Triple Test Cross Analysis of Seed Cotton (*Gossypium hirsutum* L.) Yield and its Components Grown in Salinized Conditions

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ABSTRACT

The triple test cross analysis has been applied to the study of additive, dominance and epistatic components of genetic variance for some plant performance traits, namely plant height, number of bolls, boll weight, number of seeds per boll and seed cotton yield in (*Gossypium hirsutum* L.) under two NaCl concentrations (10 & 20 dSm^{-1}). The NaCl salinity caused significant reduction in the characters studied. The results of the genetic analysis revealed that the epistatic component was an important element for all the characters studied.

Key Words: Triple test cross; Genetics; (*Gossypium hirsutum* L.); NaCl; Salinity; Epistasis; Additive; Dominance

INTRODUCTION

Environmental stresses limit the yield potential of all the crops (Wittwer, 1980). Although biotic stress has significant adverse effect on plant production, the presence of abiotic stresses such as soil salinity and acidity, drought and high temperature, also cause major losses to the productivity of agricultural crops throughout the world (Clark & Duncan, 1993). Of the different crop production constraints, soil salinity is the most serious stress prevalent in arid and semi-arid regions of the world (Shannon, 1985; Ashraf, 1994; Lin *et al.*, 1997). In Pakistan, about 5.7 x 10^6 ha of arable land had been affected by salinity to varying degrees (Mujtaba *et al.,* 2003) and thus crop plants do not exhibit their potential under these conditions. In order to reclaim the salt affected area in Pakistan, the government has done a good deal of work through engineering approach under "Salinity Control and Reclamation Project" (SCARP), but such projects were not continued due to escalating cost of labour and energy in the country. Another approach i.e. biological fix, which aims at improving salt tolerance potential of crops, has also been suggested as a sure and cost effective means to utilize the huge salt affected area (Shannon, 1984; Qureshi *et al.,* 1990; Foy, 1997; Hollington, 1998). The success of developing such a useful plant material through selection and breeding depends on two basic components i.e. the existence of variability in a crop species for salt stress and secondly this variability must have a significant genetic component. The previous work on salt tolerance reveals that variability in salinity tolerance does exist both between and within plant species (Hassan *et al.*, 1999; Azhar & Ahmad, 2000; Ali *et al.,* 2002; Khan *et al.,* 2003).

Cotton (*Gossypium hirsutum* L.) is the main cash crop of Pakistan and is grown in the areas having salts in the root zone. Cotton had been described as moderately tolerant to salinity (Maas, 1986). Therefore, further development of salinity tolerance within the species would be of great value to the growers, but information on the genetic control of variation in cotton for salt tolerance is not extensively available. There are few studies reported, which show that the character is heritable (Liu *et al.*, 1998; Noor *et al.*, 2001; Bhatti & Azhar, 2002). Therefore, keeping in view the economic and social importance of cotton crop in our society, the present research work was carried out in order to investigate the role of additive, dominance and epistatic variation for salinity tolerance in cotton. In the present genetic investigations, Triple Test Cross technique (Kearsey & Jinks, 1968) was used. This biometrical method provides the workers un-ambiguous estimates of epistasis and in the absence of epistasis, un-biased estimation of the additive and dominance components also remains un-affected by differences in allele frequencies, degree of inbreeding and gene correlation and therefore was used here.

MATERIALS AND METHODS

In previous studies on salt tolerance, CIM-1100 (P$_1$) has been rated as susceptible (Noor *et al.*, 2001) and MNH-93 (P$_2$) as tolerant to salinity (Qadir & Shams, 1997) and therefore the two cultivars of (*Gossypium hirsutum* L.) were hybridized to develop F$_1$ seed. From F$_2$ population, 25 plants were randomly selected as males and crossed back to three testers, P$_1$, P$_2$ and F$_1$, to generate L$_{1i}$ (P$_1$ x F$_2$), L$_{2i}$ (P$_2$ x F$_2$) and L$_{3i}$ (F$_1$ x F$_2$) families as suggested by Kearsey and Jinks (1968). Thus 75 families (crosses) were obtained for genetic

studies on salt tolerance in the species.

The families were grown in earthen pots of 30 cm diameter lined with polythene sheet and each family consisted of four pots each having two plants. The response of the families to NaCl salinity was assessed under 3 treatments i.e. 10 dSm^{-1}, 20 dSm^{-1} and control (without salt). The experimental layout was a completely randomized design with three replications. The pots were filled with 8.5 Kg soil each and seeded in appropriate moisture conditions. When the seedlings emerged, all the pots were treated once with ½ strength Hoagland nutrient solution (Hoagland & Arnon, 1950). Salt treatment was started four weeks after sowing and the salinity levels were maintained up to maturity following U.S. Salinity Laboratory Staff (1954). At plant maturity, the data were recoded on plant height, number of bolls per plant, boll weight, number of seeds per boll and seed cotton yield per plant. The response of each family to 10 and 20 dSm^{-1} NaCl stress was compared with that in control called relative salt tolerance or indices of salt tolerance (Maas, 1986).

The means of indices of salt tolerance of 75 genotypes in 10 and 20 dSm^{-1} NaCl stress were subjected to ordinary analysis of variance technique. The test of epistasis ($L_{1i} + L_{2i} - 2L_{3i} = 0$) was carried out according to Perkins and Jinks (1970). Variances of the sums ($L_{1i} + L_{2i}$), and differences ($L_{1i} - L_{2i}$) were used to estimate additive (D) and dominance (H) components respectively (Singh & Chaudhary, 1985). The estimates of degree of dominance ($\sqrt{H/D}$) were also worked out (Wigan, 1944).

RESULTS

Analyses of variance of indices of salt tolerance showed that plant height, number of bolls per plant, boll weight, number of seeds per boll and seed cotton yield measured between 75 TTC families in 10 and 20 dSm^{-1} NaCl levels differed significantly (P≤0.01) (Table I). The two NaCl salinities were also significantly different (P≤0.01) in terms of their effects on all the plant characters. The significant (P≤0.01) interaction term (families x salinities) revealed that the 75 families were affected differently under the two salinity levels. Results of triple test cross analyses revealed the presence of epistasis in all the characters, but the magnitude varied; it was highly significant (P≤0.01) for plant height, boll weight and number of seeds in both the salinities, but for seed cotton yield it occurred in 10 dSm^{-1} only, whilst the level reduced to significant (P≤0.05) for boll number in 10 dSm^{-1} (Table II). However, further partitioning of epistasis revealed that i type epistasis (additive x additive interaction) was non-significant for all the characters measured in low and high NaCl stresses except plant height in 10 dSm^{-1}. The presence of 'j' (additive x dominance) and 'l' (dominance x dominance) type epistasis appeared to be highly significant (P≤0.01) in the inheritance of all the characters except

number of bolls in 20 dSm^{-1}, where it was absent (Table II).

The mean squares for sums ($L_{1i} + L_{2i}$) and differences ($L_{1i} - L_{2i}$) were highly significant (P≤0.01) for all the characters in both the salinity levels (Table III) except number of bolls in 10 dSm^{-1}, where the significance level reduced to significant (P≤0.05). The estimates of additive (D) and dominance (H) components were found to be almost consistent at the two salinity levels. Low magnitude of the ratio ($\sqrt{H/D}$) indicated the predominant role of additivity in controlling plant height in both the salinities and number of seeds in 20 dSm^{-1}, whilst this ratio was almost equal to unity for number of bolls and boll weight in 10 and 20 dSm^{-1} and for number of seeds in 10 dSm^{-1}, however this ratio was greater than 1 for seed cotton yield (Table III).

DISCUSSION

The expression of salt tolerance in any crop species is a complex character and is the manifestation of both physiological and morphological characters (Shannon, 1984). However, when a specific and readily quantifiable physiological mechanism conferring salt tolerance is not available, the assessment of plant material based upon other characters of agronomic importance i.e. plant yield and its components had been suggested to be practical alternative method (Noble *et al.*, 1984). In the present investigations, the genetic material was subjected to constant NaCl stress of 10 and 20 dSm^{-1} from fourth week of sowing till the picking of plants. Therefore, such a procedure provided better evaluation of potential salinity tolerance, as had been done in corn (Kaddah *et al.,* 1964), bean (Meiri & Poljakoff-Mayber, 1970) and sorghum (Azhar & McNeilly, 1989).

Genetic analyses of the data revealed that epistasis affected plant height, boll weight and number of seeds in 10 and 20 dSm^{-1} salinity regimes and for number of bolls and seed cotton yield it was present in 10 dSm^{-1}, whilst in 20 dSm^{-1} it was absent in these two characters. Thus the presence of epistasis complicated the inheritance of plant height, boll weight and number of seeds in low and high salinities (Kumar & Raveendran, 2001). The presence of additive x additive interaction was absent in the inheritance of all the characters except plant height in 10 dSm^{-1}, however additive x dominance and dominance x dominance epistasis (j + l type) appeared to complicate the inheritance of these characters measured in low and high salinity levels except number of bolls in 20 dSm^{-1} (Kumar & Raveendran, 2001).

The TTC analyses further showed that although both additive (D) and dominance (H) components of variation appeared to predominantly affect all the characters measured in both the salinities, the ratio of ($\sqrt{H/D}$) indicated that plant height in low and high salinities and number of seeds in 20 dSm^{-1} were influenced predominantly by the additivity of the genes as ($\sqrt{H/D} < 1$), whilst number of bolls

Table I. Mean squares from analyses of variance of five plant traits measured on 75 families in 10 dSm^{-1}, and 20 dSm^{-1} NaCl salinities

Sources of variation	d.f	Plant height	No. of bolls	Boll weight	No. of seeds/boll	Seed cotton yield
Families	74	125.15**	539.80**	370.05**	337.49**	509.90**
Salinities	1	23338.08**	78206.70**	41107.81**	891.80**	122483.8**
Families x Salinities	74	85.43**	269.90**	112.61**	63.38**	253.40**
Error	300	11.92	132.90	11.98	12.21	64.40

** denotes highly significant (P≤0.01) differences.

Table II. Test of epistasis using (L$_{1i}$ +L$_{2i}$ − 2 L$_{3i}$) comparisons for five plant characters measured in two NaCl salinities

Item	d.f.	Plant height		Number of bolls		Boll weight		Number of seeds		Seed cotton yield	
		10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}
Total epistasis	25	851.90**	566.05**	3896.10*	1390.30[NS]	2322.78**	1439.28**	1232.95**	783.4**	3912.50**	705.70[NS]
i type epistasis	1	11403.90*	839.48[NS]	39088.80[NS]	2327.90[NS]	4712.24[NS]	2049.27[NS]	2552.55[NS]	193.96[NS]	34427.10[NS]	2112.05[NS]
j+l type epistasis	24	412.30**	554.66**	2429.70**	1351.20[NS]	2223.22**	1413.87**	1177.97**	807.96**	2641.00**	647.10**
Total epistasis x replicates	50	118.90	195.79	1799.50	1596.40	109.85	74.89	201.14	127.1	1111.30	430.39
i type epistasis x replicates	2	1524.40	2638.86	25584.40	21099.60	1501.79	945.07	2777.8	1672.04	15196.70	6045.82
j_l type epistasis x replicates	48	60.30	93.99	808.50	783.80	51.85	38.63	93.78	62.73	524.50	196.41

NS = Non-significant (P≥0.05); * = Significant (P≤0.05); ** = Highly significant (P≤0.01)

Table III. Mean squares due to sums (L$_{1i}$ + L$_{2ii}$) and differences (L$_{1i}$ - L$_{2i}$), estimates of additive (D) and dominance (H) components, and degree of dominance (√H/D) based upon relative values of five plant characters measured in two NaCl salinities

Item	d.f.	Plant height		Number of bolls		Boll weight		Number of seeds		Seed cotton yield	
		10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}	10 dSm^{-1}	20 dSm^{-1}
Sums	24	300.27**	239.39**	837.96**	537.88**	543.03**	258.56**	474.69**	332.73**	901.57**	263.03**
Sums x replicates	50	18.03	25.34	429.58	187.46	25.88	20.52	27.69	24.71	274.92	64.32
Differences	24	151.41**	124.39**	588.15**	522.29**	531.26**	277.71**	550.52**	209.36**	1137.14**	362.19**
Differences x replicates	50	22.27	24.56	244.55	210.16	39.51	20.29	23.29	13.94	169.99	69.72
D		376.31	285.39	544.51	467.23	689.54	317.39	596.01	410.69	835.54	264.95
H		172.19	133.11	458.13	416.17	655.67	343.24	702.97	260.56	1289.54	389.96
√H / D		0.68	0.68	0.92	0.94	0.98	1.04	1.08	0.79	1.24	1.21

*, **, denote significant (P≤0.05) and Highly significant (P≤0.01) differences respectively.

and boll weight in 10 and 20 dSm^{-1} and number of seeds in 10 dSm^{-1} NaCl showed the importance of both additive and dominance properties of genes since degree of dominance here was almost equal to unity (Table III). The higher degree of dominance (√H/D > 1) for seed cotton yield in both low and high salinities showed over-dominance. It had been reported that inheritance of salinity tolerance in *Sorghum bicolor* (Azhar & McNeilly, 1988), quantitative trait loci (QTL) in soybean (Lark *et al.*, 1995), salt tolerance in maize (Khan & McNeilly, 2005) seed cotton yield, boll weight and number of bolls in up-land cotton (Kumar & Raveendran, 2001), was affected by the involvement of epistatic genetic component. Therefore, the information reported here appeared to agree to the previous findings. The presence of epistasis at additive x dominance loci suggests that salt tolerance in (*Gossypium hirsutum* L.) is a complex phenomenon.

The present results suggest that simultaneous improvement for all the traits studied requires the population improvement programme, however for the characters that involve predominantly additive gene effects in their inheritance e.g. plant height and number of seeds, may be improved through selection in early generations as

suggested by Tripathi and Singh (1983) and Kumar and Raveendran (2001). For other characters like seed cotton yield, showing the presence of dominance gene effects, the population should be handled by bulk selection forwarded by individual plant selection in later generations.

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