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GENETIC MECHANISMS CONTROLLING SALT TOLERANCE IN *GOSSYPIUM HIRSUTUM* L. SEEDLINGS

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Abstract

Inheritance of salt tolerance at the seedling stage was studied in 5 varieties/lines (both local and exotic) of *Gossypium hirsutum* L. The five parents were crossed in all possible combinations. The 20 F_1 hybrids and their parents were grown in a mixture of sand and soil in 2:1 ratio, following completely randomised design with three replications. The response of the growing seedlings was examined to salinized (250 mM NaCl) and non-salinized (control) conditions. After three weeks, the longest roots of 25 families were measured under the two conditions. Indices of salt tolerance (relative root length) were used for genetic analysis following Hayman-Jinks approach. The regression coefficient ($b = 0.566 \pm 0.161$), and analysis of variance of (W_r+V_r) and (W_r-V_r) showed that the data were fit for genetic analysis. It was revealed that genes acted both additively and non-additively for controlling root length in salinized conditions and estimate of h^2_{ns} was 0.37. These results suggest that salt tolerance in the species may be improved further by selecting the plants having longer roots from the segregating generations in salinized media.

Introduction

Soil salinity is a limiting factor in allowing exploitation of crops in many parts of the world and the phenomenon is of frequent occurrence in arid and semiarid areas. After the development of the concept of breeding crop cultivars suitable for the saline soils (Epstein *et al*., 1980; Shannon, 1985), the geneticists and breeders started to improve salinity tolerance of existing crop species, through selection and breeding. During the past two decades there has been marked increase in attempts to overcome the problem of salinity through the adoption of biological approach. The work done on different crops showed that variation for salinity tolerance within the species existed in maize (Maas *et al*., 1983; Noora *et al*., 1995; Rao & McNeilly, 1999; Khan *et al*., 2003), sorghum (Azhar & McNeilly, 1987, 2001), wheat (Ashraf & McNeilly, 1988; Ahsan *et al*., 1996; Noori & McNeilly, 2000), rice (Ahmad *et al*., 1990), triticale and barley (Salim, 1991), lucerne (Al-Khatib *et al*., 1995), and millets (Kebebew & McNeilly, 1996). All these studies revealed that the variation may be exploited through hybridisation followed by selection in order to improve salinity tolerance in these crops.

Soils of Pakistan present the best illustration of the advance of salination, and according to an estimate about 5.7×10^6 ha of arable land have been affected by salinity in varying degrees (Mujtaba *et al*., 2003). Cotton (*Gossypium hirsutum* L.) is the major crop of the area affected and thus the development of salinity tolerance within the species would be of a value to the growers. Previous studies of salinity tolerance in cotton are relatively few, but they do suggest that variation exists within the species (Gossett *et al*., 1992; Jafri & Ahmad, 1994, Malik *et al*., 1994; Khan *et al*., 1995). Recently, Azhar &

Raza (2000), Akhtar & Azhar (2001) and Noor *et al*. (2001), found that varieties of Upland cotton responded differently to NaCl salinity. However, there is no study reported, which could explain the genetic basis of salinity tolerance in *Gossypium* species. Therefore, the present study was conducted in order to examine the genetic basis of variation in NaCl tolerance in *Gossypium hirsutum* L.

Materials and Methods

Plant materials: The plant material used in the present study was produced by crossing five varieties/lines viz., CIM-435, CIM-443, CIM-448 and B-876 (all four local) and Coker-CQ (exotic), according to diallel mating system. In order to produce sufficient number of F_1 seeds, maximum number of buds were emasculated in the evening and pollinated with the desired pollen the following morning. During emasculation and pollination necessary precautionary measures were made to avoid alien pollen contamination.

Assessment for salinity tolerance: The response of 20 F_1 hybrids and their parents to NaCl salinity was assessed in a glasshouse. The seeds were sown in polythene bags (15cm x 20cm) filled with mixture of sand and soil in 2:1 ratio. The bags were washed with plenty of distilled water. At proper moisture content, 5 seeds of each genotype were planted in each bag. Two NaCl treatments, containing standard and 250 mM were used for comparing the responses of genotypes. Each treatment for each entry was replicated thrice following completely randomised design. One week after germination (at two leaved stage), NaCl solution having 250 mM concentration was applied to 25 entries in each replication, and this was continued for two weeks at the interval of two days. Before applying the solutions, the treatment bags were thoroughly washed with deionised water. After three weeks growth, the longest root of five seedlings grown in the two treatments in each of the three replicates was measured. Thus mean root length of each family was based upon a total of 15 progeny measurements. The mean relative root length of each family measured in NaCl treatment was compared with control treatment (without salt) as suggested by Maas (1986). The data on relative salt tolerance were used to examine the genetic basis of differential genotypic responses to salinity.

Statistical analysis: Before analysing the data for genetic interpretation following Hayman-Jinks model it was necessary to see whether genotypic differences for root length are significant. Therefore the data of 20 F_1 hybrids and their parents were subjected to ordinary analysis of variance technique.

The validity of estimates and inferences derived from the simple additive-dominance model is dependent upon fulfilment of the basic assumptions underlying the model. Thus to assess the validity of some of the assumptions, and to determine fitness of the data to the genetic model, preliminary analysis of the data was carried out following Hayman (1954b). From the diallel data, variances of the component of each array (V_r), and covariances of all the offspring included in each parental array with the recurrent parent (W_r), variance of parental means (V_0L_0), variance of array means (V_0L_1), means of array variances (V_1L_1), and mean array covariences (W_0L_0) were calculated. All these statistics are given in Table 3.

Table 1. Analysis of variance of relative root length of 25 families of *G. hirsutum* L., measured under 250 mM NaCl salinity.

Sources of variation	D.F.	Sum of squares	Mean squares
Genotypes	24	2007.70	83.85**
Error	50	364.53	7.29

**, Denotes differences among genotypes highly significant

Table 2. Mean relative root length (over replications and reciprocals) of five parents and 20 F_1 hybrids of *G. hirsutum* L., and variance (V_r) and covariance (W_r).

Parents	CIM-435	CIM-448	CIM-443	B-876	Coker-CQ	V_r	W_r
1. CIM-435	71.00	74.78	84.51	81.59	83.60	35.01	19.66
2. CIM-448		81.04	84.96	85.44	82.61	18.45	14.82
3. CIM-443			87.52	85.60	81.99	3.99	6.95
4. B-876				73.41	77.42	27.81	22.99
5. Coker-CQ					76.89	9.70	4.27
Means	79.10	81.77	84.92	80.69	80.50	18.99	13.74

Table 3. Statistics calculated from the diallel data, and components of genetic variation in relative root length of five parents and 20 F_1 hybrids of *G. hirsutum* L.

Statistics / component of genetic variation	Estimates
V_0L_0	42.79
V_0L_1	4.78
V_1L_1	18.99
W_0L_0	13.74
E	1.22±2.02
D	41.57±5.08
H_1	60.62±4.64
H_2	55.40±12.45
F	29.16±12.70
H	12.90±8.40
H_1- H_2	5.22
$H_2/4H_1$	0.23
$(H_1/D)^{0.5}$	1.21
$½F /\{D (H_1-H_2)\}^{0.5}$	0.99
h^2 ns	0.37

Results

The results of analysis of variance of relative salt tolerance of parents and 20 F_1 hybrids revealed highly significant differences between the genotypes (Table 1). The significant differences allowed the use of the simple additive dominance model for genetic analysis of the data. The mean values of relative salt tolerance (over replications and reciprocals) of 25 genotypes are given in Table 2.

To test the fitness of the data to the genetic model and the validity of some of the assumptions underlying the model, two scaling tests i.e., joint regression analysis and analysis of variance of (W_r+V_r) and (W_r-V_r) were carried out. The unit slope of the regression line (b = 0.566±0.161, Fig. 1) and highly significant differences between the arrays (W_r+V_r) and non-significant differences within the arrays (W_r-V_r) provided no

evidence of the presence of non-allelic interaction in the inheritance of salt tolerance. The unit slope of the regression line further indicated that all the assumptions have been met, since according to Hayman (1954a) the regression co-efficient (b) is expected to deviate significantly from zero, but not from unity if all the assumptions are fulfilled.

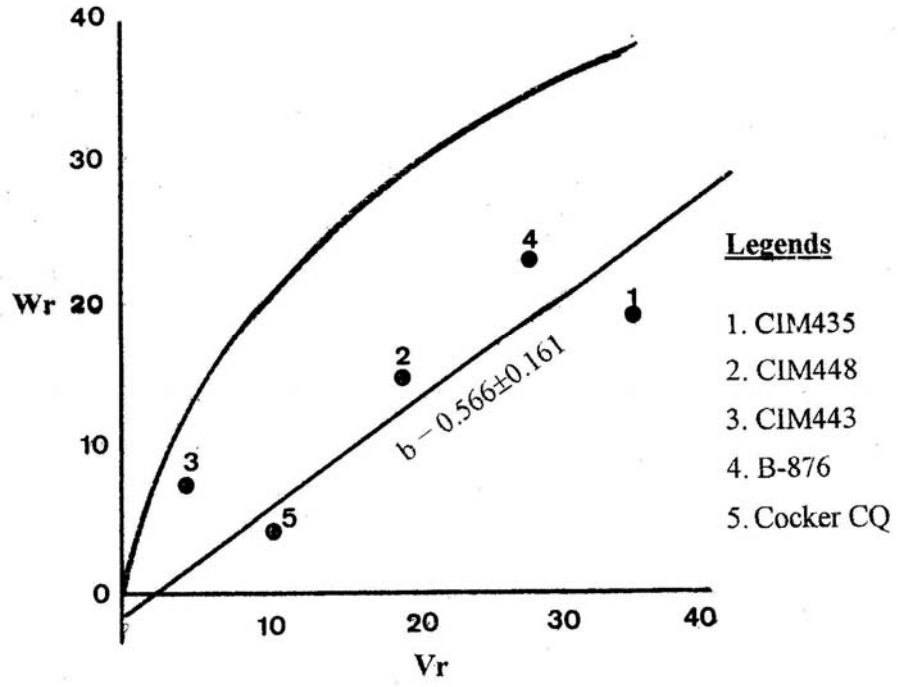

Fig. 1. Wr/Vr regression for relative root length of *G. hirsutum* L., grown in NaCl salinity.

Estimates of genetic components of variation in salt tolerance: The estimates of five components of variation, D, H_1, H_2, F and h and their standard errors in salinity tolerance of *Gossypium hirsutum* L., are given in Table 3. Although both additive and dominance properties of genes are significant, the greater magnitude of H_1 than D revealed that genes with non-additive effects were more important in controlling variation in the material. The positive value of H_1-H_2 indicates that negative and positive alleles were not equally distributed in the parents. Further evidence of this unequal distribution of alleles over loci is provided by the relative value of $H_2/4H_1$ (0.23). The upper limit of the actual value of $H_2/4H_1$ is 0.25, which arises when $H_1 = H_2$ i.e. increaser (positive) and decreaser (negative) alleles at the loci are in equal proportion in the parents. The positive value of F indicates that there were more dominant alleles for high salt tolerance than for low tolerance in the parents. The positive sign of h (mean F_1- parental means) was positive, and revealed the trend of dominance being towards the parents showing high NaCl salt tolerance. The degree of dominance estimated from the ratio $(H_1/D)^{0.5}$ is greater than 1, indicating the presence of overdominance. The ratio of $½F/\{D\ (H_1-H_2)\}^{0.5}$ indicates whether dominance varies from one locus to another, and in the present case this estimate is nearly 1, which suggests that dominance was constant over all loci affecting salt tolerance. The estimate of narrow sense heritability is low, 0.37.

The relative distribution of the array points along the regression line in Fig. 1 revealed that variety CIM-443 and Coker-CQ (exotic) being in close proximity to the point of origin, appear to contain the greatest number of dominant genes for salt tolerance. In contrast CIM-435 and B-876 being farthest away from the origin carried the maximum number of recessive genes, and CIM-448 being located midway contained 50% dominant and 50% recessive alleles for this character.

Discussion

For rapid progress in the development of salinity tolerance in *Gossypium* spp., variation in the plant material to be subjected to selection must be conditioned by a significant genetic component. To collect information on the genetic mechanisms affecting salinity tolerance in the plant material examined here, indices of salt tolerance based upon root length data were analysed. Root length is a reliable indicator of the genotypic response to salinity, and had been used previously in a number of plant species e.g., in sorghum (Azhar & McNeilly, 1987, 1988), in wheat (Ashraf & McNeilly, 1988; Noori & McNeilly, 2000), in maize (Rao & McNeilly, 1999; Khan *et al*., 2003) and in many others, and therefore, the measurement of roots to examine the genetic basis of salt tolerance in cotton is justified.

The simple additive-dominance model of Hayman (1954a, b) and Jinks (1954) was found adequate for analysing indices of salt tolerance. Effects of genes having both additive and non-additive properties were significant in affecting variation in root length (Table 3), but due to greater magnitude of H_1 than that of D, the influence of the genes acting non-additively appeared to be more pronounced. There is evidence of the presence of overdominance and absence of non-allelic interaction in controlling variation in the character. Dominance was directional towards greater NaCl tolerance. This pattern of inheritance of salinity tolerance in the plant material studied here may be useful to a research worker looking for genotypes with enhanced salt tolerance. From the genetic material the hybrids showing increased salt tolerance may be identified and evaluated continuously under salt stress for developing breeding lines, as has been suggested in sorghum (Wilson *et al*., 1978).

It has been suggested that magnitude of additive variance component and heritability estimates increases as the stress increases (Kacser & burns, 1981; Blum, 1988; Hoffmann & Parsons, 1991). However in other studies this prediction did not occur; the additive variance was suppressed as level of NaCl stress increased from 100 mM to 150 mM in sorghum (Azhar & McNeilly, 1988) and in maize from 0 mM to 60 mM (Rao & McNeilly, 1999); Thus the low magnitude of additive variance noted in the present plant material (Table 3) agree with the earlier studies, and suggest that pattern of inheritance of salinity tolerance in cotton is complex.

The estimates of heritability are expected to vary because offsprings examined are subjected to varying environmental conditions (Falconer & Mackay, 1996), and therefore, these may be interpreted and used with care for plant improvement exercise. Since variation in salt tolerance in *Gossypium hirsutum* L., was shown to be affected primarily by non-additive loci, the estimated narrow sense heritability is low, 0.37. Previously narrow sense heritability estimates for salinity tolerance in other crop species have been reported to vary. Thus in *Medicago sativa*, Noble *et al*., (1984) reported 0.5, while in sorghum these were 0.51 and 0.19 under low (100 mM) and high (150 mM)

concentration of NaCl, respectively (Azhar & McNeilly, 1988), and in maize 0.48 (0 mM) and 0.33 (60 mM) (Rao & McNeilly, 1999). Clearly the estimate of narrow sense heritability for salt tolerance in cotton assessed under 250 mM NaCl stress generally agree with those reported in other species, and suggest that further improvement in salinity tolerance in the species may be possible. Based upon the heritability estimates in *Medicago sativa*, Noble *et al*., (1984) made significant improvement in salinity tolerance selecting tolerant individuals from F_2 and F_3 generations.

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