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GLYCINEBETAINE ACCUMULATION AND ITS RELATION TO YIELD AND YIELD COMPONENTS IN COTTON GENOTYPES GROWN UNDER WATER DEFICIT CONDITION

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Abstract

An experiment was conducted to investigate the genotypic variability and relationship between accumulation of glycinebetaine and productivity traits under field induced water stress at the flowering and boll maturation stage. Twenty cultivars/genotypes were evaluated for seed cotton yield (SCY), number of bolls per plant (BN), boll weight (BW) and glycinebetaine (GB) content under well watered (W_1) and water limited regimes (W_2) during the cropping season 2006. Glycinebetaine level in 20 genotypes / cultivars under water stress conditions ranged from 9 to 21 µmol g^{-1} FW while in well watered conditions, this ranged from 0.5 to 1.5 µmol g^{-1} FW. Genotypes BH-160, FH-87, MNH-552, CIM-1100, FH-901 and NIAB-Karishma had the higher concentration of GB during water stress as compared to other genotypes, whereas RH-510, NIBGE-160 and FH-1000 had relatively lower concentration of GB. Genotypes with low accumulation and better yielder under water stress may be attributed to their long root system and short life cycle of these genotypes. GB was positively and significantly correlated with seed cotton yield and boll number, while a positive and non-significant correlation was also recorded for GB with the boll weight (BW) under stress condition. Highly significant correlation was observed between BN and SCY. Significant differences in reduction of SCY, BN and BW were observed in W2. Genotypes with high GB level showed a significant increase in SCY, BN and BW under water-limited regime (W_2). Results indicated that selection for higher glycinebetaine has the potential to speed up breeding for drought tolerance in cotton.

Key words: Glycinebetaine, Seed cotton yield, Boll weight, Boll numbers, Drought

Introduction

Cotton, a natural leading fibre crop, is grown on arid and semi-arid regions of the world. Future gains in cotton production are nearly impossible because the yield potential has reached to plateau (Rahman *et al.,* 2002). It is vital to boost the cotton production to meet the upcoming challenges of high population rate, deterioration of arable land, depletion of water resources and environmental stresses. It is therefore mandatory to devise new strategies to sustain or increase yield in water-depleted situations (Boyer, 1982).

Glycinebetaine balances the osmotic pressure between outside and the inside of cells to cope with osmotic stress and hence maintains turgor (Ashraf & Foolad, 2007). Moreover, glycinebetaine also protects physiological processes such as photosynthesis and protein synthesis under drought conditions (Larher *et al.,* 1996; Sulpice *et al.*, 1998).

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Endogenous glycinebetaine accumulation in plants varies from species to species. Cotton is one of those species that accumulate higher amount of GB than others (Gorham, 1996; Blunden *et al.,* 2001). It is also observed that GB in plants accumulated under different types of stresses, e.g., salinity stress, cold stress and water stress (Kishitani *et al.* 1994; Khan *et al.,* 1995; Colmer *et al.,* 1995; Ashraf & Foolad, 2007).

The breeding of crop plants for tolerance to abiotic stresses especially the drought has been difficult and slow. Identification of stress-tolerant lines is a challenge, in part, because of the quantitative inheritance of environmental stress tolerance as well as problems associated with developing suitable testing environments, where stress can be reproducibly applied.

A better understanding of plant stress tolerance can be developed by identifying and characterizing traits which contribute to stress tolerance and by determining their relative importance. Information gained from such an approach can then be used to develop focused breeding programs to improve stress tolerance. Complex quantitative traits such as osmotic stress tolerance can be studied by identifying individual components and then by using traditional breeding methods to select plants that possess the specific trait (Eslick & Hockett, 1974). This approach has been employed to study glycinebetaine (GB) accumulation in various genetic backgrounds of barley and maize (Grumet *et al.,* 1985; Yang *et al.,* 1995).

The primary objective of the present study was to determine that up to what extent, water stress could cause changes in glycinebetaine accumulation in cotton, and how far the resultant changes in leaves affect metabolic phenomena in the whole plant. The other objective of this study was to know the genotypic differences for glycinebetaine accumulation and its relationship with yield/growth components in cotton under well watered and water limited conditions.

Materials and Methods

The experimental material consisted of 20 upland cotton (*Gossypium hirsutum* L.) cultivars and promising breeding lines. Seed of the cultivars was obtained from different research institutes located at different ecological regions of Pakistan.

The genotypes of cotton were evaluated under two irrigation regimes, well-watered (W_1) and water-limited (W_2) in the field during 2006 at the research area of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. The experimental design was a quadruplicated split-plot with water regimes assigned in main plot and cultivars in sub-plots. Cottonseeds were delinted with sulfuric acid and soaked in water for 12 h before planting. The sowing was completed during the 1st week of April. Four rows 6 m long and 0.75 m apart of each cultivar were sown with a hand drill. 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. After germination 4 plants m^{-2} were maintained by thinning. Uniform appropriate control measures were adopted for insect-pest and weed infestation for all the treatments.

Seed cotton yield (*SCY*) was measured on central two rows from both regimes and transformed to per plant for harvest index (*HI*) estimation.

Seed cotton was hand picked from all the plots 180 days after sowing (DAS) and sun-dried for one day after removing trash and dry carpels before weighing. The number of bolls picked in three pickings was recorded from each individual plant. When final

picking was over, the total number of bolls was calculated; average number of bolls was calculated afterward. Boll weight was worked out by dividing the total yield of a plant by the total number of bolls picked from that particular plant. The average boll weight per plant was then calculated.

For glycinebetaine, fully expanded upper most leaves were taken from the plants grown under normal and water stressed conditions, and analysis was carried out according to the method of Grieve & Grattan (1983). Leaf extract was prepared in 20 mL test tubes by chopping 0.5 g leaves in 5 mL of toluene-water mixture (0.05% toluene). All the tubes were mechanically shaken for 24 h at 25°C. After filtration 0.5 mL of extract was mixed with 1 mL of 2 *N* HCl solution then and 0.1 mL of potassium tri-iodide solution (containing 7.5 g Iodine and 10 g Potassium iodide in 100 mL of 1 *N* HCl) was added and shaken in an ice cold water bath for 90 min and then 2 mL of ice-cooled water was added after gentle shaking 10 mL of 1,2 dichloroethane (Chilled at -10^{o}C) was pour in it. By passing continuous stream of air for 1-2 minutes two layers were separated, upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm. The concentrations of glycinebetaine was estimated by using standard curve developed with different concentration of GB.

All the collected data were subjected to analysis of variance and correlation coefficient (Steel & Torrie, 1980). The relationships among different traits were determined by using the computer programme Excel 2000 following the Snedecor & Cochran (1980) method.

Results and Discussion

Analysis of variance showed that mean square values of water regimes in respect to GB, BN, and BW were significantly influenced by water regimes (Table 1). Cotton genotypes also exhibited highly significant variations for GB, BN, SCY and BW.

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

SOV	d.f	*GB*	*SCY*	*BN*	*BW*
Replication	3	14.875	565423.73	0.028	0.006
Water Regime	1	9076.361**	32114880.85**	2805.625**	3.963**
Error A	3	14.748	646275.377	0.094	0.002
Cultivar	19	25.876**	2282147.535**	203.328**	0.264**
Water Regime x Cultivar	19	26.998**	799094.833	46.21**	0.036**
Residual	114				
CV %		17.86	41.78	2.29	2.65

*$P\leq0.05$, **$P\leq0.01$ and *** $P\leq0.001$. ns -Non-significant

GB= Glycinebetaine; SCY= Seed cotton yield; BN= Boll numbers; BW- Boll weight A= water

Glycinebetaine levels in all the tested genotypes are presented in Table 2. Among GB accumulating genotypes, there was a significant variation in the level of GB, which varied from 9 to 21 μmol g^{-1} FW. Mean betaine levels ranged from as low as 9 µmol g^{-1} FW to as high as 21 μmolg^{-1} FW during the crop season 2006. The highest accumulation was recorded in FH-901 under control conditions, it was the lowest in FH-87, under

drought conditions, BH-160 maintained the highest accumulation of GB while it was the least in NIBGE-160. Genotypes BH-160, FH-87, MNH-552, CIM-1100, CIM-499, FH-901 and NIAB-Karishma had the higher concentration of GB during water stress as compared to other genotypes whereas RH-510 and NIBGE-160 and FH-1000 accumulated minimum amount of GB. While genotypes MNH-554, CIM-473,VH-142, NIAB-111, FH-900, NIBGE-2 and FH-930 on the other hand, showed a medium response for GB accumulation 15.7 or 17 μmol g^{-1} FW. Similar results for maize and sorghum were also reported by several researchers (Rhodes *et al.*, 1987; Rhodes *et al.,* 1989; Rhodes & Rich, 1988). Genetic studies for sorghum suggest that recessive allele of a single locus is the cause of non-accumulation of GB in sorghum genotypes IS-2319 (Grote *et al.,* 1994) that may be the case for low accumulation of GB in RH-510, NIBGE-160 and FH-1000 under water stress conditions. The above mentioned genetic and biochemical marker can be utilized in devising breeding strategies to develop near isogenic lines differing solely for the GB traits. These genotypes could then be used to test the contribution of this trait to drought tolerance. A significant increase of GB accumulation in water limiting environment has been found in all the cotton genotypes.

Table 2. Glycinebetaine concentration in different cotton genotypes under two water regimes 2006.

Genotype	Glycinebetaine level μmol g^{-1} FW				
	W 1*	W2*	Genotype	W1	W2
BH-160	0.93±0.023	19.62±1.656	MNH-552	1.08 ± 0.002	19.57±0.690
CIM-1100	0.59±0.005	18.70±1.449	MNH-554	1.46 ± 0.016	15.48±0.811
CIM-473	0.89±0.004	14.86±1.454	NIAB-111	0.60 ± 0.009	15.70±1.885
CIM-499	0.89±0.005	19.67±1.007	NIAB-78	0.45 ± 0.007	15.95±1.094
FH-1000	0.67±0.009	10.24±1.000	RH-510	1.25 ± 0.023	8.42±0.728
FH-87	0.40±0.014	20.45±0.685	NIBGE-1	0.94 ± 0.010	17.66±1.346
FH-900	1.40±0.065	16.00±1.565	NIBGE-160	0.88 ± 0.005	8.18±0.506
FH-901	1.58±0.055	18.73±0.856	NIBGE-2	0.99 ± 0.004	14.20±1.504
FH-930	0.98±0.008	16.58±1.578	NIBGE-4	0.69 ± 0.006	15.36±1.359
VH-142	1.52±0.069	15.13±1.0759	NIAB-Karishma	0.83 ± 0.005	19.75±0.578

W1* = well watered; W2* = water limited

Genotype BH-160 produced the highest seed cotton yield (3220 kg ha^{-1}) followed by FH-900 (3143 kg ha^{-1}). The lowest seed cotton yield was observed in genotype CIM-473 (1362 kg ha^{-1}) and NIBGE-160 (3221 kg ha^{-1}) under normal watering conditions (Table3). In drought conditions, genotype FH-900 produced the highest seed cotton yield (1952 kg ha^{-1}) followed by NIAB-111 (1922 kg ha^{-1}) and NIBGE-2 (1910 kg ha^{-1}). Numbers of bolls $plant^{-1}$ were highest (32.2) in case of FH-1000 under normal and in case of drought genotype NIBGE-2 had the maximum number of boll (22). While for boll weight, differences were observed both under normal and drought conditions but differences among genotypes were not high and the range was very narrow.

Glycinebetaine was positively and significantly correlated with the seed cotton yield and boll number, whereas a positive correlation of glycinebetaine with boll weight was found under water deficit conditions (Table 4). Significant correlation existed between number of bolls $plant^{-1}$ and seed cotton yield $plant^{-1}$ (Table 1). The correlation of average boll weight with seed cotton yield (Table 4) was also highly significant (r = 0.900**), reflecting its effectiveness for selection of high yielding genotypes based on more

Table 3. Seed cotton yield and yield components in cotton genotypes grown under two water regimes.

Genotypes	Seed cotton yield kg ha^{-1}		Bolls plant^{-1}		Boll weight (g)	
	W_1	W_2	W_1	W_2	W_1	W_2
BH-160	3220.8 ± 22.36	1853.5 ± 4.46	29.5±0.612	19.0±0.082	3.3±. 008	3.0±0.009
CIM-1100	2109.8 ± 65.460	1177.0 ± 3.329	22.5±0.545	14.4±0.082	2.9±0.055	2.5±0.015
CIM-473	1362.1 ± 16.779	1164.9 ± 5.051	15.2±0.816	14.9±0.050	2.8±0.015	2.4±0.006
CIM-499	2698.3 ± 34.168	1827.1 ± 5.030	25.6±0.283	18.6±0.082	3.3±0.577	3.0±0.004
FH-1000	2746.7 ± 10.002	876.7 ± 1.747	32.2±0.141	10.7±0.058	2.6±0.008	2.53±0.004
FH-87	2930.4 ± 57.658	1806.2 ± 8.152	31.2±0.141	19.8±0.082	2.9±0.004	2.75±0.004
FH-900	3143.8 ± 8.631	1952.5 ± 3.282	31.0±0.081	21.0±0.374	3.1±0.007	2.86±0.007
FH-901	1947.0 ± 4.741	1239.7 ± 1.924	18.8±0.141	13.8±0.082	3.2±0.004	2.75±0.004
FH-930	2471.7 ± 22.725	1037.3 ± 4.191	23.8±0.816	10.6±0.216	3.2±0.019	3.08±0.004
VH-142	2114.4 ± 1.688	744.7 ± 0.063	12.9±0.041	10.5±0.05	2.9±0.004	2.2±0.408
MNH-552	2487.1 ± 7.199	823.9 ± 2.899	24.2 ±0.816	8.7±0.577	3.2±0.011	2.86±0.009
MNH-554	1901.9 ± 5.770	950.4 ± 3.409	19.0±0.082	10.4±0.082	3.1±0.011	2.86±0.004
NIAB-111	2531.1 ± 3.788	1922.8 ± 5.537	25.0±0.115	21.9±0.058	3.1±0.004	2.75±0.004
NIAB-78	2098.8 ± 2.726	1309.0 ±10.52	20.4±0.082	14.6±0.082	3.2±0.006	2.75±0.008
RH-510	2322.1 ± 3.066	1085.7±0. 857	23.3±0.129	11.9±0.058	3.1±0.007	2.75±0.006
NIBGE-1	1542.2± 5.212	840.4±1.655	16.3±0.058	9.6±0.0812	3.0±0.011	2.75±0.004
NIBGE-160	1316.7 ± 2.801	507.1±2.102	12.1±0.058	14.6±0.082	3.3±0.108	2.86±0.004
NIBGE-2	2666.0 ± 3.674	1910.0±2.638	28.0± 0.082	22.0±0.115	3.0±0.013	2.75±0.009
NIBGE-4	1762.2 ± 3.009	1009.8±0.564	16.7±0.058	11.2±0.082	3.2±0.004	2.75±0.006
NIAB Karishma	2709.3 ± 3.182	1673.1±2.602	26.7±0.058	18.6±0.082	3.1±0.007	2.75±0.012

*Standard error * W1* = well watered; W2* = water limited

Table 4. Correlation coefficient of different characters of cotton grown under two different water regimes.

Traits	Glycinebetaine		Seed cotton yield kg/ha		Boll number/plant	
	W1	W2	W1	W2	W1	W2
Glycine betaine	-----	-------				
Seed cotton yield kg/ha	-0.116	0.455*	-------	--------		
Boll number/plant	-0.293	0.292*	0.918*	0.900**	--------	--------
Boll Weight (g)	0.168	0.203	0.054	0.291**	-0.107	0.1909

* = Significant, **= Highly significant W1 = well watered; W2 = water limited

Table 5. Distribution of genotypes for leaf glycinebetaine.

Glycinebetaine (μmol g^{-1} FW)	Cultivars within class
8 to 10	NIBGE -160, RH-510
10 to 12	FH-1000
12 to 14	----------
14 to 16	NIBGE-2
16 to 18	CIM-473, MNH-554, VH-142, NIBGE-4, FH-900, NIAB-78, NIAB-111
18 to 20	FH-930, NIBGE-1, FH-901
20 to 22	MNH-552, NIAB-Karishma, FH-87, CIM-1100, BH-160, CIM-499

number of bolls. Positive correlation of glycinebetaine with the studied yield parameters measured (Table 4) showed a positive effect on yield during growth and development under water deficit conditions. A substantial increase in accumulation of glycinebetaine level under water stress was observed in many folds and there was a significant difference in glycinebetaine level in well-watered and water-limited conditions in all the genotypes. Some genotypes with low accumulation of glycinebetaine showed better seed cotton yield boll weight and boll number under water limited conditions. This may have been due to their long root system and short life cycle. Due to short crop duration,

drought escape /avoidance mechanism works for the plant. In other genotypes, high glycinebetaine accumulation showed a positive association with high seed cotton yield, boll number and boll weight. Glycinebetaine accumulation in cotton under water deficit conditions helps maintain osmotic potential and minimum water loss, which leads to better yield of cotton. This study showed the extent of variability for glycinebetaine accumulation in water limiting and control conditions of different cotton genotypes. Genotypic differences for glycinebetaine accumulation were found significant among cotton genotypes. The cotton genotypes used in the present study, synthesized higher amount of GB in water-limited environment than under normal conditions. The frequency of distribution of the glycinebetaine in the leaves in 20 cotton genotypes from the samples taken during the crop season 2006 is presented in Figure 1. Individual glycinebetaine levels of all the tested genotypes are presented in Table 5. Six genotypes of cotton exhibited GB levels ≥ 20 μmol g^{-1} FW.

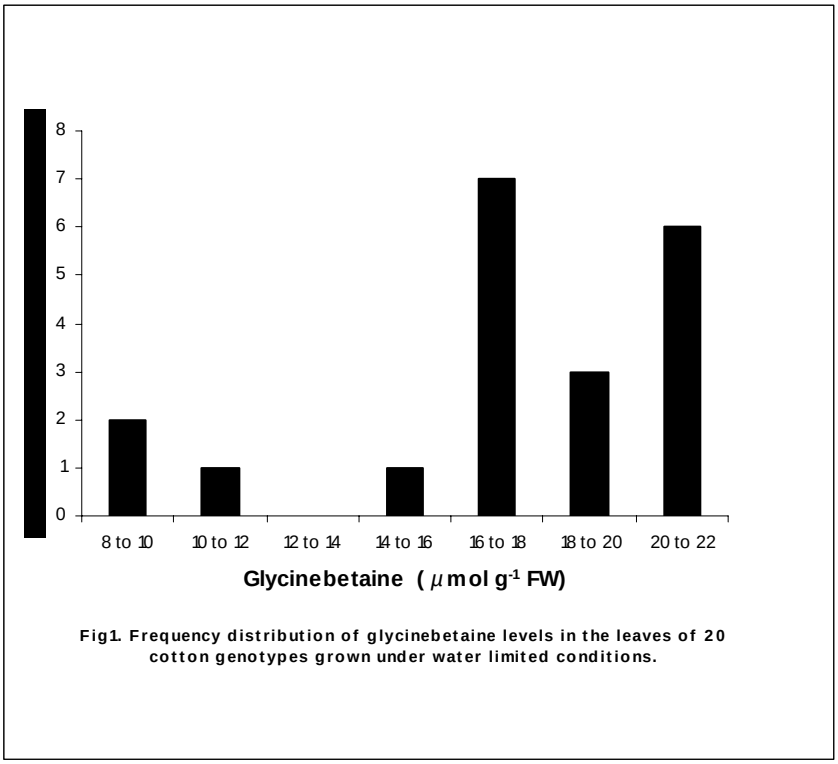

Fig1. Frequency distribution of glycinebetaine levels in the leaves of 20 cotton genotypes grown under water limited conditions.

Conclusion

From the results presented here it is amply clear that a considerable genotypic variation for glycinebetaine (GB) accumulation exists in the set of cotton cultivars examined and a clear relationship of *GB* accumulation in the leaves with plant productivity under water stress environment was observed. Therefore, *GB* accumulation in the leaves can be used as an indirect selection criterion for *SCY, BN, and BW* under water-limited environments. However, it requires information on heritability and

combining ability effects for its efficient use in breeding programmes. The association between *SCY* and *BN* under water stress suggests that *BN* is also a primary determinant of *SCY* under water stress conditions. Genetic improvement of *BN* under water stress may also improve seed cotton yield. Moreover, MNH-552, NIAB-Karishma, FH-87, CIM-1100, BH-160, and CIM-499 were found in the higher range of GB accumulation under water stress conditions and they could be exploited in breeding for drought tolerant cotton cultivars, and for cotton genomic studies. An understanding of the metabolic and genetic basis of this genotypic variation in GB accumulation in cotton may assist in devising breeding strategies to develop near-isogenic lines differing solely for the GB trait. These lines could then be used to test the contribution of this trait to drought tolerance.

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References

Ashraf, M. and M.R. Foolad 2007. Improving plant abiotic stress resistance by exogenous application of osmoprotectants glycinebetaine and proline. *Env. Exp. Bot*., 59:206-216.

Blunden, G., A.V. Pata, N.J. Armstrong and J. Gorham. 2001. Betaine distribution in the Malvaceae. *Phytochemistry*, 58: 451-455

Bohnert, H. J., D. E. Nelson and R.G. Jensen 1995 Adaptations to environmental stresses. *Plant Cell.*, 7: 1099-1111.

Boyer, J. S.1982.Plant productivity and environment. *Science,* 218: 443-448.

Colmer, T. D., E. Epstein and J. Dvorak. 1995. Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and salt-tolerant wheat x Lophopyrum elongatum (Host) A. Love amphiploid. *Plant Physiol*., 108: 1715-1724.

Gorham, J. 1996. Glycinebetaine is a major nitrogen containing solute in the Malvaceae. *Phytochemistry,* 43: 367-369

Grieve, C.M., and S.R. Grattan. 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

Grote, E. M., G. Ejeta and D. Rhodes. 1994. Inheritance of glycinebetaine deficiency in sorghums. *Crop Sci*., 34:1217-1220

Grumet, R., T. G. Isleib and A. D. Hanson .1985. Genetic control of glycinebetaine level in barley. *Crop Sci*., 25: 618-622.

Khan, A.H., M.Y. Ashraf, S.S.M. Naqvi, B.Khanzada and M. Ali**.** 1995. Growth, ion and solute contents of sorghum grown under NaCl and Na_2SO_4 salinity stress. *Acta Physiol. Plant.,* 17:261-268.

Kishitani, S, K. Watanabe, S. Yasuda, K. Arakawa and T. Takabe. 1994. Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants. *Plant Cell Environ*., 17: 89-95.

Larher, F., G. N. Rotival, P. Lemesle, M. Plasman and A. Bouchereau. 1996. The glycine betaine inhibitory effect on the osmo-induced proline response of rape leaf discs. *Plant Sci*., 113:21-31.

Lerma, C., P. J. Rich, G. C. Ju, W. Yang, A. D. Hanson and D. Rhodes. 1991. Betaine deficiency in maize. Complementation tests and metabolic basis. *Plant Physiol*., 95: 1113-1119.

Rahman, M., D. Hussain and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton cultivars. Genotypes by DNA fingerprinting technology. *Crop Sci*., 42: 2137-2144

Rhodes, D., P. J Rich, A. C. Myers, C.C. Reuter and G.C Jamieson. 1987. Determination of betaines by fast atom bombardment mass spectrometry: Identification of glycinebetaine deficient genotypes. *Plant Physiol*. 84 (3): 781-788

Rhodes, D., P.J. Rich, D.G. Brunk, G.C. Ju, J.C. Rhodes, M.H. Pauly and L.A. Hanson. 1989. Developing of two isogenic sweet corn hybrids differing for glycine contents. *Plant Physiol*. 91: 1112-1121.

Snedecor, G.W. and G.W. Cochran.1980. Statistical Methods, 7th edition, lowa State University Press, Ames, lowa.

Steel, R.G.D. and J.S. Torrie, 1980. Principles and procedures of statistics: A biological approach. 2nd edition. McGraw-Hill Book Company Inc. New York. Toronto, pp 633.

Sulpice, R., Y. Gibon, A. Bouchereau, and F. Larher. 1998. Exogenously supplied glycinebetaine in spinach and rapeseed leaf discs: compatibility or non-compatibility? *Plant Cell Environ*., 21: 1285-1292.

Yang, W. J., A. Nadolska- Orczyk, K.V. Wood, D.T. Hahn, P.J Rich, A.J. Wood, H. Saneoka, G.S. Premachandra, C.C. Bonham, J.C. Rhodes, R.J. Joly, Y. Samaras, P.B. Goldsbrough and D. Rhodes. 1995. Near-isogenic lines of maize differing for Glycine betaine. *Plant Physiol*., 107: 621-630

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