GENETIC BASIS OF ION UPTAKE AND PROLINE ACCUMULATION IN *GOSSYPIUM HIRSUTUM* L.

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Abstract

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Inheritance of Na^+, K^+/Na^+ uptake and proline accumulation at maturity was studied in six, *Gossypium hirsutum* L. cultivars using diallel analysis. Parents and F_1s were grown in 0, 17.5 and 20 dSm^{-1} salt in iron containers. At maturity, leaf samples were collected to measure Na^+, K^+/Na^+ and proline accumulation in the plant material. Indices of salt tolerance (relative values) of Na^+, K^+/Na^+ and proline accumulation were used for genetic analysis following Hayman-Jinks approach. Joint regression coefficients (b) were used to assess the validity of data sets to the simple genetic model, and the model was found to be adequate for analyzing all data sets. It was revealed that genes controlling variation are additively controlled, and hence the estimates of h^2 were high under low and high salinity. These results suggest variation in these characters and potential for improving salinity tolerance in cotton. The plants with low Na^+, greater K^+/Na^+ ratio and greater proline accumulation may be selected from segregating population of the examined material.

Key words: diallel analysis, narrow sense heritability, salinity, proline

Abbreviations: D - additive gene effects; dSm^{-1} - deci Siemens per meter; E - environmental component; EC - electrical conductivity; F - estimation of relative frequency of dominance and recessive alleles in the parents; $(H_1/D)^{0.5}$ - average degree of dominance; H_1 and H_2 - dominance effects of genes; $H_2/4H_1$ - proportion of gene with positive and negative effects in the parents; h2 - overall dominance effect; h^2 - heritability in narrow sense; NaCl - sodium chloride; K – Potassium; TSS - total soluble salts.

Introduction

Among the environmental stresses, soil salinity has devastating effects on crop production than others stresses. Salts near the soil surface result in highly stressful condition for plant growth, and ultimately limit yield or result in plant death. The development of this menace is of greater magnitude in arid and semi-arid areas, and in Pakistan the extent of salt limiting production area has been estimated to the extent of 5.7×10^6 ha of arable land (Mujtaba et al., 2003). In addition to the adoption of engineering approaches to ameliorate the effects of soil salinity, another possibility which appears to be more feasible is the development of crop cultivars suitable for the areas affected, also referred to as the biological approach (Qureshi et al., 1990; Hollington, 1998). Breeders and geneticists have made tremendous efforts to explore the existence of variation in different crop species and its potential for salt tolerance e.g. in *Zea Mays* (Rao and McNeilly, 1999; Khan et al., 2003), *Sorghum bicolor* (Azhar and McNeilly, 1987, 2001a; Igartua et al., 1995), *Triticum aestivum* (Ashraf and McNeilly, 1988; Noori and McNeilly, 2000; Ali et al., 2002), *Glycine max* (Shereen et al., 2001; Kamal et al., 2003), *Oryza Sativa* (Ahmad et al., 1990), triticale and *Hordeum vulgare* (Salim, 1991) and *Pennisetum typhoides* (Kebebew and McNeilly, 1996). These workers used morphological characters to distinguish salt tolerant and susceptible plants under hydroponic condition and at plant maturity.

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In addition to utilizing genetic variation in morphological character, physiological information about salinity tolerance may increase genetic gain in tolerance. Ion exclusion is the basic criterion to study the response of most of crop species to salinity. Variation in plant species showing glycophytic adaptation is related to the efficiency with which they exclude Na^+/or Cl^- from the leaves (Azhar and McNeilly, 2001b). Selection of varieties with low Na^+ transport has been reported in *O. sativa* (Yeo et al., 1988), and *T. aestivum* (Rashid et al., 1999; Munns et al., 2000). Salinity tolerance in *Solanum lycopersicum* was due to the exclusion of Na^+ and Cl^-, and higher concentration of K^+ in stem and leaves (Shaaban et al., 2004), while in salt stressed soybean, Na^+ concentration increased, but K^+ concentration decreased (Shereen et al., 2001; Kamal et al., 2003). Higher salt tolerance in *Z. mays* was associated with significantly lower Na^+ concentration in shoots and a significantly higher K^+/Na^+ ratio (Eker et al., 2006). Similarly in *G. hirsutum*, high K^+ and higher K^+/Na^+ ratio was reported by Ashraf and Ahmad (2000) and Bhatti et al. (2006). These studies indicate that most of the investigations on ion uptake have been focused on Na^+ and major cations like K^+ (Gouia et al., 1994), and it is clear that tolerant cultivars have higher K^+/Na^+ than susceptible cultivars (Goudarzi and Pakniyat, 2008).

In addition, plants under stress undergo osmotic adjustments by accumulating one or more organic solutes, called osmolytes (Naidu et al., 1992), and these play an important role in counteracting the effect of osmotic stress (Yoshiba et al., 1997). Proline is one of the osmolytes which is accumulated in various plants that are subjected to salinity stress, and such accumulation improves their growth (Mohammadkhani and Heidari, 2008; Turkan and Demiral, 2009; Aziz and Khan, 2000, 2001).

These physiological mechanisms are under genetic control (Tudge, 1988) but little is known about their inheritance or variation. The study reported herein was designed to determine Na^+, K^+/Na^+ and proline accumulation in six varieties of cotton. The parents were crossed in a full diallel system of mating. The thirty F_1 progenies and the parental lines were analyzed using a simple additive-dominance model (Hayman, 1954a; Hayman, 1954b; Jinks, 1954). The information reported in this paper may be useful for continued improvement in salinity tolerance of crop species examined.

Materials and Methods

Hybridization of parents

Obsolete and indigenous cultivars, NIAB78, B557, MNH522, Qalandri and MNH147 along with BP52NC63, an exotic line were screened for seedling tolerance using NaCl solution (Nabi et al., 2011). Three varieties/lines, NIAB78, B557, and MNH522 were salt tolerant while Qalandri, MNH147, and BP52NC63 were salt sensitive. These parents were grown in pots, 30 × 35 cm (height and upper diameter respectively), in a glasshouse. Sixteen plants of each genotype, two plants per pot, were grown with 0.25 g urea fertilizer (46% N) applied to each pot every 15 days after planting, and plants were watered daily. The six parents were crossed in all possible combinations using hand emasculation and pollination. Maximum numbers of pollinations were attempted to produce sufficient quantity of F_1 seeds, while some of the buds were also covered with glassine bags to produce selfed seed. All precautionary measures were adopted during crossing to avoid foreign pollen contamination of the genetic material. At maturity, seed cotton from crossed and the selfed bolls was picked, and ginned to obtain seeds.

Responses of the genetic material to NaCl salinity

In order to examine the genetic basis of responses to salinity, the genetic material comprising 30 F_1 hybrids and the six parents were planted in NaCl salinity at 0, 17.5, 20 and dSm^{-1}. The response of plant material to three salt treatments was tested by growing the material in 54 iron containers, each measuring 157.5 cm × 90 cm × 45 cm (length, width and height respectively). The experimental design was a randomized complete block design with three replications. The seeds of 36 entries were sown in six containers, each having six genotypes with five plants spaced 18 cm within the row and 25 cm between the rows.

After the emergence of the seedlings, all the containers were watered once with ½ strength Hoagland nutrient solution (Hoagland and Arnon, 1950). The desired NaCl salinity having electrical conductivity (EC) of 17.5 and 20 dSm^{-1}, considering the saturation % of soil in the containers, were prepared in the nutrient solution and applied to the growing plants. The plants in control containers were fed with only nutrient solution. The salinity levels in the containers were monitored weekly, using the EC meter, and desired concentration was maintained adding salt solution (USDA, 1954). The containers were continuously irrigated with salinized solution until plant maturity. The plant material was sprayed, when required, to save the plants from the attack of sucking pests and boll worms. The samples of leaves were obtained from the mature plants and stored separately in micro-tubes for one week in commercial freezer. The cell sap was extracted using the standard technique of centrifugation (Gorham et al., 1984). Flame photometer was used to measure the concentration of Na^+ and K^+ ions. Proline was calculated according to the following formula given by Bates et al. (1973).

$$\text{Proline (μmol/g f. wt)} = \frac{\text{μg proline ml}^{-1} \times \text{ml of toluene /115.5}}{\text{Grams of sample}}$$

Indices of salt tolerance

Mean absolute values of Na^+, K^+/Na^+ ratio and proline accumulation measured in the 30 F_1 hybrids and six parents grown under two salinities were compared in percentage with those of control, called relative values (Maas, 1986). This was done in three replicates. These values were used for further analysis.

Statistical analysis

Simple analysis of variance technique was used to analyze relative values to determine if genotypic differences were significant (Steel et al., 1997). Only significant differences validate the data for genetic analysis. To determine the adequacy of simple additive- dominance model and to assess the validity of some of the assumptions underlying the genetic model analysis, data were analyzed following Hayman (1954a,b). From the analysis, variance of the components of each array (Vr) and covariance (Wr) of all offspring included in each parental array with non-recurrent parents, variance of parental means (V_0L_0), variance of array means (V_0L_1), means of array variance (V_1L_1) and mean array covariance (W_0L_0) were calculated. These statistics were used for the estimation of four genetic components, D, an additive component; H_1 and H_2, measure of variation due to dominance effects and F, provides an estimate of relative frequency of dominant to recessive alleles in the parental lines. Joint regression coefficient (b) analysis was done using variance (Vr) and covariance (Wr). According to Hayman (1954a), the regression co-efficient (b) must deviate significantly from zero, but not from unity, if all the assumptions underlying the genetic model were met.

Assumptions and Adequacy of Hayman and Jinks Model

The validity of the genetics information describing the genetical properties of a group of genotypes obtained by the diallel cross method is dependent, to some extent, upon the following assumptions (Crumpacker and Allard, 1962)

- Homozygous parents
- Normal diploid segregation of the chromosomes
- No differences between reciprocal crosses
- Independent action of non-allelic genes
- No multiple allelism
- Independent distribution of genes among the parents

Although the diallel cross method was originally devised to analyze data collected from parental material meeting all the above conditions, the work on potato (Kaminski, 1977) showed the testing of these assumptions unnecessary. However, a brief examination of some of these conditions fulfilled is given here.

Cross-pollination in cotton varies from 5-6% or more, depending upon the population of insects in the locality (Poehlman and Sleper, 1995). In the present investigation the parental material was maintained through self pollination each year. Therefore, the patents involved in the crossing scheme were assumed to be homozygous.

G. hirsutum is an amphidiploid derived from diverse diploid species with A and D genomes but the studies of Endrizzi (1962) and Kimber (1961) showed that the chromosomes of the tetraploid segregate in diploid manner.

The reciprocal differences in the characters examined here were removed by taking the means of direct and reciprocal crosses.

The other assumptions of the simple genetic model such as independent actions of non-allelic genes, no multiple allelism, and independent distribution of genes were tested by conducting the formal analysis of variance of the data.

Results

The results of the analysis of variance of relative values of Na^+, K^+/Na^+ and proline accumulation in 30 F_1 hybrids and 6 parents revealed highly significant differences among the genotypes (Table 1). Thus the use of additive dominance model for analyzing the data was valid.

Adequacy of additive-dominance model to the data set

The results of joint regression analysis, Na^+, Na^+/K^+ ratio and proline accumulation in both the salinities are given in

Table 1
Mean squares due to ion uptake and proline accumulation in *Gossypium hirsutum* L.

Source of variation	d. f.	Na^+		K^+/Na^+		Proline accumulation	
		17.5 dS m^{-1}	20 dS m^{-1}	17.5 dS m^{-1}	20 dS m^{-1}	17.5 dS m^{-1}	20 dS m^{-1}
Genotypes	35	1923.9**	1704.4**	253.0**	0.022**	4490.2**	11481.0**
Error	72	29.9	165.9	4.9	0.001	228.1	578

**, shows genotypic differences significant at P<0.001

Table 2. The regression co-efficients of Na$^+$ in 17.5 dSm^{-1} (b = 1.11 ± 0.07), and K$^+$/Na$^+$ ratio (b = 0.92 ± 0.17) deviated from zero and are of unit slop, but b = 0.63 ± 0.27 did not deviate from zero for proline accumulation. At 20 dSm^{-1} regression co-efficients for Na$^+$ (b = 0.91 ± 0.11), K$^+$/Na$^+$ (b = 0.98 ± 0.07) and proline accumulation (b = 0.74 ± 0.10) deviated significantly from zero, and all are of unit slope. These results suggest that all the assumptions underlying the genetic model have been fulfilled (Hayman, 1954a), and the data were analyzed following Hayman's (1954a) genetic model. The array points being closer to the regression lines in figure 1A, 1B, 2A, 2B and 3A provided evidence of the absence of epistasis in the inheritance of uptake of these ions and proline accumulation, a suggestion given by Hayman (1954a).

Estimation of genetic components of variation
Na$^+$ Contents

From the relative sizes of D, H$_1$ and H$_2$ items when plants were grown in 17.5 dSm^{-1} salinity some important inferences about the genetic causes of variation in Na$^+$ content can be

Table 2
Regression co-efficients (with standard error) of ion uptake and proline accumulation in *Gossypium hirsutum* L.

Ions	17.5 dS m^{-1}	20 dS m^{-1}
Na$^+$ contents	1.11 ± 0.07	0.91 ± 0.11
K$^+$/ Na$^+$ ratio	0.92 ± 0.17	0.98± 0.07
Proline accumulation	0.63 ± 0.27	0.74± 0.10
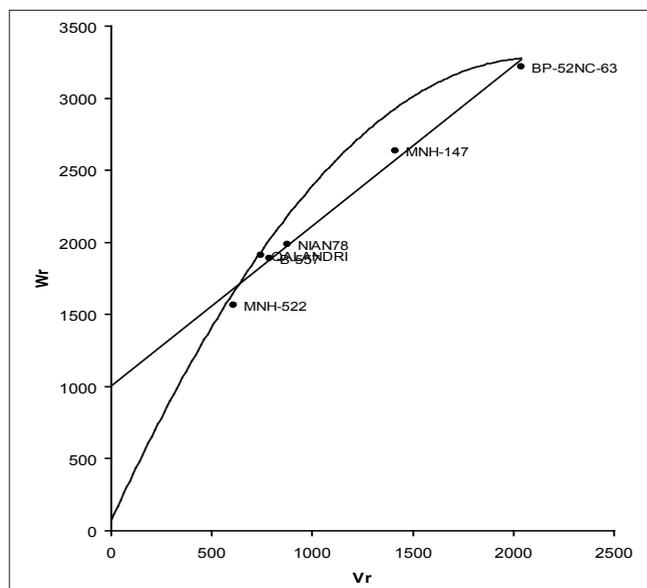
made. Although both additive and non-additive affects were shown to effect the control of Na$^+$ uptake (Table 3), because D$_1$ > H$_1$, the genes with cumulative properties appeared to be pronounced. Similarly significant positive F value revealed that there were more dominant genes than recessive genes in the parents. The degree of dominance was measured by (H$_1$/D)$^{0.5}$ which is less than one, indicating the presence of partial dominance, and this was supported by the intercept of regression line above the origin on Wr axis (Figure 1A). The positive value of H$_1$- H$_2$ indicates that increasing and decreasing genes were not equally distributed in the parents. Further evidence of this unequal distribution of alleles over loci is provided by the ratio H$_2$/4H$_1$ = 0.20 in 17.5 dSm^{-1}. With equal distribution of genes, this value would have been 0.25, which arises when H$_1$= H$_2$ i.e. increasing (positive) and decreasing (negative) alleles at all loci are in equal proportion in the parents. The positive sign of h^2 in the 17.5 dSm^{-1} salinity treatment suggested that dominance acted towards the parents with greater Na$^+$ content. Narrow sense heritability of Na$^+$ uptake in 17.5 d Sm^{-1} was 0.91. Examination of Figure 1A indicates that cultivar MNH522 contained the maximum number of dominant genes, and in contrast BP52NC63 in the 17.5 dSm^{-1} treatments and MNH147 in the 20 dSm^{-1} possessed maximum number of recessive genes for Na$^+$ uptake.

In the 20 dSm^{-1} salinity treatment only D item was significant while H$_1$, H$_2$ and F appeared to be non-significant, suggesting the importance of additive genes controlling variation in Na$^+$ content. The degree of dominance was exhibited by the ratio (H$_1$/D)$^{0.5}$ which was lesser than one suggesting
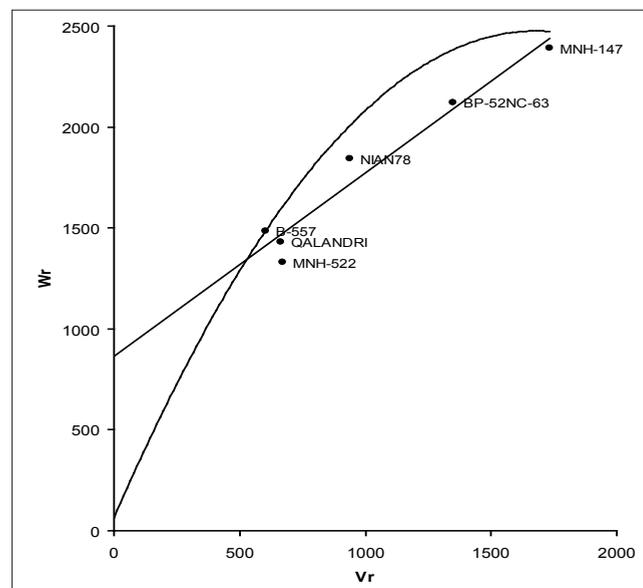

Fig. 1. (A) Wr/Vr graph for Na+ contents in 17.5 dS/m salinity

Fig. 1. (B) Wr/Vr graph for Na$^+$ contents in 20 dS/m salinity

partial dominance. This was verified by the position on intercept of the regression line above the origin of the Wr axis, which may also be an indication of partial dominance (Figure 1B). The negative sign of h^2 for the 20 dSm^{-1} indicated dominance towards the parents with low Na^+ contents. Estimate of h^2 under higher salinity treatment was 0.90. From the distribution of array points along the regression line in Figure 1B, the identification of those parental lines carrying the most dominant genes and those processing maximum recessive alleles were possible. From the comparison cultivar MNH522 being in close proximity to the point of origin, appear to contain the greater number of dominant genes for Na^+ content

and in contrast MNH147 being away from the origin appears to carry the maximum number of recessive alleles.

K^+/Na^+ ratio

At 17.517.5 dSm^{-1}, the greater magnitude of D than H_1 revealed that genes acted additively in controlling variation in K^+/Na^+ ratio (Table 3). The magnitude of H_1 and F appear to be non-significant. The estimate of the ratio $(H_1/D)^{0.5}$ was less than one, indicating varying degree of dominance and this situation is verified by the position of the regression slope in Figure 2A. The estimate of h^2 was 0.88. The relative position of array points along the regression line in Figure 2A indi-

Table 3
Genetic components of variation in ion uptake and proline accumulation in *Gossypium hirsutum* L.

Components	Estimates of Na^+		Estimates of K^+/Na^+		Estimates of proline
	17.5 dSm^{-1}	20 dSm^{-1}	17.5 dSm^{-1}	20 dSm^{-1}	20 dSm^{-1}
D	5281.35±70.16	3653.68±77.75	45.21±1.03	40.55±0.47	5633.82±435.84
H_1	746.29±161.99	345.60±179.54	5.94±3.09	2.84±1.09	3305.80±1006
H_2	593.79±140.94	241.18±156.20	8.72±2.62	2.35±0.95	2987.70±875.58
F	1771.17±166.51	317.96±184.54	0.94±2.09	0.49±1.12	-2680.72±1034.44
h^2	47.29±94.29	-19.18±104.50	0.48±1.75	0.01±0.63	428.89±585.76
E	25.74±23.49	123.86±26.03	0.58±0.43	0.75±0.16	209.92±145.93
$(H_1/D)^{0.5}$	0.38	0.31	0.46	0.26	0.77
$H_2/4H_1$	0.2	0.17	0.23	0.21	0.23
Heritability$_{ns}$	0.91	0.9	0.88	0.94	0.82

NB, value is significant when it exceeds 1.96 after dividing by its standard error
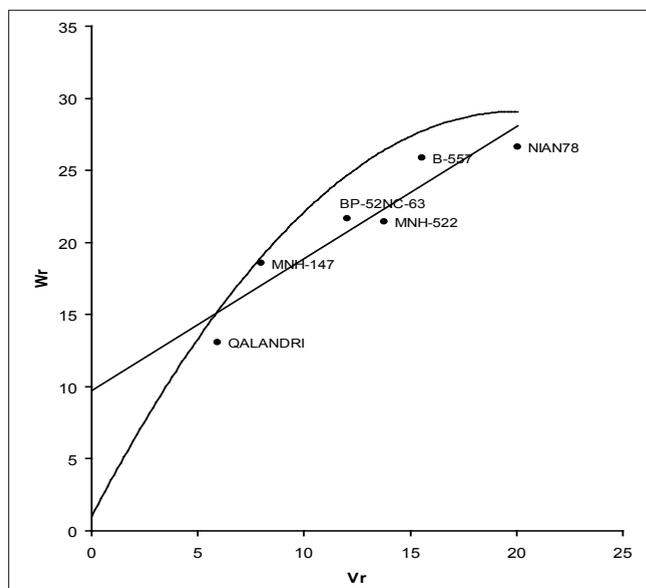

Fig. 2. (A) Wr/Vr graph for K^+/Na^+ ratio in 17.5 dS/m salinity
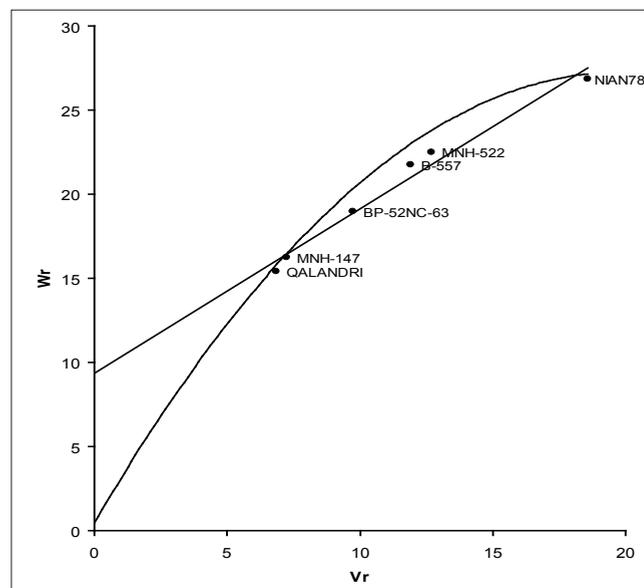

Fig. 2. (B) Wr/Vr graph for K^+/Na^+ ratio in 20 dS/m salinity

cate that Qalandri carried the maximum number of dominant genes, while NIAB78 contained the more recessive alleles for K^+/Na^+.

The relative estimates of components of variation in K^+/Na^+ in the 20 dSm^{-1} treatment are given in Table 3. Although D, H_1 and H_2 are significant, the greater magnitude of D than H_1 suggests the importance of additive genes in the inheritance of K^+/Na^+. Since magnitude of H_1 is almost equal to H_2, the genes were equally distributed in the parents and therefore the ratio $H_2/4H_1 = 0.21$ which is almost equal to the theoretical maximum of 0.25. The ratio of $(H_1/D)^{0.5} = 0.26$ showed the presence of partial dominance. The value of F is non-significant and the estimate of h^2 is 0.94. The location of array points in Figure 2A indicated that Qalandri contained a greater number of dominant genes, and NIAB78 contained more recessive genes.

Proline accumulation

When plants were grown in 20 d Sm^{-1} NaCl, the magnitude of D, H_1, H_2 and F appeared to be significant. Although both additive and non-additive genes appeared to control proline accumulation in the plant material, the greater magnitude of D than H_1 and H_2, indicates that additive gene effects were more pronounced than dominance (Table 3). As magnitude of H_1 is nearly equal to H_2, equal distribution of genes in the parents was evidenced, and the ratio of $H_2/4H_1 = 0.23$ strengthened this conclusion. The ratio of $(H_1/D)^{0.5} = 0.77$ which is less than 1, suggested the presence of partial dominance, and
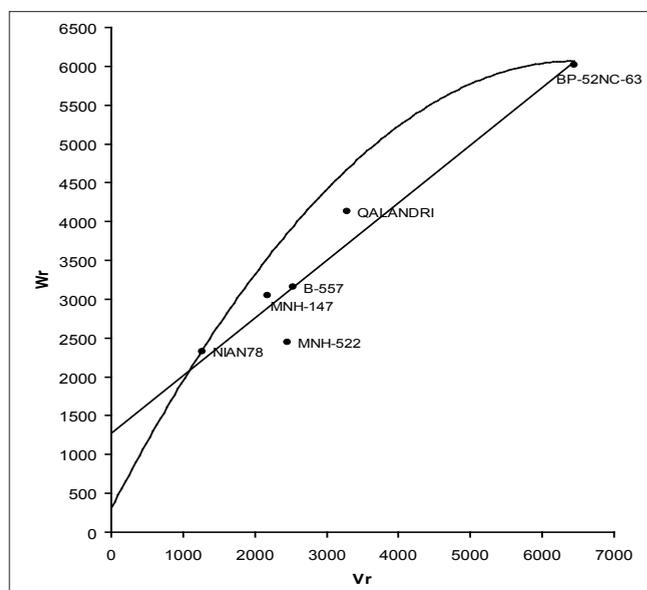
this was substantiated by the slope of regression line in Figure 3A. The negative F value revealed that recessive genes were more frequent than dominant genes. Estimate of narrow sense heritability was high, 0.82. The distribution of cultivar points for proline accumulation suggested that NIAB78 possessed maximum number of dominant genes, while reverse was true for BP52NC-63.

Discussion

Development of crop cultivars adapted to saline soils either by selection from existed cultivars or using general crop variability, requires an understanding of physiological basis of salinity tolerance in plants, and it has been argued that these basis are genetically controlled (Tudge, 1988). The plant material examined here for Na^+, K^+/Na^+ ratio and proline accumulation provided an understanding of the genetic basis of the mechanism controlling salinity tolerance in *G. hirsutum* L.

Preliminary analysis revealed the existence of variation in Na^+, K^+/Na^+ and proline accumulation in the six parents used in this study. Adequacy of the simple genetic model to the data sets provided evidence of the absence of non-allelic interaction in the inheritance of ion uptake and proline accumulation. Genes with additive effects were predominant for controlling the uptake of uptake of Na^+, K^+/Na^+ and proline accumulation in *G. hirsutum* L., when six parents and their full sib F_1's were grown under low and high salinities, although dominance properties of genes were also present. Lawrence (1984) had argued that populations subjected to strong selection pressure showed reduced additive components for these characters. In the literature there is no information which could show that cotton had been subjected to directional selection pressure in the past, either in the wild or cultivated forms, for enhancing salinity tolerance. Thus it seems likely that additive variation for increasing salinity tolerance may be available to the breeders. The availability of greater additive component suggests that selection of plants with low Na^+, greater K^+/Na^+ and greater porline accumulation for increasing salinity tolerance in upland cotton.

It has been suggested that magnitude of additive variance and heritability estimates increases as stress increases (Blum, 1988 and Hoffmann and Parsons, 1991). However in some other studies, additive variance was suppressed as NaCl stress increased e.g. in *S. bicolor* (Azhar and McNielly, 1988) and *Z. mays* (Khan et al., 2003). Thus the low magnitude of additive variance for Na^+ and K^+/Na^+ under increased salinity in this study agrees with theses earlier studies, but accumulation of greater proline under 20 dSm^{-1} was found to increase additive variance and hence heritability.

Fig. 3. (A) Wr/Vr graph for proline accumulation in 20 dS/m salinity

Since variation in Na^+ and K^+/Na^+ uptake in both the salinities and proline accumulation in increased salinity was due to genes acting additively, the estimated h^2 appeared to be inflated. Falconer and Mackay (1996) had suggested that these estimated are subject to environmental variation, and therefore must be used with great care for selecting the desired plants in segregating generations. The higher estimate of h^2 for Na^+, K^+/Na^+ and proline accumulation in both the salinities might be due to the expression of genes associated with salinity tolerance or a small environmental variation (Saranga et al., 1992; Bhatti et al., 2006). It had been argued that hidden variation, previously unselected could be uncovered when the material is grown under stress, thus possibly increasing the heritability (Bradshaw and Hardwick, 1989). Nonetheless the estimates of heritability of Na^+, K^+ and proline accumulation seem to be encouraging for making direct selection for improving NaCl tolerance in upland cotton. However further studies are needed to substantiate the present information and formulating appropriate breeding strategy for the development of NaCl tolerant material.

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