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GENETIC MECHANISM CONTROLLING SELECTED WITHIN BOLL YIELD COMPONENTS AND PHYSIOLOGICAL TRAITS OF *GOSSYPIUM HIRSUTUM* L. UNDER SALINITY STRESS

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ABSTRACT

An experiment was conducted to elucidate the genetic governance of within boll yield components and physiological trait of cotton under control and salinity stress. Ionic concentration varied in all of the genotypes at both salinity levels, ie., higher Na+ concentration was observed in stress condition. Male, female interaction was significant in lint mass per unit seed surface area, chlorophyll content, K^+/Na^+ ratio, concluding that within boll yield components and ionic concentration in cell are controlled by non-additive type of gene action. High broad sense heritability and mild narrow sense heritability estimates revealed that within boll yield components and physiological traits are probably controlled by additive and non-additive gene action with pronounced effect of non-additive gene action under control and salt stress condition. Genotypic and phenotypic coefficient of variability was vigorous in stress condition due to the interaction of salinity tolerant genes of tolerant genotypes. Genetic governance is influenced due to specific environmental factors therefore care should be taken in the entitlement of genetic governance of particular traits.

Key words: abiotic stress, genetic mechanism, *Gossypium hirsutum* L, physiological traits, salinity stress, within boll yield components,

INTRODUCTION

Crop plants cope with different types of environmental stresses during their life cycle. Among them salinity is major abiotic stress which causes serious damage to agricultural productivity (Hameed, 2010). Impact of salinity include reduced agricultural productivity, low economic return and erosion of soil. It affects the plants in many ways such as ion toxicity, osmotic stress, oxidative stress and deficiency of essential nutrients in plants, which in result reduces the water uptake from the soil (Shrivastava and Kumar, 2015). Worldwide area under saline and sodic condition is about 397 mha and 494 mha respectively which is about 6% of total world land area.

In Pakistan 10 mha of land is badly affected by the salt stress, which is 12.9% of the country's land (FAO, 2015). Due to the salinity stress there will be 30% land degradation in next 25 years and 50% up to 2050 (Wang et al., 2003). Salt stress is due to the presence of large amount of soluble salts in water and soil like SO_4^-, Cl^- and HCO_3^-. Among these salts, NaCl is the most soluble and present in the abundant quantity in the soil (Munns and Tester, 2008). Salt affected area increases in the developing countries due to the more evapotranspiration, less rainfall and use of saline water for irrigation (Arzani, 2008). It is the most important problem in global agriculture and seriously affecting the crop production (Williams, 1999). In irrigated areas agriculture crop production is seriously damaged by salinity stress (Aragues et al., 2011).

Cotton (*Gossypium hirsutum* L.) belongs to family *Malvacea* and is considered as the most important world fibre crop grown in many countries with annual production of 113.9 m bales (Abbas, 2011a). It is important cash crop of Pakistan, and known as the "White Gold". Cotton crop contribute about 1.4% in GDP and about 7.1% as a value addition in agriculture. It is important source of raw material to the textile industry. In 2013-14 during the month of July to March textile industry of Pakistan earned foreign exchange of US$ 10.385 billion. Cotton covers about 2.80 million hectares area of Pakistan and production is about 12.8 million bales. Pakistan is the 4th largest producer and 3rd largest consumer of seed cotton in the world (Govt of Pakistan, 2014-15).

To overcome the salinity problems different approaches are being used such as physical manipulation of soil to drain or leach down salts out of the root zone. But, due to high input this approach is limited to few areas (Qureshi and Lennard, 1998). Hence alternative approach also called as “Biosaline agriculture” is most effective, durable and economic for development of salt tolerant cultivars (Flowers and Yeo, 1995; Khan et al., 2001; Ashraf and Akram, 2009). Sufficient genetic variation has been reported in the cotton germplasm for salt tolerance (Khan et al., 1998; Ashraf and Ahmad, 2000b; Noor et al., 2001, Bhatti and Azhar, 2002). Genetic mechanism controlling within boll yield components and physiological traits have been reported (Imran et al., 2012, Allah et al., 2015), but the genetic mechanism controlling within boll yield components and physiological traits under salinity stress are not reported yet. Available variation for these traits can be utilized in the development of salt tolerant varieties after determining its genetic mechanism under salinity stress. The information about genetics of plant traits related to salinity tolerance helps the plant breeder in the development of salinity tolerant varieties. Environment has strong influence on the genetic governance of various traits. Gene action might be changed along with the change in the environment (Perkins et al., 1984, Hoffmann and Merila, 1999; Saranga et al., 2001). Therefore present study was planned to estimate genetic mechanism controlling within boll yield components and physiological traits under normal and salt stress condition to determine the level of environmental influence conceived by genetic components at NaCl @ 15 dSm^{-1}.

MATERIALS AND METHODS

The present experiment was carried out in the experimental area of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan, (Latitude = 31 O- 26' N, Longitude = 73O- 06' E, Altitude = 184.4m) during 2013-14. Breeding material comprised of eight parents having contrasting morphological, fibre quality and yield related traits and were developed by different breeding stations by using hybridization methods. Three male parents (MNH-886, CIM-598 and FH-142) and five female parents (PB-39, PB-899, BH-95, MNH-129 and CIM-473) were sown in the glass house. MNH-886 is early maturing variety having medium compact growth habit with 4.5 gm boll weight, seed index (gm) 8.1-8.2, lint percentage 40.77% and fibre length 28.6 mm. FH-142 possesses plant height 140-160 cm with boll weight of 3.8-4.4 gm, lint percentage of 39-40%, seed index 7.8-8.2 gm, staple length 28mm. PB-899, a medium statured elite line with small boll size (3.2 gm) and medium seed index = 8.0 gm, evolved by field crossing and with good fiber quality. PB-39 another short statured elite line with low boll weight (2.9-3.2 gm) and lint percentage of 39% evolved by field crossing. CIM-598 possesses 2.9 gm boll weight, 40.1% lint percentage, 28.3 mm staple length and 125 cm plant height. CIM-473 also possess 3.0 gm boll weight, with semi compact growth habbit having staple length of 28.12 mm and lint percentage of 39.54%. MNH-129 has boll weight of 2.8-3.5 gm, lint percentage of 39.35% and staple length of 28.13mm. Crosses were made among male and female parents in 2013 to produce F_1 seeds. At maturity, crossed and selfed-bolls were picked and ginned separately to get the F_1 seed. In May 2013, 23 genotypes including 15 hybrids and 8 parents were sown in normal and saline condition (NaCl @ $15dsm^{-1}$) in randomized complete block design (RCBD) in split plot arrangement with 2 replications in iron pots (12″ × 10 ″ × 8 ″). Salinity levels were placed in main plots while genotypes were placed in subplots. As soon as seedlings germinated each pot was irrigated with distilled water keeping in view the need of the treatment. Control pots were supplied with distilled water resultantly having EC 2.3 dSm^{-1} while salinity treatments were irrigated with 2.5mM NaCl solution so as to make the EC equals to 15 dSm^{-1}. Salinity level was measured twice a week throughout the experimental duration with EC meter and maintained with the addition of proper quantity of 2.5M NaCl solution. Fertilizer (NPK) was applied to whole bulk of soil before pot filling @ 120, 60 and 60 Kg hac^{-1}. All of the fertilizer was applied at the time of sowing so that equal amount of nutrients were distributed to all of the pots.

Uniform crop husbandry practices from seed sowing to seed cotton picking were ensured to keep a healthy crop. Whether data of the experimental site is shown in figure 1. Field capacity was maintained by supplementary irrigations when needed. Insect pest attack was controlled by using the recommended pesticides. At the time of maturity 5 plants of each genotypes were tagged in each replication in both the treatments (normal and saline) and the data were collected for the lint percentage (LP) = weight of lint/weight of seed cotton × 100, seed index (SI) = 100 seed weight, lint index (LI) = (seed index) × (lint percentage)/(100-lint percentage), seed/boll = seeds in sample/number of bolls, seed mass per boll (SM/B) = seed mass of sample/number of bolls, lint mass per unit seed surface area (LM/SSA) = Fibre mass/surface area × mean length × converted mic. Sodium (Na^+) and Potassium (K^+) concentrations were measured by flame photometer, K^+/Na^+ ratio was calculated by dividing K^+ with Na^+, while chlorophyll contents were recorded with CCM-200 chlorophyll meter (Opti-Sciences, Inc) at noon to obtain chlorophyll content index CCI.

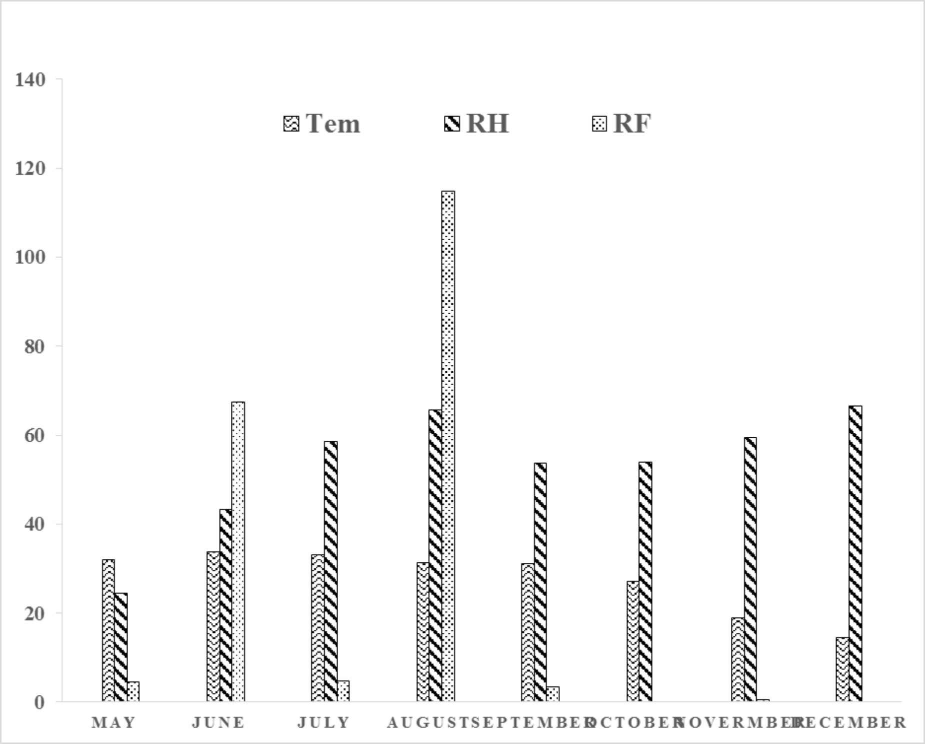

Figure 1. Weather data (Average temperature, Relative humidity RH, rain fall RF) during the experimental period of the cite

Statistical Analysis

The collected data were subjected to the analysis of variances in RCBD with split plot arrangement in suggested by Steel et al., (1997). Data showing significant differences under normal and salt stress condition were further analyzed by North Carolina Design II mating scheme (Comstock and Robinson, 1948, 1952) to estimate the gene action, components of variations and heritability. Broad sense heritability was calculated by using genotypic, phenotypic and environmental variance according to Burton, 1951.

RESULTS AND DISCUSSION

Analysis of Variance

Combined analysis of variance (normal and salinity stress) showed highly significant differences ($P \leq 0.01\%$) for all of the traits in case of genotypes and genotype × treatment interaction while highly significant and significant differences were found for all of the traits in case of treatments (Table 1). Traits showing significant as well as highly significant differences for genotypes were further analyzed according to North Carolina Design II. Mean performance of the parents and their crosses for within boll and physiological traits were conspicuously different under salinity stress and control condition with pronounced effects for K+, Na+ concentrations and K+/Na+ ratios. (Data not shown).

Table 1. Mean squares from analysis of variance for various traits of 23 cotton genotypes grown under control and salinity stress of NaCl @ 15 dSm^{1} (combine analysis at all levels).

SOV	**Df**	**Seed Index**	**Lint Index**	**Seed Number/boll**	**Seed Mass/boll**	**Lint mass/ unit seed surface area**	**K^{+} conc.**	**Na^{+} conc.**	**K^{+}/ Na^{+} ratio**	**CCI**
Replications	1	0.15$^{n.s}$	0.04$^{n.s}$	2.05$^{n.s}$	0.0002$^{n.s}$	0.00036$^{n.s}$	1.249$^{n.s}$	0.55$^{n.s}$	0.04$^{n.s}$	5.15$^{n.s}$
Genotypes	22	8.95**	8.03**	14.26**	12.14**	11.8**	12.284**	13.74**	7.98**	62.3**
Error 1	22	0.16	0.196	2.49	0.0148	0.0046	1.553	0.743	0.13	4.55
Salinity Level	1	0.46$^{n.s}$	1.450*	6.94*	0.1599**	0.00031$^{n.s}$	377.067**	120.1*	26.67**	433.3**
Gen x Tret	22	12.4**	18.1**	15.27**	8.59**	9.18**	7.86**	11.17**	8.13**	31.06**
Error 2	23	0.28	0.25	1.32	0.01995	0.00426	2.138	0.93	0.1455	5.434

n.s.= non-significant, *=significant at P<0.05, **= highly significant at P<0.01.

The Analysis of Variance Through North Carolina Design II

Character showing significant differences for genotypes was further analyzed by splitting the source of variation into its components i.e males, female and their interaction in North Carolina design II. In this design parents were ignored and only their crosses were considered. Analysis of variance for different traits revealed highly significant differences for lint index in replication, seed index and K^+/Na^+ ratio in males, for K^+/Na^+ ratio and CCI in females, seed index, Na^+ conc., K^+/Na^+ ratio in male × female interaction under normal condition. Similarly seed index in replication, lint index, seed per boll and seed mass per boll showed significant differences under normal condition (Table 2). In case of salinity stress condition highly significant differences were observed for seed per boll, seed mass per boll, lint mass per unit area in case of male parents while in case of male × female interaction K^+ conc, K^+/Na^+ ratio showed highly significant differences (Table 3).

Table 2. Mean squares values from NCD-II for various traits of 15 cotton genotypes under control condition.

SOV	**df**	**Seed Index**	**Lint Index**	**Seed Number/ boll**	**Seed Mass/boll**	**Lint mass/ unit seed surface area**	**K^+ conc.**	**Na^+ conc.**	**K^+/ Na^+ ratio**	**CCI**
Replication (R)	1	0.97*	1.71**	0.842$^{n.s}$	0.02$^{n.s}$	0.0002$^{n.s}$	0.047$^{n.s}$	0.99**	0.174$^{n.s}$	0.32$^{n.s}$
Males (M)	2	98.66**	71.01*	4.521$^{n.s}$	64.1*	0.006$^{n.s}$	0.012$^{n.s}$	0.20$^{n.s}$	159.1**	0.08$^{n.s}$
Females (F)	4	0.32$^{n.s}$	0.16$^{n.s}$	4.926$^{n.s}$	0.02$^{n.s}$	13.5*	2.023$^{n.s}$	8.07*	84.7**	111.9**
Male × Female (M×F)	8	15.1**	0.13$^{n.s}$	5.11$^{n.s}$	0.03$^{n.s}$	0.007$^{n.s}$	6.55*	12.8**	63.9**	19.102*
Error	15	0.12	0.15	2.263	0.0123	0.0030	1.593	0.64	0.129	6.087

n.s.= non-significant, *=significant at $P<0.05$, **= highly significant at $P<0.01$.

Table 3. Mean squares values from NCD-II for various traits of 15 cotton genotypes under salinity stress (NaCl @ 15 dSm^{-1}).

SOV	**df**	**Seed Index**	**Lint Index**	**Seed Number/boll**	**Seed Mass/boll**	**Lint mass/ unit seed surface area**	**K^+ conc.**	**Na^+ conc.**	**K^+/ Na^+ ratio**	**CCI**
Replication(R)	1	1.45*	0.64$^{n.s}$	0.236$^{n.s}$	1.07*	0.005$^{n.s}$	0.17$^{n.s}$	0.94$^{n.s}$	0.17$^{n.s}$	0.68$^{n.s}$
Males(M)	2	19.1*	99.45**	119.8**	112.1**	103.12**	5.01$^{n.s}$	23.3*	0.177$^{n.s}$	24.7*
Females(F)	4	0.48$^{n.s}$	0.177$^{n.s}$	16.8*	0.019$^{n.s}$	0.004$^{n.s}$	8.04*	0.33$^{n.s}$	0.166$^{n.s}$	1.6$^{n.s}$
Male × Female (M × F)	8	0.39$^{n.s}$	0.247$^{n.s}$	5.474*	0.013$^{n.s}$	0.002$^{n.s}$	12.8**	1.28$^{n.s}$	46.4**	8.47$^{n.s}$
Error	15	0.214	0.154	1.539	0.015	0.004	2.15	0.83	0.06	4.34

n.s.= non-significant, *=significant at $P<0.05$, **= highly significant at $P<0.01$.

Estimation of Variability, Heritability and Gene Action

Under control condition variance due to male, female and male x female interaction ranged from -1.903-0.095, -0.42-15.478 and -0.01-6.58 respectively. Broad sense heritability was highest for K^+/Na^+ ratio (90.8%) and minimum for lint index (35.9%). Maximum genotypic, phenotypic and environmental coefficient of variability was observed for K^+/Na^+ ratio (Table 4). Under salt stress, variance due to male, female and interaction of male × female interactions ranged from -0.77-1.618, -0.78-0.523 and -0.001-5.29 respectively. Broad sense heritability ranged from 0.308- 0.916. Higher and lower broad sense heritability's were observed for lint index and lint mass/unit seed surface area in salt stress in contrast to normal condition. Upper limit of coefficient of variabilities were higher than the respective trait's variabilities under normal condition (Table 5).

Combined analysis of variance for within boll yield components and physiological traits have shown that genotypes possesses diverse variability which is manifested as a result of observation collected at normal as well as salt stress condition. In past very limited comparative research has been reported especially on within boll yield components as well as physiological traits.

Table 4. Estimation of variance, heritability and gene action for various traits in 15 F_1 crosses under control condition.

Components	Seed Index	Lint Index	Seed Number/boll	Seed Mass/boll	Lint mass/ unit seed surface area	K^+ conc.	Na^+ conc.	K^+/Na^+ ratio	CCI
σ^2m	0.045	0.058	-0.089	0.004	-0.0025	-0.453	-0.260	0.095	-1.903
σ^2f	-0.030	0.005	-0.081	0.000	0.001	-0.420	-0.121	0.035	15.478
σ^2mf	0.192	-0.010	1.574	0.006	0.002	1.476	1.079	0.255	6.508
h^2ns	0.233	0.132	-	0.159	0.167	-	-	0.185	0.458
h^2bs	0.672	0.559	0.725	0.704	0.751	0.723	0.847	0.908	0.897
PCV	13.896	10.004	14.050	14.458	22.369	27.926	42.913	68.729	14.501
GCV	12.974	5.996	11.961	12.131	19.382	23.746	39.496	65.485	13.736
ECV	4.978	8.009	7.372	7.866	11.168	14.696	16.781	20.865	4.647

Table 5. Estimation of variance, heritability and gene action for various traits in 15 F_1 crosses under salinity stress (NaCl @ 15 dSm^{-1}).

Components	Seed Index	Lint Index	Seed Number/boll	Seed Mass/boll	Lint mass/ unit seed surface area	K^+ conc.	Na^+ conc.	K^+/Na^+ ratio	CCI
σ^2m	0.072	0.130	0.650	0.010	0.003	-0.772	0.174	-0.029	1.618
σ^2f	0.014	-0.012	0.217	0.001	0.000	-0.782	-0.159	-0.050	0.523
σ^2mf	0.091	0.046	1.968	-0.001	-0.001	5.292	0.228	0.202	2.062
h^2ns	0.232	0.412	0.156	0.557	0.123	-	0.217	-	0.262
h^2bs	0.715	0.732	0.862	0.693	0.308	0.894	0.533	0.916	0.743
PCV	12.977	16.520	17.147	13.938	15.402	110.401	19.072	132.766	8.306
GCV	10.971	14.137	15.919	10.730	8.554	104.376	13.925	127.049	7.158
ECV	6.930	8.547	6.372	8.896	12.808	35.974	13.033	38.542	4.214

NCMDII analysis of variance revealed that variance due to male and female parents for all of the traits showed similar trend both in normal and salinity stress condition (Table 2,3). Interaction of male and females revealed non-significant variation for all of the traits. Sodium (Na^+) ion concentration varied in salt tolerant and susceptible genotypes i.e., there were low Na^+ ion concentration in salt tolerant genotypes while high concentration were observed in salt susceptible genotypes. Generally there is significant uptake of sodium by cotton plant in the salt stress condition. The passive movement of sodium ions into the roots destroyed the membrane of root cell (Leidi and Saiz, 1997; Akhtar et al., 2010). The result of present study were according to earlier researchers and confirmed that under the salt stress Na^+ ion concentration increases in the plant which inhibits the uptake of potassium (K^+) whose deficiency ceases vital function in the plant which may eventually leads to death (James et al., 2011). Plant become salt tolerant when there was exclusion of Na^+ ion from cell. So the reduction in concentration of Na^+ ion may be used as selection criteria for selection of tolerant genotypes. As it was reported earlier, in barley (Storey and Jones, 1978), in wheat (Ali et al., 2007), in rice (Aslam et al., 2013) and in cotton (Ashraf, 2002; Abbas, 2011a; Nabi, 2010).

K^+ ion concentration decreased in all the cotton genotypes under the salt stress as compared to normal condition, however there were little reduction in K^+ ion concentration in some genotypes in the salinity stress. The decrease in K^+ ion concentration was due to the more Na^+ ion concentration in the saline medium that creates the hindrance for the absorption of K^+ ions. These results are same as the earlier researcher study (Kent and Lauchli, 1985; Ashraf and Ahmad, 2000a; Akhtar et al., 2010; Abbas et al., 2011b).

There was decrease in K^+/Na^+ ratio in the leaves under the salt stress, the reduction is due to the replacement of some ions like Ca^{++} from the membrane. As a result there were disturbance in membrane integrity and K^+ ion movement takes placed out of the cell which result in reduction of K^+/Na^+ ratio (Kent and Lauchli, 1985). The results are same as find by the earlier researcher in cotton (Qadir and Shams, 1997; Akhter et al., 2010) and in wheat (Akhtar et al., 1998; Ali et al., 2009).

For all of the traits male and female parents showed wide range of variability. Generally variance due to male × female interaction was greater in magnitude for all of the traits except Chlorophyll Content Index in which variance due to female parent was higher in control condition while in stress condition variance due to male was at par. The performance of F_1 genotypes showed that highly significant variation was observed for lint index, seed mass/boll and K^+/Na^+ ratio due to male parent in control and salt stress condition (Table 2). Male × female interaction contribution for within boll yield components and physiological traits were due to the maternal and paternal contribution was significant for chlorophyll content, K/Na ratio, K and Na concentrations as has been reported by (Ashraf and Ahmad. 2000b, Akhter et al., 2010). It can be concluded that within boll yield component and ionic concentration traits are probably significantly governed by dominance type of gene action as the same is inferred from the heritability estimates.

Heritability is the inherited portion of variability. From high broad sense heritability it is depicted that within boll yield components such as seed index, lint index and physiological traits (K^+, Na^+ and K^+/Na^+ ratio) showed predominant additive effects in their inheritance as already reported by (Khan et al., 1998, Gregorio and Senadhira, 1993, Murtaza et al., 1995, Neelima et al., 2004) because of higher broad sense heritability as has been reported by earlier researcher in cotton (Salam, 1993, Rumbaugh et al., 1989, Dhiva et al., 2002; Azhar et al., 2007; Ashraf and Ahmad 2000a; Abbas et al., 2011a). But taking in consideration, mild narrow sense heritability in the governance of within boll yield components, K^+, Na^+ and K/Na ratio both in control condition and salinity stress it is revealed that genetic component governing these trait is other than additive as have been found by (Mahmood et al., 2004, Ali and Shahid 2009) in cotton and by (Gopikannan and Ganesh 2013) in rice. Lower narrow sense heritability found in normal and in salt stress condition shows that selection could not be done for the genetic improvement under normal as well as salt stress condition in earlier generations and it must be postponed until later generations as suggested by Tripathi and Singh (1983), or heterosis breeding can be brought under consideration for these traits (Melchinger et al., 2007). Also recurrent selection procedure could be taken on, as it accumulates both additive and non-additive effects of genes (Coyle and Smith, 1997. Bulk method of selection will be more suitable as compared to pedigree method for belated selection.

Low variability existed for all ionic concentration traits at control level while there has been observed a little increase in genotypic and phenotypic coefficient of variance under saline conditions. This is probably due to the fact that when there is abundance of salts in soil susceptible plants, intake lot of sodium than tolerant plant. The net amount of salt in salinity susceptible genotypes reaches lot more than tolerant genotypes. Although high heritability values under salt stress were observed but selection on the basis of ionic concentration traits may still not be effective due to low variability. The earlier researchers had also observed high heritability under stress conditions (Blum, 1988; Hoffman and Parsons, 1991) while, some researchers found low heritability for few traits (Rumbaugh et al., 1984) and suggested that increase or decrease in heritability under stress conditions is because of diversified interaction of various genes under different environments and heritability values are specific to a particular population under particular environment (Comstock and Moll, 1963). Interpretation of heritability values should be made with somber care because a salt tolerant genotype will be more vigorous in salinity stress as compared to susceptible variety, however in normal conditions susceptible genotype may perform better than salt tolerant. Therefore if an experiment is conducted in a single environment the genetic inference may not be attributed to overall conditions as there are chances of false positive estimates. Verification in all types of environments is advisable prior to any conclusion.

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