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GENETIC VARIABILITY IN DIFFERENT BIOCHEMICAL TRAITS AND THEIR RELATIONSHIP WITH YIELD AND YIELD PARAMETERS OF COTTON CULTIVARS GROWN UNDER WATER STRESS CONDITIONS

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Abstract

Water scarcity is an important factor limiting cotton production worldwide particularly in Pakistan. To identify drought tolerant genotypes, it is vital to understand their genetic variation for different biochemical traits under water limited conditions. In the present study, 24 genotypes of cotton (*Gossypium hirsutum* L.*)* were evaluated under two irrigation regimes viz., well watered (W_1) and limited water (W_2) conditions. Before physiological maturity, cotton leaves were collected and analyzed for nitrate and nitrite reductase activities, and total free amino acids. At maturity, data regarding yield and yield parameters were recorded. Significant reduction in case of all the activities of nitrate and nitrite reductase, and yield parameters was observed under W_2 condition in all the genotypes; however, total free amino acids were substantially increased under W_2 condition. Correlation between the yield parameters of cotton and biochemical traits was determined. Non-significant correlation between nitrate reductase activity and yield parameters was observed under limited water condition. The genotypes evaluated exhibited decrease in the activities of nitrate and nitrite reductase whereas total free amino acids accumulation was higher under drought conditions that showed comparatively higher yield. This study shows that these biochemical traits were regulated genetically and environmentally in the tested cotton genotypes. It was concluded that these biochemical traits can be used as biochemical markers for screening cotton germplasm for drought tolerance as well as for evolving high yielding drought tolerant varieties of this crop. The findings are useful in bridging plant biochemistry and molecular biology for identifying and selecting genes involved in conferring drought tolerance in cotton.

Introduction

Cotton (*Gossypium hirsutum* L.) is the leading natural fiber crop and is grown on 33.9 million hectares worldwide which produces 120.3 million bales. It contributes more than 60% of the total foreign exchange in the economy of Pakistan (Anon., 2007). Like other crops, the cotton production has reached a plateau because of narrow genetic base (Rahman *et al.*, 2002; 2008). In the present scenario, cotton production fluctuates substantially because of abiotic and biotic stresses. Among the abiotic stresses, drought is recognized as the most devastating which limits the cotton production markedly. Due to quantitative expression of drought tolerance trait, breeding efforts have not met the expectations (Blum, 1988; Pasioura, 2002).

To understand the drought tolerance mechanism and its nature in different plants, multiple investigations have led to develop understanding of different physiological, biochemical and morphological features conferring drought tolerance for getting insight into the molecular basis of tolerance. The role of glycinebetaine and proline in conferring drought tolerance has been explored in cereals and other crop plants (Chandrasekar *et al.,* 2000; Xing *et al*., 2001; Sulpice *et al.,* 2002; Mickelbart *et al*., 2003; Diouf *et al*., 2004; Iqbal *et al*., 2005; Wahid *et al*., 2006; Shirasawa *et al., 2006;* Sankar *et al.,* 2007). In cotton, the role of glycinebetaine and its association with drought tolerance has been elucidated (Blunden *et al*., 2001; Sarwar *et al.,* 2006). These physiological factors mitigate the water stress by lowering the osmotic potential of the cell sap for preventing its outward movement (Stewart *et al.,* 1966*;* Storey & WynJones, 1977). Similarly, the other organic compounds such as total free amino acids which accumulate in water stressed plants also play a significant role in osmotic adjustment of the cell sap (Good & Zaplachinski, 1994; Mattioni *et al.,* 1997; Parida *et al.,* 2007). In another investigation, accumulation of high nitrite and nitrate reductase in response to limited water supply has been associated with drought tolerance (Ashraf *et al*., 1995). Parida and his co-workers found an increase in total free amino acids during their study in cotton and suggested that this may be one aspect for drought tolerance in cotton (Parida *et al.,* 2007).

In cotton, drought stress affects the crop by limiting fiber yield and deteriorating lint quality (McWillium, 2003). Keeping in view the importance of cotton crop and water scarcity in Pakistan, it is essential to initiate research activities for improving drought tolerance in cotton by employing physiological strategies to overcome cotton production losses under drought conditions. The present work aimed at studying the genotypic variations in the cultivated cotton species for physiological parameters such as total free amino acids, nitrate reductase and nitrite reductase activity, and their association with the yield components. Such study was expected to provide useful information which would not only help conventional breeding program but would also help identifying DNA markers associated with QTLs conferring drought tolerance and also could lead to initiate gene cloning programs in Pakistan to develop drought tolerant varieties of cotton.

Materials and Methods

The experimental material consisted of 24 upland cotton genotypes collected from different research institutes located in different ecological regions of Pakistan. These genotypes were evaluated in the field under two irrigation regimes at the research area of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. The water regimes were as follows:

W_1 (well watered) = One irrigation at planting and 5 subsequent irrigations as required for normal crop growth and development.

W_2 (Limited water) = One irrigation at planting and one supplemental 40 days after planting. Daily rainfall during the growing season was recorded at the experimental site.

A split-plot design with four replications was used with water regimes in the main plot and genotypes in the sub plots. Cottonseed was delinted with sulfuric acid and soaked in water for 12 h before planting. In growing season, planting was completed during the 1st week of April. Four 6-m rows spaced 0.75 m apart were sown with each genotype using a hand drill. Commercial chemical fertilizers were applied at the rate of 100-50-50 kg N-P_2O_5-K_2O ha^{-1}, respectively at the time of seedbed preparation. Plant population of 4 plants m^{-2} was established by hand thinning 25 days after germination. Throughout the season, appropriate control measures for weeds and insect pests were followed uniformly in all the plots.

Measurement of productivity traits: Seed cotton yield was measured as kg ha^{-1} on the basis of two central rows from furrows of plots of both the regimes. Seed cotton was hand-picked from all the plots 180 days after planting, and before weighing, the cotton was sun-dried for one day and the trash and dry carpals were removed. Average seed cotton weight of 40 bolls picked from each plot was used to estimate boll weight.

Analysis of variance and correlation coefficients were calculated for all the possible character combinations with the objective to derive information about the relationship among different character combinations following Steel & Torrie (1980) and Snedecor & Cochran (1980).

Total free amino acids: For the estimation of total free amino acids, 1 mL from each sample (extracted in 0.2 M phosphate buffer pH 7.0) was taken in test tubes and 1 mL of 10% pyridine, and 1mL of 2% ninhydrin solution were added into each tube. The tubes were then heated in boiling water bath for about 30 minutes. The contents of each tube were then made to 50 mL with distilled water. The optical densities of these colored solutions were then read at 570 nm using spectrophotometer (Hitachi-200) and free amino acids were calculated according to Hamilton & Slyke (1943).

Nitrate reductase activity (NRA): NRA in leaves was recorded using the method of Sym (1984). Potassium nitrate was used as a substrate. At first, 0.02 *M* KNO_3 solution was prepared in phosphate buffer of pH 7.0. Fresh plant (leaves) material (0.5 g) was homogenized in 5 mL phosphate buffer (pH 7.0) containing 0.02 *M* KNO_3. Samples were incubated in dark at 32^oC for 1 h. After incubation, 1 mL of reaction medium was taken in another test tube and mixed with 0.5 mL sulfanilamide prepared in 2*N* HCl. After this, 0.5 mL of 1-Naphthyl-ethylene diamine dihydrogen chloride, was added. Pink diazo colour complex was produced due to NO_2 formation. Absorbance was read at 542 nm against a set of standards developed with $NaNO_2$ on a spectrophotometer (Hitachi-220).

Nitrite reductase activity (NiRA): NiRA in leaves was determined following the method of Ramarao *et al*. (1983). Sodium nitrite was used as a substrate. Sodium nitrite solution (0.02 *M*) in phosphate buffer of pH 5.0 was prepared. Fresh plant material (0.5 g) was homogenized in 4.5 mL phosphate buffer (pH 7.0) and 0.5 mL of $NaNO_2$ (0.02*M*). Sample was incubated at 30^oC in water bath with gentle shaking for 30 minutes. The sample was then transferred in boiling water for two minutes to terminate the reaction and then cooled the reaction mixture. One mL of cooled extract was taken, treated it with 1% sulfanilamide prepared in 2*N* HCl and 0.02% of aqueous solution of *N* 1-Naphthyl-ethylene diamine dihydrochloride; colour was developed and then the optical density was read at 542 nm on a spectrophotometer (Hitachi-220). Standard curve with $NaNO_2$ was developed. The activity of NiRA was determined as NO_2 utilized $g^{-1}F.W\ h^{-1}$.

Results

Analyses of the data (ANOVA) exhibited that mean square values of water regimes with respect to total free amino acids (TFAA), nitrate reductase activity (NRA), nitrite reductase activity (NiRA), seed cotton yield (SCY), boll number (BN), and boll weight (BW) were significantly influenced by water regimes (Table 1). Least significant test was used to check the differences among the genotypes at 5% probability level. Significant differences were observed for SCY, BN and BW under both the regimes. Genotypes also significantly differed in accumulating TFAA, NiRA and NRA.

Total free amino acids (TFAA): The frequency distribution of the levels of TFAA is shown in Fig. 2. Genotypic differences were found for TFAA under both the water regimes. The individual TFAA levels of all tested genotypes are presented in Table 2. Mean TFAA levels ranged from 2.2 to 4.37 µg/g fresh weight under limited water conditions (Table 2). The highest accumulation (2.89 µg/g fresh weight**)** was recorded in FH-1000, while a sharp reduction in TFAA accumulation was observed in NIAB-Karishma under W_1 condition (Table 2). Under limited water condition, NIAB-111 with the mean value of 4.37 µg/g fresh wt showed a marked increase in TFAA accumulation among all the cotton genotypes tested in this study whereas CIM-443 was found with the lowest mean value of 2.2 µg/g fresh weight for TFAA accumulation under W_2 conditions (Table 2). After NIAB-111, seven genotypes (FH-87, FH-1000, FH-2000, CIM- 473, CIM-497, FH-900, and NIAB-Karishma) evidenced the highest range of TFAA pool i.e., 3.2 to 3.6 µg/g, whereas six genotypes (CIM-443, FH-925, CIM-499, CIM-707, FH-634 and NIBGE-1) showed the minimum range of TFFA concentration of from 2.2 to 2.6 µg/g **(**Table 4, Fig. 2). However the eight genotypes (NIBGE-2, MNH-552, CIM-501, RH-510, BH-160, MNH-554, MNH-642, and FH-901) responded moderately for TFAA under limited water condition (Table 4, Fig. 2). Six genotypes viz., CIM-443, FH-925, CIM-499, CIM-707, FH-634, and NIBGE-1 exhibited the lowest accumulation of TFAA (< 2.6 µg/g fresh wt, Table 4).

Table 1. Mean square values of different traits in cotton under two water regimes.

Source of variation	df	NRA	NiRA	TFAA	SCY	BW	BN
Replications	3	0.437	0.011	0.012	1304.838	0.012	0.385
Water regime	1	147.876**	190.204**	66977**	4549682.287**	4.441**	3439.16**
Error A	3	0.083	0.009	0.006	905.850	0.015	0.528
Genotypes	23	17.223***	10.219***	0.688***	1328133.149**	0.571**	118.869**
Water X genotypes	23	5.855**	1.593**	1.083**	438602.387**	0.068**	57.458**
Residual	138	0.388	0.011	0.012	462.386	0.011	0.425
Coefficient of variability %		22.10	1.98	4.53	1.16	3.79	3.28

*p≤0.05, **p≤0.01, and ***p≤0.001. NS= Non–significant, NRA= Nitrate reductase activity, NiRA= Nitrite reductase activity, SCY= Seed cotton yield, BW = Boll weight, BN= Boll number and A= Water

Table 2. Total Free amino acids, nitrite reductase activity, nitrite reductase activity, seed cotton yield and yield components in cotton genotypes grown under two water regimes.

Genotype	TFAA		NRA		NiRA		SCY		BN		BW	
	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
BH-160	1.69	3.05	8.06	3.83	6.37	5.45	3220.80	1853.50	29.50	19.00	3.30	2.97
CIM-443	1.73	2.2	2.87	1.09	6.70	5.21	2109.35	1177.00	22.50	18.75	2.63	2.58
CIM-473	1.40	3.29	8.10	4.64	7.50	3.83	1362.13	1164.90	27.61	14.95	2.75	2.42
CIM-497	1.67	3.97	2.47	0.71	4.29	3.29	1363.00	1164.90	15.75	14.75	2.75	2.40
CIM-499	1.85	2.46	6.08	1.60	8.30	4.83	2698.30	1827.10	52.60	18.60	3.30	2.97
CIM-501	2.50	2.83	6.46	3.63	4.45	3.35	2332.75	1383.08	21.00	14.50	2.55	2.20
CIM-707	2.04	2.49	1.45	0.60	5.04	3.11	2698.00	1827.08	24.25	18.25	3.03	2.80
FH-1000	2.89	3.25	1.89	1.02	5.59	4.04	2746.70	876.70	32.20	10.70	2.64	2.53
FH-1200	2.33	3.49	3.13	2.36	6.47	4.58	2169.23	1645.55	18.92	18.50	3.01	2.73
FH-2000	1.60	3.25	2.15	0.78	5.05	4.09	3213.25	973.50	34.54	12.90	3.20	2.83
FH-634	1.86	2.52	1.36	1.35	5.47	4.10	2272.60	1346.40	24.86	18.00	2.60	2.10
FH-87	1.63	3.20	5.94	4.18	5.34	3.15	2930.40	1806.20	31.20	19.80	2.86	2.75
FH-900	2.25	3.41	2.30	1.74	6.09	3.57	3143.80	1952.50	31.00	21.00	3.08	2.86
FH-901	2.10	3.10	2.32	1.65	7.86	5.51	1947.00	1239.60	18.81	13.80	2.40	2.33
FH-925	1.51	2.40	2.25	1.60	8.54	4.85	1947.00	1239.60	18.81	13.80	2.40	2.33
MNH-552	1.72	2.74	1.43	1.23	6.86	5.24	2487.10	823.90	24.20	8.70	3.19	2.86
MNH-554	1.80	3.05	2.07	1.39	4.96	2.95	1901.90	950.40	19.00	10.40	3.08	2.86
MNH-642	1.67	2.99	2.23	1.66	4.15	2.74	1901.90	950.45	19.00	10.25	2.80	1.82
NIAB-111	1.37	4.37	2.21	1.03	7.52	6.29	2531.10	1922.80	25.00	21.90	3.08	2.75
NIAB-78	1.53	3.47	5.47	1.33	5.03	3.10	2098.80	1309.00	20.40	14.00	3.19	2.75
NIBGE-1	2.21	2.55	5.51	1.38	7.97	3.97	1542.20	840.40	16.30	9.60	2.97	2.75
NIBGE-2	2.49	2.69	2.26	1.39	5.75	3.63	2666.00	1910.00	28.00	22.00	3.00	2.75
N-Karishma	0.67	3.56	4.32	1.95	6.72	4.96	2709.30	1673.10	26.70	18.60	3.08	2.75
RH-510	1.59	2.84	2.40	0.45	8.07	6.41	2322.10	1085.70	23.30	11.90	3.08	2.75

W_1= Well watered regime, W_2 =Limited water regime, NRA= Nitrate reductase activity, NiRA= Nitrite reductase activity, SCY=Seed cotton yield, BW =Boll weight, BN=Boll number.

Positive correlation of TFAA with seed cotton yield (SCY, r=0.234), boll number (BN, r=0.205), boll weight (BW, r=0.39) was observed (Table 3). Results obtained for the standard curve showed non-significant association of TFAA with SCY (R^2=0.054), and BN (R^2=0.042). A significant association (R^2=0.15) of BW with TFFA was found (Fig. 1).

Table 3. Correlation coefficient (r) of different characters of cotton grown under limited water regime.

Traits	TFAA	NRA	NiRA	SCY	BN	BW
TFAA	-					
NRA	0.126	-	-			
NiRA	0.082	-0.57	-			
SCY	0.233^{NS}	0.266^{NS}	0.0845^{NS}	-		
BN	0.205^{NS}	0.2^{NS}	0.125^{NS}	0.92**	-	
BW	0.39*	0.046^{NS}	0.15^{NS}	0.0674	0.0424	-

* = Significant, NS= Non-significant TFAA= Total free amino acids, NRA= Nitrate reductase activity, NiRA= Nitrite reductase activity, SCY= Seed cotton yield, BN= Boll numbers and BW= Boll weight

Table 4. Distribution of genotypes with respect to leaf total free amino acids under water limited regime (W_2).

Total free amino acids (µg/g fresh wt)	Cultivars within class
2.2 to 2.6	CIM-443, FH-925, CIM-499, CIM-707, FH-634, NIBGE-1
2.7 to 3.1	NIBGE-2, MNH-552, CIM-501, RH-510, BH-160, MNH-554, MNH-642, FH-901
3.2 to 3.6	FH-87, FH-1000, FH-2000, CIM-473, CIM-497, FH-900, NIAB-78, FH-1200, NIAB-KARISHMA
3.7 to 4.1	-
4.2 to 4.6	NIAB-111

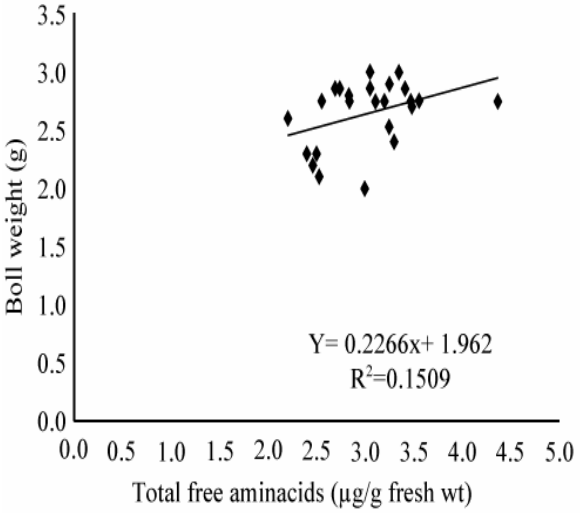

Fig. 1. Relationship between boll weight and total free amino acids in limited water regime (W_2).

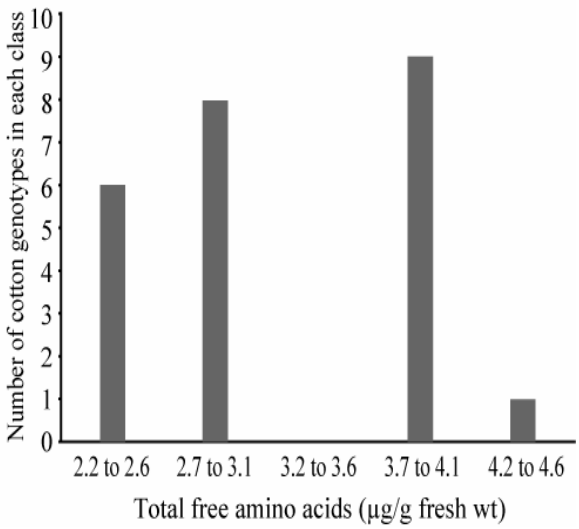

Fig. 2. Frequency distribution of total free amino acids levels in the leaves of 24 genotype grown under in limited water regimes (W_2).

Nitrate reductase activity: In all the tested cotton genotypes, the nitrate reductase activity (NRA) decreased due to water stress (Table 2). Individual NRA levels of all tested genotypes are presented in Table 5. Genotypes showed substantial variation in NRA under W_2 condition that ranged from 0.45 in RH-510 to 4.64 µ mol NO_3 formation g^{-1} f.wt.h^{-1} in CIM-473 (Table 2). Highest concentration of NRA under W_1 condition was found in CIM-473 while it was lowest in FH-634 (Table 2). Genotype CIM-473 exhibited the highest level of NRA under both the water regimes (8.1 and 4.64 µmol NO_2 g^{-1} F.W. h^{-1} under W_1 and W_2, respectively). Minimum activity of the enzyme (0.45) was observed in genotype RH-510 (Table 2). Minute differences in NRA were observed in FH-634 for NRA under W_1 and W_2 conditions. Maximum variation for NRA was found in genotype CIM-499 under the two water regimes (Table 2). A positive but non-significant correlation of the enzyme activity with yield parameters was observed (Table 3).

Nitrite reductase activity (NiRA): Limited water supply led to an abrupt reduction in leaf NiRA of the cotton genotypes (Table 2). Individual NiRA levels of all tested genotypes are presented in Table 6. Significant decrease was observed for NiRA under W_2 regime as compared to W_1 (Table 2). However, the cotton genotypes showed a continuous variation for NiRA (Table 2). Under W_1 regime, FH-925 and RH-510 had higher of NiRA which was 8.54 and 8.07 µmol NO_2 utilized g^{-1}F.W. h^{-1}, respectively. While lower level of NiRA was found in MNH-642 (4.15) under W_1 regime. The highest NiRA was recorded in RH-510 (6.41) under W_2 regime, while it was the least in MNH-642 (2.74µmol NO_2 utilized g^{-1} F.W. h^{-1}). The genotypes MNH-554, BH-160, FH-901, NIAB-111 and RH-510 showed a higher NiRA under water stress, while genotypes MNH-642, MNH-554, NIAB-78, CIM-707, FH-87, CIM-497 and CIM-501 showed minimum NiRA. However, rest of the cotton genotypes exhibited a moderate level of NiRA (Table 2). Correlation of NiRA with SCY, BN and BW was positive but non-significant.

Discussion

In the present study, genotypic differences in different biochemical traits under well watered (W_1) and limited water (W_2) regimes were investigated in 24 cotton genotypes, followed by establishing their association with yield parameters. Experimental results showed a reduction in yield under W_2 condition. Yield is influenced by a range of internal and external factors (Ullah *et al*., 2008). Genetic potential of cotton genotypes with regard to different biochemical traits is an important tool for screening and assessing crop production under limited water conditions. Drought stress is observed as overwhelming factor causing decrease in seed cotton yield (SCY) of all the genotypes evaluated in this experiment. Similar results under water stress environment have also been found in cotton under limited water conditions (Sarwar *et al*., 2006; Ullah *et al*., 2006; Rahman *et al*., 2008; Ahmad & Jabeen. 2009).

In all the 24 genotypes, reasonably high but similar amount of NRA and NiRA were found under W_2 regime with substantial reduction in their activities. These findings are congruent with the previous reports (Vyas *et al.,* 1996; Chandra *et al.,* 2004; Ulfat *et al.*, 2007). Nitrate reductase is involved in post translational regulation including inactivation through protein phosphorylation and subsequent Mg^{2+} dependent proteins (Kaiser *et al*., 1999; Kaiser & Huber 2001; Kaiser *et al*., 2002; Lillo *et al*., 2004). Nitrate reduction is well known for its sensitivity to external stress conditions (Ashraf *et al.,* 1995). Water stress which ultimately results in a decrease in leaf water potential caused a marked inhibition in NRA (Heur *et al*., 1979; Larson *et al*., 1989; Ashraf, 1994). Similarly, water stress also affected the nitrite reductase activity (Heuer *et al*., 1979). Nitrate reduction activity showed positive correlation with yield parameters under water limited conditions and all genotypes showed

significant differences in the activity that may be useful for cotton breeding program. NiRA which decreased significantly under limited water regime in almost all the cotton genotypes confirmed the findings of the earlier studies on wheat under drought conditions (Ashraf *et al.,* 1995; Ashraf, 1998). Total free amino acids (TFAA), showed an increase under limited water condition in cotton has been complemented by several investigations conducted on different crop plants (Good & Zaplachinski, 1994; Mattioni *et al*., 1997; Parida *et al.,* 2007). Increase in levels of TFAA also reflected the mode of adjustment to drought in cotton crop (Parida *et al*., 2007**).** Higher accumulation of TFAA helps plants to cope drought stress by executing different protective/defensive functions like, osmotic adjustment, protection of cellular macromolecules, maintaining cellular pH, storage of nitrogen, scavenging of free radicals and detoxification of the cells. A positive correlation of total free amino acids with yield parameters was found significant which could be utilized for future cotton breeding programs.

Table 5. Distribution of genotypes with respect to nitrate reductase activity under water limited regime (W_2).

Nitrate reductase activity (μ mol NO_2 g^{-1}f.wt.h^{-1})	Cultivars within class
0 to 0.45	RH-510
0.46 to 0.91	CIM-707, CIM-497, FH-2000
0.92 to 1.37	FH-1000, NIAB-111, CIM-443, MNH-552, NIAB-78, FH-634
1.38 to 1.83	NIBGE-1, NIBGE-2, MNH-554, FH-925, CIM-499, FH-901, MNH-642, FH-900
1.84 to 2.29	NIAB-KARISHMA
2.30 to 2.75	FH-1200
2.76 to 3.21	-
3.22 to 3.67	CIM-501
3.68 to 4.13	BH-160
4.14 to 4.59	FH-87

Table 6. Distribution of genotypes with respect to nitrite reductase activity under water limited regime (W_2).

Nitrite reductase activity (μmol NO_2 utilized g^{-1} fwt.h^{-1})	Cultivars within the class
2.0 to 2.7	MNH-662
2.8 to 3.5	MNH-554, NIAB-78, CIM-707, FH-87, CIM-497, CIM-501
3.6 to 4.3	FH-900, NIBGE-2, CIM-473, NIBGE-1, FH-1000, FH-2000, FH-634
4.4 to 5.1	FH-1200, CIM-499, FH-925, NIAB-KARISHMA
5.2 to 5.9	CIM-443, MNH-554, BH-160, FH-901
6.0 to 6.7	NIAB-111, RH-510

Conclusion

Keeping in view the variation and response of genotypes under two water regimes, the present study suggests that drought stress can cause changes in total free amino acids, nitrate reductase activity, nitrite reductase activity and yield parameters. Biochemical parameters studied are important indices of the tolerance of cotton crop genotypes to drought stress. A positive association of biochemical traits with yield parameters under water limited conditions would be helpful for plant breeders for screening and selection of cotton germplasm to develop a variety evolving programme for drought tolerance. This information will be utilized for further study and could prove a good basis for research on molecular aspect of drought stress in cotton crop. Significant association of one of the yield trait i.e. boll weight with plant amino acids could then be used to test the contribution of this trait to drought tolerance. Correlation estimation between biochemical and yield traits showed no good association except between amino acids and boll weight, which suggests that they may contribute towards drought tolerance indirectly and in combination with other environmental and genetic factors as their association is positive but these findings seem of little value for further investigation and use in breeding programme. An understanding of the metabolic and genetic basis of this genetic variation in biochemical trait i.e., amino acid level in cotton could assist in devising breeding strategies to develop near iso-genic lines differing solely in the amino acid trait. Such a study that considers amino acid as a target trait may also provide information to prepare genetic maps.

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