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Genetic Mechanisms Controlling Variation for Salinity Tolerance in Upland Cotton at Plant Maturity

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ABSTRACT

The genetic basis of salt (NaCl) tolerance at maturity stage was examined in six accessions of *Gossypium hirsutum* L. at plant maturity. The six accessions were crossed in all possible combinations. The NaCl tolerance of 30 F_1 hybrids and their six parents were assessed at maturity level in the iron containers by subjecting to constant NaCl treatments i.e., 0, 17.5, and 20 dS/m salinity. Indices of salt tolerance (relative salt tolerance) were analyzed using diallel method. Both additive and dominance effects appeared to be important for the expression of variation under low and high salinity levels. Estimates of narrow sense heritabilities for salt tolerance were remarkable. This suggested that rapid improvement in (NaCl) tolerance in *G. hirsutum* using high selection pressures in the F_2 population may be made through selection and breeding. © 2010 Friends Science Publishers

Key Words: Upland cotton; NaCl salinity; Heritability; Relative salt tolerance

INTRODUCTION

The problem of soil salinity is of frequent occurrence in arid and semi arid regions (Ashraf & Fatima, 1994; Lin *et al.*, 1997; Khan *et al.*, 2001). In Pakistan, due to continuous use of low quality irrigation water for agriculture purpose, about $5.7x10^6$ ha of arable land had been rendered saline (Mujtaba *et al.*, 2003). Although the engineering approach has been effective in decreasing the concentration of salts to a greater extent, the escalating cost of labor and energy, have become uneconomical in developing countries like Pakistan. The alternative strategy, 'the genetic approach' which appears to be feasible and practicable for the development of crop cultivars suitable for the areas affected. This approach had been emphasized by many research workers (Qureshi *et al.*, 1990; Azhar & Khan, 1997; Hollington, 1998; Shannon *et al.*, 1998; Rao & McNeilly, 1999; Khan *et al.*, 2003; Madidi *et al.*, 2004).

For bringing evolutionary change in any plant character e.g., in salt tolerance there are two important components. Firstly, there must be significant variation for the character to be improved and secondly, the character must be affected by genetic component. In the previous reports, presence of variation in salt tolerance had been observed in different crops, as for example, in wheat (Akhtar *et al.*, 2003; Bhatti *et al.*, 2004; Ali *et al.*, 2007), maize (Rao & McNeilly, 1999; Khan *et al.*, 2003), sorghum (Azhar & McNeilly, 1987, 2000 & 2001), sunflower (Bhutta *et al.*, 2004), barley (Czembor, 2000; Dakir *et al.*, 2002; Madidi *et al.*, 2004), rice (Ahmad *et al.*,1990; Shannon *et al.*, 1998; Lee *et al.*, 2003).

Cotton (*Gossypium hirsutum* L.) is an important cash crop grown in the area mostly affected by salinity in the country. Thus different researchers conducted the studies on salinity tolerance in cotton showed that variation at seedling stage exists within *Gossypium hirsutum* L. (Ashraf & Ahmad, 1999; 2000a & b; Noor *et al.*, 2001; Saqib *et al.*, 2002; Bhatti & Azhar 2002; Khan *et al.*, 2004; Akhtar *et al.*, 2005; Ali *et al.*, 2005; Bhatti *et al.*, 2006; Azhar *et al.*, 2007). However, a few studies explored the genetic mechanism controlling salinity tolerance at early plant development ((Liu *et al.*, 1998; Bhatti *et al.*, 2006; Azhar *et al.*, 2007). In vie of the fact that measurement of salinity using agronomic characters at plant maturity is the most dependable method (Noble *et al.*, 1984), the present investigation is an attempt to provide information regarding genetic basis of salinity tolerance in *Gossypium hirsutum* L.

MATERIALS AND METHODS

Hybridization of parents: For the development of plant material for genetic studies, six parents i.e., NIAB 78, B557, MNH 522, Qalandri, MNH 147 and BP52NC63 were grown in earthen pots in glasshouse during October to November, 2005. Each parent had eight pots and seedlings were thinned to two plants per pot. For good health, growth and development of plants, 0.25 g Urea fertilizer (46% N) was supplied to each pot every 15 days after planting, and plants were watered daily. When the parents started to flower, these were crossed in all possible combinations

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using hand emasculation and pollination. Maximum number of pollinations were attempted to produce sufficient quantity of F_1 seeds, whilst some of the buds were also covered with glaycine bags to produce selfed seed. All the precautionary measures were adopted during crossing to avoid foreign pollen contamination of the genetic material. At maturity, hybrid seed cotton from crossed as well as selfed bolls were picked and ginned to obtain seeds.

Assessment of response of F_1 hybrids and their parents to NaCl salinity at plant maturity: In order to study the genetic basis of responses of accessions/lines to salinity, 30 F_1 hybrids and six parents were planted under 17.5 and 20 dS/m and a control. For planting the material 54 iron containers measuring 157.5 cm × 90 cm × 45 cm were used for experimentation. The plant material was sown following completely randomized design with three replications; thus there were 18 containers in each replication. In each container six genotypes each having five plants spaced 18 cm within the rows at 25 cm apart from other row were grown. After the emergence of seedlings, all the containers were watered once with ½ strength Hoagland nutrient solution (Hoagland & Arnon, 1950). The desired NaCl salinity i.e., electrical conductivity (EC) of 17.5 and 20 dS/m considering the saturation % of soil in the containers were prepared in the nutrient solution and applied to the plants. The experimental units to be treated as control were fed with only nutrient solution. The salinity levels were checked weekly, using the EC meter and maintained by adding proper quantity of salt solution to the container. The experiment was conducted during cropping season i.e., April-May 2006. The containers were continuously watered till plant maturity, as and when needed to keep them at field capacity on the basis of visual observation and experience. The plant material was sprayed, when required, to save the plants from the attack of sucking pests and boll worms. Yield of seed cotton (g), number of bolls per plant and boll weight (g) were measured to see the response of the genetic material to the salinized conditions at maturity.

Indices of salt tolerance: The responses of all the entries to increasing NaCl salinity were compared on relative basis (Maas, 1986). Relative salt tolerance may be defined as % growth of a genotype in salinized condition compared with that in control. Relative salt tolerance was computed according to the following formula:

$$\text{Relative salt tolerance (Salt tolerance index)} = \frac{\text{Value of a character in NaCl}}{\text{Value of a character in control}} \times 100$$

Statistical analysis: Ordinary analysis of variance technique was run on all the data to see whether the genotypic differences are significant (Steel *et al*., 1997). Only significant differences among genotypes to validate the data for genetic analysis (mean squares are omitted from the text).

The adequacy of simple additive- dominance model to account for the data set on three plant characters was determined by joint regression (b) of variance (V_r) and co-variance (W_r). According to the suggestions of Hayman (1954), the regression co-efficient (b) must deviate significantly from zero, but not from unity, if all the assumptions underlying the genetic model were met.

RESULTS

Joint regression analysis was carried out using indices of salt tolerance of F_1 generation under 17.5 and 20 dS/m and the results are given in (Fig. 1a b, 2a b & 3a b). The regression co-efficient of yield of seed cotton obtained under low salinity, 17.5 dS/m (b = 0.770±0.192), number of bolls (b = 0.843±0.134) and boll weight (b = 0.723±0.145), deviated significantly from zero and are of unit slope, at high salinity the regression co-efficient of yield of seed cotton (b = 0.951±0.306), number of bolls (b = 1.223±0.218) and boll weight (b = 0.794±0.155), appeared to deviate significantly from zero, but not for unity. The results revealed that the simple genetic model was fully adequate to the data set on yield of seed cotton, number of bolls and boll weight under low and increased salinities.

Estimation of Genetic Components of Variation under Salinity

Yield of seed cotton: Genetic components of variation, estimated at low and high salinity levels are presented in Table I. The magnitude of additive effect, D under 17.5 dS/m (11.72) and 20 dS/m (51.15) are greater than the dominance variance, H_1 (5.97) and proportion of positive and negative genes in the parents, H_2 (5.20), suggesting the importance of cumulative gene in the inheritance of yield of seed cotton. The negative sign of relative frequency of dominance and recessive alleles in the parents, F at 17.5 dS/m indicated that number of recessive alleles was more frequent than dominant alleles in the parents, while the reverse was true in 20 dS/m, which indicated the presence of dominant alleles in the parent. A positive sign of over all dominance effects of heterozygous loci, h^2 at 17.5 dS/m indicated the trend of dominance towards the parents towards more yield of seed cotton, but reverse was observed at 20 dS/m. Estimate of narrow sense heritability of yield of seed cotton was almost equal 0.82 and 0.85 in 17.5 and 20 dS/m, respectively. Variety BP52NC63 contained the maximum number of dominant genes in 17.5 dS/m, whilst Qalandri possessed more recessive genes (Fig. 1a). At higher salinity 20 dS/m variety B557 contained the maximum number of dominant genes, and BP52NC63 possessed the maximum number of recessive genes (Fig. 1b).

Number of bolls per plant: Relative size of D, H_1 and H_2 showed that the effect of additive genes was important in affecting variation in the number of bolls, the plant material tested at both the salinity levels. The unequal estimates of H_1 and H_2, at both the salinities, revealed unequal distribution of genes in the parents, which was verified by the ratio of $H_2/4H_1$ i.e., 0.21 (for equal distribution the maximum value is 0.25). The negative sign of F at 17.5 dS/m suggest that number of recessive genes were more

Table I. Estimates of components of variation in three plant characters

Components	Yield of seed cotton		No. of bolls		Boll weight	
	17.5 dS/m	20 dS/m	17.5 dS/m	20 dS/m	17.5 dS/m	20 dS/m
D	11.72±1.02	51.15±2.21	285.35±16.63	407.33±7.53	154.53±15.78	291.25±23.90
H_1	5.97±2.37	18.95±5.10	129.59±38.39	76.27±17.39	240.01±36.45	126.93±55.19
H_2	5.20±2.06	11.59±4.44	109.06±38.39	52.41±15.14	215.18±31.71	103.13±48.02
F	-2.51±2.44	12.71±5.25	-24.64±39.47	168.46±17.88	19.42±37.47	0.64±56.73
h^2	0.25±1.38	-0.46±2.97	0.969±22.35	-1.39±10.13	73.08±21.22	3.51±32.13
E	0.37±0.34	0.99±0.74	8.02±5.57	6.10±2.52	6.16±5.29	6.75±8.00
$(H_1/D)^{0.5}$	0.71	0.61	0.67	0.43	0.75	0.66
$H_2/4H_1$	0.22	0.15	0.21	0.17	0.22	0.200
$Heritability_{ns}$	0.82	0.85	0.82	0.87	0.57	0.83

Fig. 1a: Wr/Vr graph for yield of seed cotton in17.5 dS/m

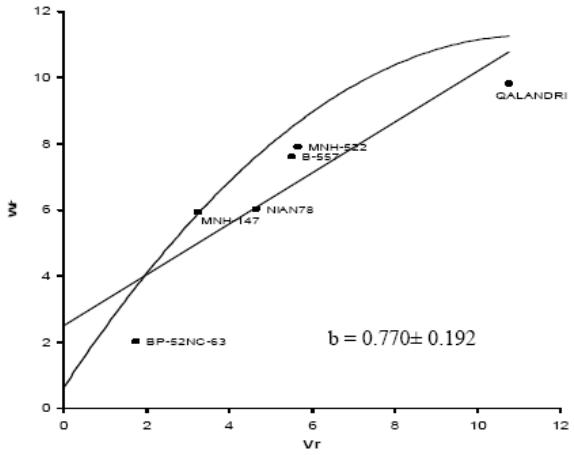
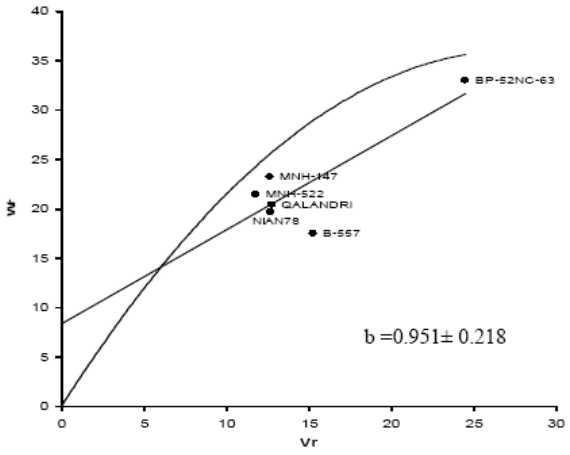


Fig. 1b: Wr/Vr graph for yield of seed cotton in 20 dS/m



frequent than dominant genes but at 20 dS/m reverse was true. The positive sign of h^2 indicated the trend of dominance towards the parents with greater number of bolls at 17.5 dS/m and vice versa. The estimates of narrow sense heritability at both the salinities appeared to be almost similar i.e., 0.82 and 0.87. Perusal of data showed that B 557 at 17.5 dS/m and MNH 522 at 20 dS/m manifested the greatest number of dominant genes and in contrast Qalandri and B 557carried the greater number of recessive genes for number of bolls per plant (Fig. 2a & b).

Boll weight: The estimates of genetic variation in boll weight were significant (Table I). However greater magnitude of H_1 and H_2 than that of D at 17.5 dS/m indicated that genes with non-additive properties were important in the inheritance of boll weight. The magnitude of H_1 and H_2 at both the salinity levels, indicated unequal distribution of genes in the parents and this was verified by $H_2/4H_1$ at low salinity (0.22) and high salinity (0.20). The positive value of F indicated dominant genes were more frequent than recessive genes. Positive sign of h^2 suggested that dominance appeared to be towards the parents having heavier boll weight. The degree of dominance was exhibited by the ratio $(H_1/D)^{0.5}$ = 0.75 at low salinity and 0.66 at high salinity, a partial dominance (Fig. 3a). The estimate of narrow sense heritability for the character at 17.5 dS/m was 0.57.

At 20 dS/m, genes with both additive and non-additive genes controlled variation in the boll weight since as D, H_1 and H_2 components of variation were significant ($P<0.05$). The low estimate of $(H_1/D)^{0.5}$ (0.66) revealed partial dominance for boll weight and this situation was supported by the intercept of regression line on the positive side of Wr axis (Fig. 3b). A greater difference between H_1 and H_2 and a low ratio of $H_2/4H_1$ (0.20) suggested that genes were unequally distributed in the parents. The positive sign of F indicated the presence of more dominant genes in the parents than recessive genes for boll weight. The narrow sense heritability was (0.83).

DISCUSSION

In other studies, salinity was applied at late developmental stages of for example in rice, salt treatment was started at the early tillering, late tillering and heading stages (Pearson & Bernstein, 1959), in maize at the vegetative, tasseling and grain filling stages (Maas *et al.*, 1983), in sorghum during vegetative, reproduction and maturation period (Maas *et al.*, 1986; Azhar & McNeilly, 1989) to determine inheritance for salinity tolerance. In present work on cotton, the genetic material was subjected to constant NaCl stress from transplanting of the seedling till the harvest of plant at maturity. Such a procedure would

Fig. 2a: Wr/Vr graph for Number of bolls in 17.5 dS/m

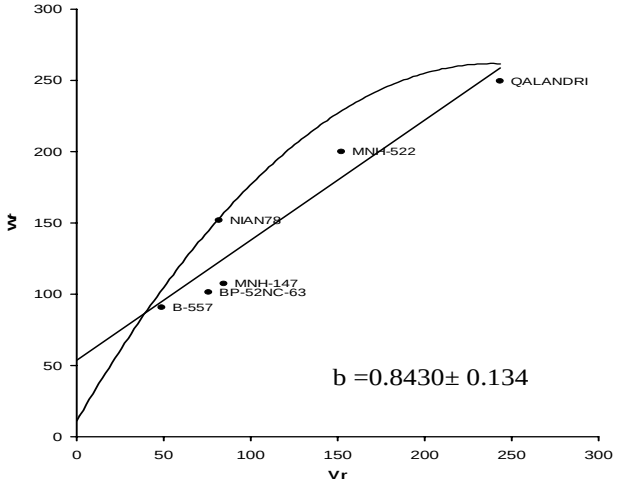


Fig. 2b: Wr/Vr graph for Number of bolls in 20 dS/m

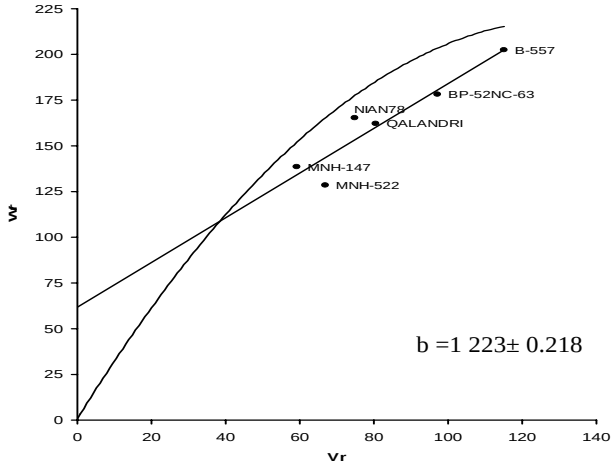


Fig. 3a: Wr/Vr graph for Boll wieght in 17.5 dS/m

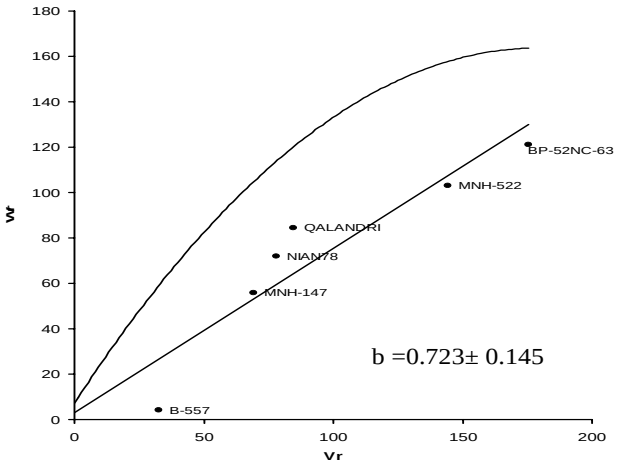
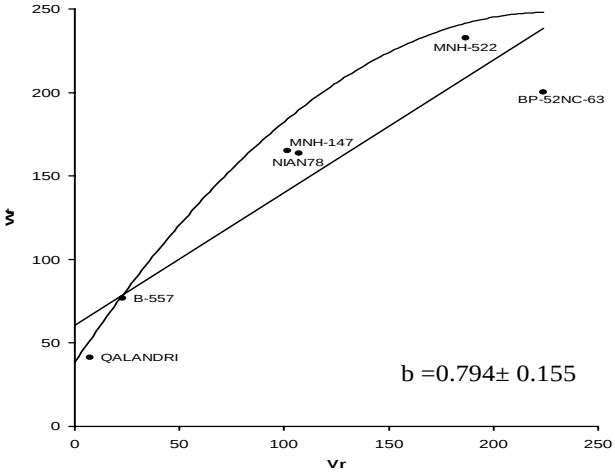


Fig. 3b: Wr/Vr graph for Boll wieght in 20 dS/m



seem to provide better evaluation of the genetic material for salinity tolerance, as suggested by Meiri and Poljakoff-Mayber (1970).

It is suggested earlier that when readily quantifiable physiological mechanism conferring salt tolerance is not available, assessment of plant material based upon the measurement of other plant characters of agronomic importance i.e., yield of green matter and grain yield appears to be practical alternative (Noble *et al.*, 1984). Thus based upon this suggestion, Azhar and McNeilly (1989) evaluated four sorghum accessions for their salinity tolerance at plant maturity and measured relative grain yield, relative grain weight and relative number of grains/spike as an indicator of the responses to salinity. In the present plant material agronomic measurements on number of bolls, boll weight and yield of seed cotton were made to study the responses as suggested by Noble *et al.* (1984). When such a useful plant material is available for improving salinity tolerance in (*G. hirsutum* L.), the use of biometric methods may provide information on genetic mechanism controlling variation in salinity tolerance becomes important. It helps to estimate relative contribution of the genetic components of variation i.e., additive, non-additive and epitasis, etc. in salinity tolerance. Of those, simple additive model was found to be fully adequate for analyzing data for yield of seed cotton, number of bolls and boll weight at 17.5 and 20 dS/m. The diallel cross analysis of salinity tolerance has been done previously in cotton (Akhtar & Azhar, 2001; Azhar *et al.*, 2007), sorghum (Azhar & McNeilly, 2001) and maize (Khan *et al.*, 2003).

From the present data it is clear that variation in responses to salinity was controlled by additive genes at low salinity (17.5 dS/m), whilst boll weight appeared to be affected by non-additive genes. At high salinity (20 dS/m), yield of seed cotton, number of bolls and boll weight were revealed to be effected by the additive genes as evident from the high values of additive variance, D i.e., 51.15±2.21, 407.33±7.53 and 291.25±23.90, respectively. Although

cumulative genes effects appeared to be important in controlling variation in salinity tolerance, dominance acted towards greater NaCl tolerance. This is clearly advantageous in a breeding program aimed at improving salinity tolerance of *G. hirsutum.* The estimates of narrow-sense heritability were also high, in both the salinity levels, due to greater magnitude of additive component. In the present investigations magnitude of additive component and magnitude of heritability narrow sense for yield of seed cotton, number of bolls and boll weight appeared to be greater at 20 dS/m, NaCl salinity as compared to those at 17.5 dS/m, as reported by Blum, (1998), Hoffmann and Parsons (1991). High estimates of heritability in narrow sense represented fixable and additively heritable variation, which indicated that selection response should be rapid for these characters. The estimates of heritability of agronomic characters used in the present investigation are high, which might be due to greater additive genetic variation, due to expression of genes associated with salinity tolerance or a smaller environmental variation (Saranga *et al*., 1992). It is argued else, where that hidden variation previously could be un-covered when moderate stress is applied, thus showing the possibility of increasing heritability estimates (Bradshaw & Hardwick, 1989).

Falconer and Mackey (1997) suggested that the estimates of heritability are subjected to environmental variation and therefore must be used with great care, while screening the breeding material. Nonetheless these estimates and mode of inheritance of salinity tolerance seem to be encouraging to a breeder and making selection for plants with enhanced salt tolerance in subsequent generations based upon the characters of agronomic importance at maturity. Thus based upon the available reports on maize (Rao & McNeilly,1999), rice (Gregoria & Senadhira, 1993; Shannon *et al*., 1998), pearl millet (Kebebew & McNeilly, 1999), lucerne (Al-Khatib *et al*., 1994) and tomato (Foolad, 1996) and results of present work, it seems likely that significant improvement in these species may be made through selection and breeding.

In conclusion, variation against NaCl salinity in cotton was controlled by additive as well as non-additive gene effects. Salinity tolerance based upon yield of seed cotton, number of bolls and boll weight appeared to be highly heritable as evident from high estimates of heritability narrow sense, thus suggesting that improvement in salinity tolerance may be made by selection of desirable plants.

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