus confirm the earlier findings that the increased accumulation of proline is mainly due to fresh biosynthesis of proline under stressed conditions (Table 7).

Table 1: Effect of levels of PEG-6000 on proline accumulation

Osmotic stress (MPa)	Proline, µmol g-1 fr.wt.	Germination,%
-0.1	41.50	91.00
-0.3	62.80	83.00
-0.5	125.3	67.50
-0.7	151.0	51.00
-1.0	153.5	42.50

Table 2: Effect of Osmotic stress induced by PEG 6000 on proline accumulation in sorghum cultivars

S. No	Cultivar	µ mol of proline g-1 fr. wt.		Fold increase
		Control	**Stress**	
A)	**Tolerant cultivars**			
1	P.Maulee	36.17	138.90	3.84
2	RSV-458	51.32	164.74	3.21
3	SPV-1546	41.15	121.63	2.95
4	RSV-658	43.06	176.06	4.10
5	SPV-1090	39.49	178.89	4.53
	Range	36.17-51.32	121.63-178.89	2.95-4.53
	Mean	42.24	156.04	3.73
	SE±	1.28	2.36	
	CD 5%	3.57	7.08	
B)	**Susceptible cultivars**			
1	RSV-491	43.07	117.67	2.73
2	RSV-613	73.10	158.95	2.17
3	RSV-214	68.50	128.10	1.87
4	SPV-1502	71.05	124.34	1.75
5	CSV-216	50.33	112.24	2.23
	Range	43.07-73.10	112.24-158.95	1.75-2.73
	Mean	61.21	128.26	2.15
	SE±	2.38	3.25	
	CD 5%	6.62	9.75	
C)	**Crosses**			
1	CSV-216XRSV-458	35.77	147.41	4.12
2	SPV-1546XRSV-658	71.75	144.23	2.01
3	SPV-1502XRSV-458	59.88	129.95	2.17
	Range	35.77-71.75	129.95-147.41	2.01-4.12
	Mean	55.80	140.53	2.77
	SE±	2.10	3.15	
	CD 5%	6.30	9.45	

Table 3: Effect of water stress induced by withholding irrigation on leaf proline accumulation in sorghum.

Sr. no	Cultivar	µ mol of proline g-1 fr. wt.		Fold increase
		Control	**Stress**	
A)	**Tolerant cultivars**			
1	P.Maulee	30.34	121.65	4.00
2	RSV-458	28.60	87.22	3.04
3	SPV-1546	32.94	103.43	3.01
4	RSV-658	32.37	127.86	3.94
5	SPV-1090	28.83	121.65	4.22
	Range	28.60-32.94	87.22-127.86	3.04-4.22
	Mean	30.62	112.36	3.67
	SE±	1.15	2.32	
	CD 5%	3.45	6.96	
B)	**Susceptible cultivars**			
1	RSV-491	44.89	117.39	2.61
2	RSV-613	43.07	103.43	2.40
3	RSV-214	37.31	60.36	1.61
4	SPV-1502	50.65	91.30	1.80
5	CSV-216	39.145	83.00	2.12
	Range	37.31-50.65	60.36-117.39	1.61-2.61
	Mean	43.01	91.10	2.11
	SE±	1.25	2.12	
	CD 5%	3.75	6.36	
C)	**Crosses**			
1	CSV-216XRSV-458	47.62	119.81	2.51
2	SPV-1546XRSV-658	47.03	68.85	1.46
3	SPV-1502XRSV-458	38.90	98.83	2.54
	Range	38.90-47.62	68.85-119.81	1.46-2.54
	Mean	44.52	95.83	2.17
	SE±	1.50	3.20	
	CD 5%	4.50	9.60	

Table 4: Effect of Osmotic stress induced by PEG 6000 (-0.5 MPa) on P_5CS activity in sorghum

S. no	Cultivar	µmol of -glutamyl hydroxamate formed g-1 tissue hr-1		Fold increase
		Control	**Stress**	
A)	**Tolerant cultivars**			
1	P.Maulee	1.13	3.50	2.45
2	RSV-458	1.67	4.10	3.09
3	SPV-1546	1.40	4.75	3.39
4	RSV-658	1.88	3.45	1.83
5	SPV-1090	1.67	4.23	2.53
	Range	1.13-1.88	3.45-4.75	1.83-3.39
	Mean	1.55	4.01	2.66
	SE±	0.019	0.027	
	CD 5%	0.053	0.075	
B)	**Susceptible cultivars**			
1	RSV-491	1.33	1.72	1.29
2	RSV-613	1.34	3.18	2.37
3	RSV-214	2.26	3.61	1.59
4	SPV-1502	1.38	2.26	1.63
5	CSV-216	2.02	4.30	2.13
	Range	1.33-2.26	1.72-4.30	1.29-2.37
	Mean	1.67	3.01	1.80
	SE±	0.039	0.044	
	CD 5%	0.109	0.123	
C)	**Crosses**			
1	CSV-216XRSV-458	1.23	5.07	4.12
2	SPV-1546XRSV-658	1.74	3.18	1.82
3	SPV-1502XRSV-458	2.23	6.75	3.02
	Range	1.23-2.23	3.18-6.75	1.82-4.12
	Mean	1.73	5.00	2.99
	SE±	0.020	0.035	
	CD 5%	0.060	0.105	

Table 5: Effect of water stress created by withholding irrigation on glycine betaine accumulation in sorghum

S. no	Cultivar	Glycine betaine (µg g-1 fr.wt.)		Fold increase
		Control	**Stress**	
A)	**Tolerant cultivars**			
1	P.Maulee	29.86	109.82	3.67
2	RSV-458	41.68	112.99	2.71
3	SPV-1546	32.77	99.63	3.04
4	RSV-658	21.01	83.65	3.98
5	SPV-1090	22.84	94.09	4.11
	Range	21.01-41.68	83.65-112.99	2.71-4.11
	Mean	29.63	100.04	3.50
	SE±	1.50	2.20	
	CD 5%	4.50	6.60	
B)	**Susceptible cultivars**			
1	RSV-491	42.94	49.60	1.15
2	RSV-613	40.17	108.89	2.71
3	RSV-214	39.24	67.66	1.72
4	SPV-1502	44.40	60.52	1.36
5	CSV-216	36.04	80.72	2.24
	Range	36.04-44.40	49.60-108.89	1.15-2.71
	Mean	40.56	73.48	1.84
	SE±	0.93	1.58	
	CD 5%	2.60	4.41	
C)	**Crosses102.93**			
1	CSV-216XRSV-458	38.12	102.93	2.70
2	SPV-1546XRSV-658	29.07	59.95	2.06
3	SPV-1502XRSV-458	29.03	98.23	3.77
	Range	29.03-38.12	59.95-102.93	2.06-3.77
	Mean	32.07	87.04	2.84
	SE±	1.05	1.60	
	CD 5%	3.15	4.80	

Table 6: Effect of water stress created by withholding irrigation on soluble protein content in sorghum.

S. no	Cultivar	Soluble protein (mg g-1 fr.wt.)		Fold increase
		Control	Stress	
A)	Tolerant cultivars			
1	P.Maulee	15.83	75.72	4.77
2	RSV-458	20.94	72.02	3.43
3	SPV-1546	13.60	59.67	4.38
4	RSV-658	10.20	51.70	5.06
5	SPV-1090	11.07	57.15	5.16
	Range	10.20-20.94	51.70-75.52	3.43-5.16
	Mean	14.33	63.21	4.56
	SE±	0.71	2.30	
	CD 5%	1.97	6.90	
B)	Susceptible cultivars			
1	RSV-491	34.18	49.85	1.45
2	RSV-613	19.53	72.31	3.70
3	RSV-214	18.46	47.46	2.57
4	SPV-1502	29.25	51.41	1.75
	Range	18.46-34.18	47.46-72.21	1.45-3.70
	Mean	24.91	55.12	2.34
	SE±	0.80	1.80	
	CD 5%	2.40	5.40	
C)	Crosses			
1	CSV-216XRSV-458	15.93	82.13	5.15
2	SPV-1546XRSV-658	23.12	64.82	2.80
3	SPV-1502XRSV-458	13.94	66.00	4.73
	Range	13.94-23.12	64.82-82.13	2.80-5.15
	Mean	17.66	70.98	4.23
	SE±	1.05	0.35	
	CD 5%	3.15	1.52	

Table 7: Correlation coefficient between proline, P_5CS activities, glycine betaine and soluble proteins in control and stressed seedlings of ten cultivars with three crosses of sorghum

S. No	Parameter	Correlation coefficient	
		Control condition	Stress condition
1	Proline x P_5CS	-0.181	0.0452
2	Proline x Glycine betaine	0.507	0.254
3	Proline x Soluble protein	0.625	0.189
4	P_5CS X Soluble protein	-0.2208	0.4020

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