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RESEARCH ARTICLE

Biomass Partitioning and Genetic Analyses of Salinity Tolerance in Sunflower (*Helianthus annuus* L.)

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Abstract

Biomass partitioning was studied in sunflower (*Helianthus annuus* L.) inbred lines and their hybrids differing in salinity resistance. Differential biomass partitioning was observed among resistant and susceptible lines as well as within resistant lines, which were grown in large pots. Traits such as number of dead leaves and total number of nodes may be used as dominant markers for understanding the mechanism of resistance to salinity. Multi-location trials differing for salinity levels showed low and non-significant heritabilities across the environment for biochemical traits showing their sensitivity to the environment and a significant G X E interaction. Thus selection could only possible within the salinity level for these traits. Contrastingly, morphological traits such as number of nodes showed significant heritabilities (narrow or broad sense) across the environment. These traits may be exploited by the selection of inbred lines across or with salinity levels. Among various salinity levels, 12 dSm^{-1} was found to be feasible for screening and selection as it promoted the additive type of gene action.

Key words : additive gene effects, avoidance, marker, salinity stress, tolerance

Abbreviations
CMI: cell membrane injury, TRL: total root length, MRL: main root length, LRL: lateral root length, LRD: lateral root density, LRN: lateral root number: SM: stem + leaf mass, HM: head mass including achenes, RM: receptacle mass, R/S: root shoot ratio, RVR: reproductive to vegetative ratio, A/R: achene to receptacle ratio, HI: harvest index, LA: leaf area, TNN: total numbers of nodes, AY: achene yield, PH: plant height, DD: degree of droopiness, HD: head diameter, AOA: active osmotic adjustment, POA: passive osmotic adjustment.

Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop supplying more than 13% of the total edible oil produced globally (Rauf et al. 2008a). Sunflower requires fewer days to complete its life cycle than other field crops, while high quantities per unit area and quality edible oil (rich in linoleic and oleic acids) are the prime factors proving its versatility which allow this crop to be grown in 68 countries (FAO 2010). Certain other characteristics such as its robustness and extensive tap root system or osmo-regulatory mechanisms have induced tolerance in the crop to perform better under water-limited conditions (Rauf and Sadaqat 2008a; Rauf et al. 2009b).

Despite having fair tolerance against drought, sunflower performs poorly under salinity stress. Different studies have shown a suppressing effect of salinity stress on sunflower (Akram et al. 2007; El-Kader et al. 2006; Noreen and Ashraf 2008). When compounded, these studies indicate that salinity causes a serious decline in yield and its related parameters. Studies aimed at understanding the partitioning of various salts in sunflower and other species and varieties assumed that having a higher ability to discriminate K^+ over Na^+ have made it a superior crop (Ashraf and Harris 2004; Wided et al. 2009). However, little information exists regarding the above-to-below-ground biomass partitioning in saline environments. Flowers (2004) reviewed very few stud-

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ies at the whole plant level while a significant proportion of studies were related with only one component, salt tolerance. There is very little documentation regarding below- and above-ground traits, possibly because of the laborious nature of recording these traits (Rauf 2008). However, the estimation of biomass accumulation in various plant organs determines the response of these traits to salinity and may be helpful in calculating mass-based ratios. For example, harvest index (HI) is derived from the ratio of achene yield to biological yield. Thus, a high HI is indicative of translocation and mobilization of photosynthates or food reserves to the reproductive organs (Rauf and Sadaqat 2008a). A comparison of the biomass partitioning and ratios of salt-sensitive or -tolerant inbred lines would assist in measuring the overall response towards salinity stress and thus improve our understanding of breeding for salinity tolerance in sunflower.

It is also important to determine the genetic variance\ and their components associated with traits of interest (Rauf et al. 2008b, 2009a). Genetic variance and its components would determine the impact of the environment and gene interactions involved in the traits (Rauf et al. 2009a) and further determine the suitability of traits to be used as selection criteria.

In the present study, three experiments were carried out on sunflower with the following objectives: (i) to estimate salt stress injury, (ii) to assess the robustness and response of the sunflower root system to a saline environment, (iii) to estimate biomass partitioning under salinity stress, (iv) to identify the possible adaptive mechanism of sunflower to salinity, (v) to establish a relationship of below-to-above-ground plant traits in association with salinity tolerance, and (vii) to determine genetic variances associated with biochemical and morphological traits.

Materials and Methods

The studies were comprised of two phases, i.e. a large pot experiment and a multi-location field trial. Large pot experiments were conducted to determine the impact of salinity on below-ground plant biomass partitioning as well as above-ground biomass partitioning. On the other hand, field experiments were conducted to determine the genotype X environment interaction, genotypic variance, heritabilities, and selection of breeding material.

Experiment 1

Selection of plant material

Breeding lines (i.e. B lines are used to maintain cytoplasmic male sterile lines which are used as the female lines) and restorer (R) lines (used as male lines (pollen parent) in commercial production of hybrid seed and also to restore fertility in hybrid seed when it is crossed to female cytoplasmic male sterile lines) were planted in fields specifically suitable for screening against salinity stress at two locations located within a 1 km radius during the summer of 2009. Fields had a salinity level (measured in terms of electric conductivity or EC) which ranged from 3 to 11 dSm^{-1}. There were 28 sunflower B lines and two rows per B line within each replication. Each row was 18 m long. Plant-to-plant and row-to-row distance was 24 and 60 cm, respectively. Within each row six competitive plants (having plants at an optimum distance on each side) were selected for measurement of various plant traits. Data from both plots were subjected to analyses of variance which indicated significant ($P \leq 0.05$) variation due to inbred lines, salinity, and inbred lines X salinity regimes. Fifteen morphological, phenological, and physiological traits were used to estimate a multivariate score index across salinity levels through computer-based software MiniTab 15. B lines were categorized into various groups on the basis of their scores. Genotypes were selected having the highest positive score or the lowest negative multivariate score.

Development of plant material

Selected material, i.e. 4 female lines and 1 male were crossed in a line X tester mating design. Line X tester is a type of mating design in which few B lines are declared as female and they are crossed to each of the declared male B lines. 4 female X 1 male lines resulted in the 4 crosses. Parents as well as their crosses were further evaluated in various experiments.

Response of root traits to salinity stress

In order to determine the response of the sunflower tap root system to salinity stress, an experiment was conducted in large plastic bags with 25 kg of soil and with a soil depth of 60 cm during October 2009 and continued until mid-February, 2010. Each bag was filled with an equal ratio of sand, silt, and organic matter (leaf litter). Five inbred lines and five crosses, including one commercial hybrid, were used in the experiment. Each treatment was replicated twice. Salinity regimes were developed by applying normal irrigation water having an EC < 0.1 dSm^{-1} as the non-saline treatment while the saline treatment consisted of irrigating the bags with brackish water, which slowly accumulated salt in the root zone and final EC at plant harvest, which reached 12 dSm^{-1}. The leaching of salts from the root zone was avoided by irrigating with 3 L of water at each interval. Previous experiments in sunflower showed that sunflower root characteristics did not show growth nor did they decline after anthesis (Rauf and Sadaqat 2007). Therefore, the experiment was terminated at anthesis at which time data was recorded for cell membrane injury and root characteristics.

The roots were carefully removed from plastic bags and placed in muslin cloth by dousing with tap water to determine the various morphological parameters of roots and roots were removed from pots at the time of floral anthesis and since yield parameters cannot be estimated at this stage. Therefore, leaf characteristics and yield parameters were measured in a separate experiment. Various traits (main root length, lateral root length, plant height, and total root length) were measured on graph paper while the number of lateral roots was counted manually. Total root length was the sum of main root length and lateral root length and expressed in cm. Mass-based root traits were measured on a digital balance. The root-to-shoot ratio was obtained by dividing the total root mass by the above-ground shoot biomass. Lateral root length density was the ratio of lateral

root number to the main root length (Rauf et al. 2008b).

Cell membrane stability

Cell membrane stability measurement was carried out according to Singh (2004). This technique has also been used in several other crop species to determine salinity resistance (Farooq and Azam 2006). To estimate cell membrane stability, leaf discs were cut from the 2nd last leaf from the top of the canopy and subsequently dipped in de-ionized water at 25°C with gentle shaking for 4 h to determine the electric conductivity (EC) of the sample. The second last leaf node was tagged for this analysis so as to have homogenous leaf age. Older leaves have deteriorated quality due to the accumulation of metabolites, therefore young leaves were preferred over older ones (Gnanasiri et al. 1987). Samples were than autoclaved for 15 min at 121 psi and EC was again determined. Cell membrane stability was computed as the ratio of EC after dipping for 4 h in de-ionized water to the EC value after autoclaving.

Experiment 2

Partitioning of above-ground biomass

A separate experiment was conducted in large plastic bags from January 15 to May, 2010 to ascertain the impact of salinity on above-ground plant traits. The procedure adopted was the same as that mentioned above. However, these plants were raised to maturity to determine the yield parameters and various above-ground plant traits. Plant height was measured with the help of a measuring tape; number of nodes was counted manually; stem girth with a Vernier caliper; stem, seed and head mass were measured on a digital balance. Leaf area was determined by outlining the leaf on graph paper; the number of small units were calculated and converted to cm^2. HI is the ratio of seed to the total above-ground mass while the reproductive vegetative ratio was obtained by dividing the reproductive mass by the total above-ground mass.

Statistical analyses

Statistical analyses of data was carried out in a completely randomized arrangement with two factors i.e. genotypes and salinity levels (non-saline 0.9 dSm^{-1} or saline 12 dSm^{-1}). The genotypes were further subdivided into inbred lines and their hybrids.
Analyses of variance showed significant ($P \leq 0.01$) variation among the genotypes, salinity levels and their interaction, i.e. genotypes X salinity level. A significant genotype X salinity level interaction indicated that genotypes had changed their relative ranking across salinity levels. Thus, mean performance of genotypes is indicated within both levels in Figs. 1-9.

Experiment 3

Field trials

Field experiments were carried out to estimate the genetic variance associated with various biochemical and morphological traits which will further help to determine the selection criteria for the development of salt-resistant hybrids and inbred lines. Furthermore, it will also be helpful in selecting useful plant material for the establishment of hybrids and inbred lines.

Development of plant material

Five female lines were crossed with four male testers in a line X tester mating design to produce 20 F_1 hybrids. These hybrids, along with their parents, were sown during February, 2010 crop year in three locations with varying soil salinity determined through electric conductivity, i.e. 2.1, 7.2, and 12 dSm^{-1}. To determine the salinity level of the specific field, random samples were collected from the field and sent to the soil testing laboratory. Field plots with homogenous levels of salinity were selected. Plants were sown on ridges with an inter-row distance of 70 cm and an inter-plant distance of 30 cm. Each genotype was assigned to two rows within each replicate. Plants were raised according to normal crop husbandry techniques adapted to those regions of Punjab. Insects were controlled with synthetic pesticides before they reached the threshold level while leaf diseases were scored as absent.

At the time of anthesis, biochemical traits were estimated while at maturity agronomic traits were estimated.

Cell membrane injury

Leaf samples of parents and hybrids were collected from field plots with an EC of 7.2 and 12 dSm^{-1}. This trait has relevance with salinity tolerance; therefore, leaf sampling was only carried out from those salinity levels which can cause significant membrane injury. Cell membrane injury was measured with the method indicated in the section above, i.e. cell membrane injury.

Osmotic adjustment

Osmotic adjustment was estimated as solute contribution to the total osmotic potential. Three organic, i.e. total soluble carbohydrates, proline and amino acids contributed to the active osmotic adjustment while three inorganic solutes (Ca^{2+}, Mg^{2+}, K^+) contributed to the passive osmotic adjustment. Active osmotic adjustment was the sum of organic solutes and passive osmotic adjustment was the sum of inorganic solutes. For the measurement of osmolytes in the cell sap, a weighed sample of 200 mg of leaves from the top of the canopy was used. Organic and inorganic solutes were extracted according to the method of de Lacerda et al. (2003). Solute measurement for each sample was carried out in triplicate.

Morphological traits

At maturity, five competitive plants having sunflower plants on either side were tagged and measured for various morphological traits, i.e. nodes $plant^{-1}$, degree of droopiness, head diameter, and yield $plant^{-1}$.

Statistical and biometrical procedures

Data was analyzed in a randomized complete block design under a factorial arrangement, i.e. salinity levels and genotypes. The genotypes sum of squares was further partitioned into parents and hybrids and parents vs. hybrids. The genotypes X salinity interaction was also determined and this interaction was also

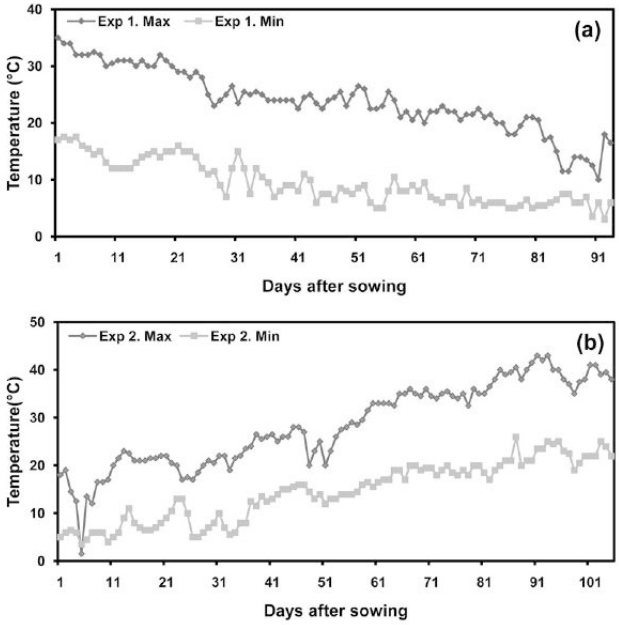

Fig. 1. Mean maximum and minimum temperatures for root characteristics (Exp. 1) and above-ground biomass partitioning (Exp. 2).

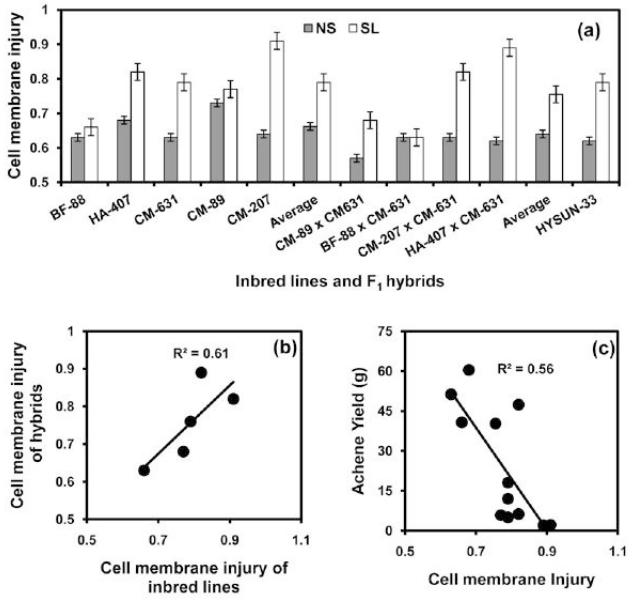

Fig. 2. Estimation of cell membrane injury in various inbred lines and hybrids under saline (SL) and non-saline (NS) regimes (a); Relationship of cell menbrane injury of inbred line and hybrids (b); Relationship of the achene yield of inbred line and cell membrane injury (c).

partitioned into parents X salinity, hybrids X salinity, and parents vs. hybrid X salinity interactions.

Traits showing significant variation due to genotypes were further analyzed to determine genetic variance associated with these traits and selection of suitable parents for the development of hybrids and breeding lines on the basis of these traits. Genetic variance and combining ability of the parents was estimated according to Kempthorne (1957) and outlined by Singh and Chaudary (1985). Two types of heritability were estimated, i.e. broad sense heritability (ratio of genotypic variance to the phenotypic variance; σ^2Genotypic/ σ^2Phenotypic) and narrow sense heritability (ratio of additive variance to the phenotypic variance; σ^2additive/ σ^2Phenotypic). Broad sense heritability was index of total genetic variation from the total variation (variation due to genotype and environment) while narrow sense heritability was index of fixable variation from the total variation.

Results

Genotype X salinity vs. genotype X temperature regimes

Plant traits, i.e. plant height, cell membrane stability, and above-ground dry matter were determined in both experiments. Growth conditions such as pot size, soil, and salinity regimes were similar in both experiments but there were differences in daily mean maximum and minimum temperature (Fig. 1). The temperature in experiment 1 declined while in experiment 2 it increased as both experiments advanced. Therefore, analysis of variance (ANOVA) was carried out to determine the differences across salinity and temperature regimes for these two traits, which showed significant differences ($P \leq 0.05$) due to genotype, temperature and salinity regime. Interactions such as genotypes X salinity were significant ($P \leq 0.05$) and high for all traits while genotype X temperature regime was significant but low compared to genotype X salinity. These results imply that performance of genotypes was more significantly affected by the salinity regime than by temperature. This is understandable since sunflower is grown throughout the year in Pakistan. Although there are significant differences in yield due to differential accumulation of heat units during different parts of the year, sunflower hybrids that give high yield in one season also remain exceptional in the other season (Khaliq and Cheema 2005; Qadir et al. 2007). Therefore, mean values for these traits were averaged across both experiments.

Status of salinity tolerance in selected plant material

Cell membrane injury (CMI) is indicative of salinity tolerance in the inbred lines (Fig. 2a). On average, parental lines, their hybrids and a single commercial hybrid showed an injury level of 18, 16, and 27%, respectively, under the salt regime. Two inbred lines, i.e. BF-88 and CM-89, showed a non-significant ($P \geq 0.05$) difference in non-saline and saline regimes in CMI (Fig. 2a). These two lines, when crossed with the salt-sensitive male inbred line CM-631 (22% injury level), produced hybrids BF-88 X CM-631 and CM-89 X CM-631 with an injury level of 1 and 19%, respectively. Hybrids from salt-sensitive female lines HA-407 (21% injury level) and CM-207 (42% injury level), when crossed with CM-631, showed an injury level of 44 and 22%, respectively. The relationship between salt injury of parental inbred lines and their hybrids was significant ($R^2 = 0.61$) (Fig. 2b), thus showing that the salt tolerance level of parental female lines is depicted in their hybrids, i.e. BF-88 X CM-631. Furthermore, the relationship between achene yield and the salt injury level of genotypes was also significant and negative (Fig. 2c).

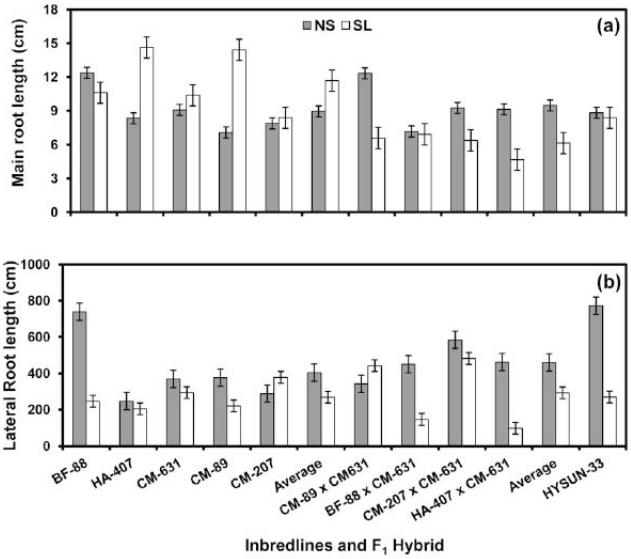

Fig. 3. Estimation of main root length (a) and lateral root length (b) under saline and non-saline regimes.

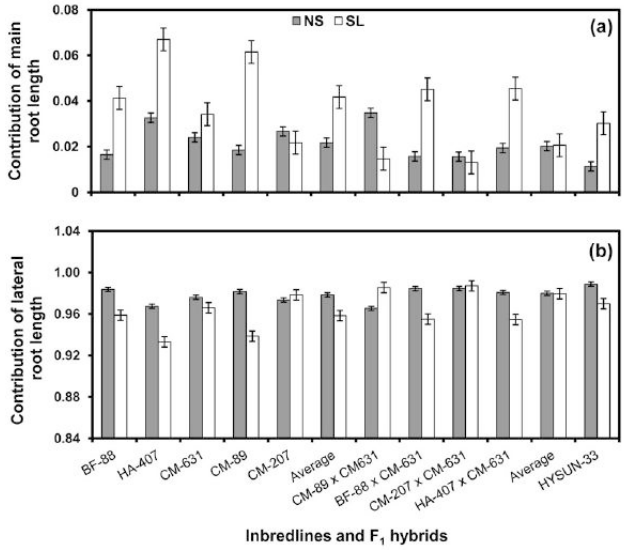

Fig. 4. Contribution of main root length (a) and lateral root length (b) to the total root length under saline (SL) and non-saline (NS) regimes.

Below-ground biomass partitioning

TRL (total root length) was partitioned into main root (MRL) and lateral root length (LRL). Inbred lines CM-207 and CM-631 showed a non-significant increase ($P \geq 0.05$) in MRL (Figure 3a). Similarly, hybrids also showed a non-significant ($P \geq 0.05$) increase for MRL under salinity stress. Among the inbred lines, HA-407 and hybrids Hysun-33 and BF-88 X CM-631 showed the highest MRL under salinity stress. HA-407 and its hybrid HA-407 X CM-631 showed the highest increase in MRL under salinity stress (Fig. 3a).

A decrease in the mean value due to salinity stress was observed for LRL (Fig. 3b). On average, hybrids and inbred lines showed significant ($P \leq 0.05$) repressing effects on LRL. However, inbred lines HA-407 and CM-631 showed a non-significant ($P \geq 0.05$) decline for LRL in a saline regime while CM-207 and CM-89 X CM-631 showed a non-significant ($P \geq 0.05$) increase for LRL. The effect of salinity significantly ($P \leq 0.05$) decreased the mean values of the resistant inbred lines BF-88 and CM-89 for LRL. Inbred line CM-207 and hybrids CM-207 X CM-631 and CM-89 X CM-631 showed the highest LRL under a saline regime (Fig. 3b).

The general contribution of MRL to TRL increased in inbred lines under salinity stress except for CM-207 while hybrids showed a non-significant change ($P \geq 0.05$) in the contribution of MRL. CM-89 X CM-631 showed a significant ($P \leq 0.05$) decrease in MRL under salinity stress (Fig. 4a). On the other hand, the contribution of LRL to TRL in inbred lines decreased under salinity stress while hybrids showed a non-significant ($P \geq 0.05$) change in the contribution of LRL except for CM-89 X CM-631, which showed a significant increase while BF-88 X CM-631 showed a significant decrease (Fig. 4b).

Despite the low contribution of MRL to TRL, the contribution of the main root mass to the total root mass was high and this contribution increased under salinity stress for most of the inbred lines and hybrids (Figs. 5a and b). BF-88 showed the highest total root mass (TRM) under a non-saline regime (Figs. 5a and b). This line showed a higher contribution of LRM to TRM in a non-saline regime. In contrast, the contribution of MRW was high in a saline for this genotype. However, inbred lines BF-88, HA-407, and CM-631 significantly contributed to LRM in saline conditions. The inbred line CM-207 showed the highest TRM under saline conditions due to the significant contribution of MRM. The TRM of inbred line HA-407 increased under salinity due to a significant increase of MRM (Fig. 5a). However, it showed non-significant changes in the contribution of MRM in both regimes which was maintained above 70% in both regimes (Fig. 5b). Among the hybrids, CM-207 X CM-631 followed by CM-89 X CM-631 showed the highest TRM while the highest contribution of MRM was seen in crosses BF-88 X CM-631 and HA-407 X CM-631 (Figs. 5a and b).

Resistant inbred lines BF-88 and CM-89 maintained their root-to-shoot ratio (R/S) under salinity by showing a non-significant ($P \geq 0.05$) increase of R/S under salinity stress in comparison with the non-saline regime (Fig. 6a). However, a significant increase in R/S was observed in salinity-susceptible inbred lines such as HA-407, CM-631, and CM-207. Hybrid CM-89 X CM-631 showed the highest R/S under the saline regime while a repressing effect were observed in cross CM-207 X CM-631. On average, inbred lines showed a higher R/S than hybrids, suggesting that hybrids prefer above-ground organs for translocation of photosynthates.

The repressing effect of salinity was evident on lateral root number (LRN) in inbred lines BF-88, CM-631 while HA-407 and CM-89 maintained ($P \geq 0.05$) their LRN and CM-207 showed a significant ($P \leq 0.05$) increase in LRN (Fig. 6b). CM-89 X CM-631 and CM-207 X CM-631 showed the highest LRN under salinity stress. On average, inbred lines showed higher LRN than hybrids under salinity stress.

All inbred lines showed a decline in lateral root density (LRD) except for CM-207, which showed a significant ($P \leq$

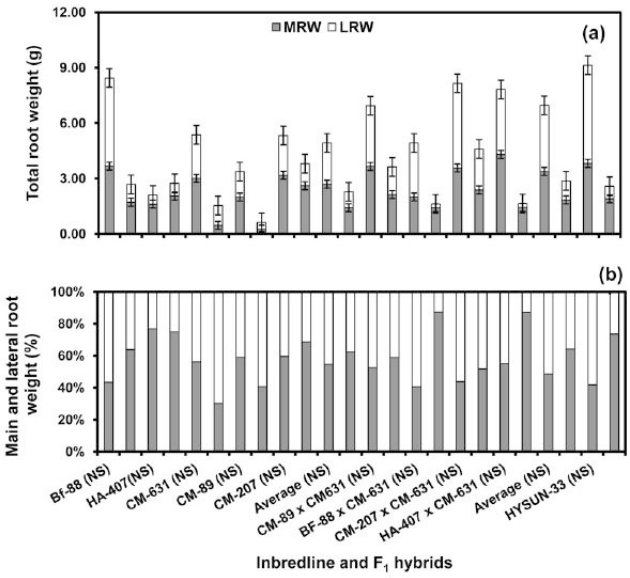

Fig. 5. Main root mass (MRW) + lateral root mass (LRW) as affected by saline regime(a) and proportion of main root length and lateral root to the total root length in percentage (b).

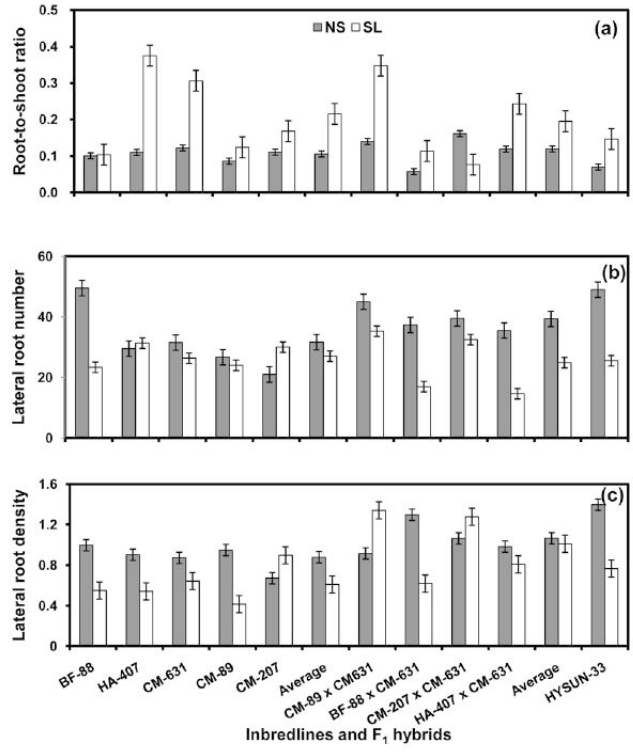

Fig. 6. Estimation of root-to-shoot ratio (a), lateral root number (b), and lateral root density (c) under saline and non-saline regimes.

0.05) increase in LRD under the stress regime. Among the hybrids, CM-89 X CM-631 and CM-207 X CM-631, LRD increased in response to salinity (Fig. 6c).

The highest LRD was observed in inbred line CM-207, which showed a significant increase in LRD under salinity stress (Fig. 6c). Among the hybrids, CM-207 X CM-631 and CM-89 X CM-631 showed a significant ($P \leq 0.05$) increase in LRD under the saline regime. Repressing effects of salinity were observed for all other inbred lines and hybrids. On average, hybrids showed higher LRD than inbred lines.

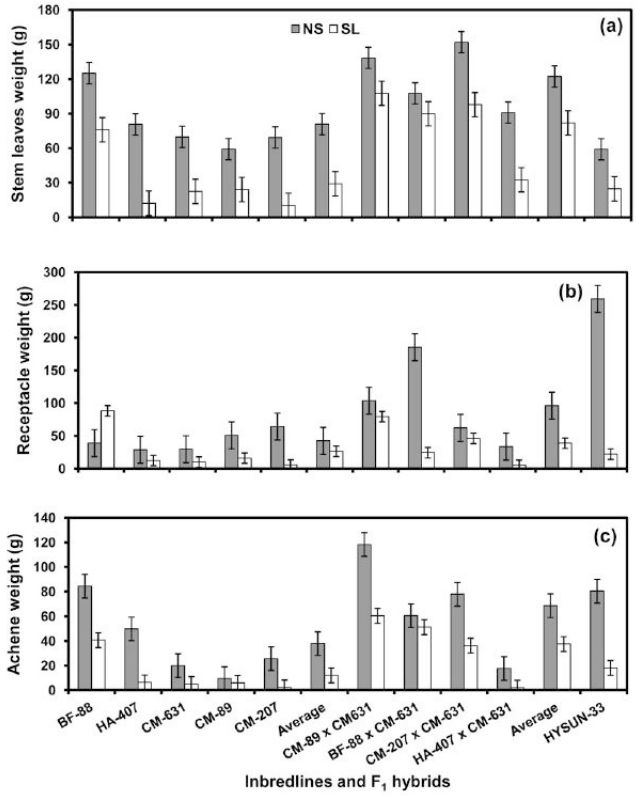

Fig. 7. Partitioning of above-ground biomass stem leave mass (a); receptacle mass (b); achene mass (c) in saline (SL) and non-saline (NS) regimes.

Above-ground biomass partitioning

The above-ground biomass of stem + leaf mass (SM), head mass including achenes (HM) and achene mass (AM) is given in Figs. 7-9.

Repressing effects were evident on SM. Resistant inbred line BF-88 showed the highest SM (Fig. 7a). Among the hybrids, CM-89 X CM-631 showed the highest SM under saline conditions. On the other hand, BF-88 X CM-631 showed a non-significant decrease ($P \leq 0$ .05) in SM under salinity stress.

Receptacle mass (RM) decreased under the salinity regime (Fig. 7b). However, BF-88 showed a significant increase in RM under saline conditions. CM-89 and HA-407 showed a non-significant ($P \geq 0.05$) decrease in RM. Among the hybrids, CM-89 X CM-631 showed the highest RM while CM-207 X CM-631 showed a non-significant repressing effect of salinity on RM.

BF-88 was the inbred line that yielded the highest number of achenes in both regimes (Fig. 7c). High yield potential of this inbred line was not observed in achene yield of its hybrid BF-88 X CM-631. This hybrid showed a non-significant ($P \geq 0.05$) change in yield under salinity. Salinity-resistant line CM-89 also showed a non-significant ($P \geq 0.05$) decrease in achene yield under a saline regime. This line produced the highest achene-yielding hybrid CIM-89 X CM-631 in both regimes. However, achene yield of this hybrid was statistically similar to that of BF-88 X CM-631 under the saline regime.

On average, inbred lines showed a non-significant ($P \geq 0.05$)

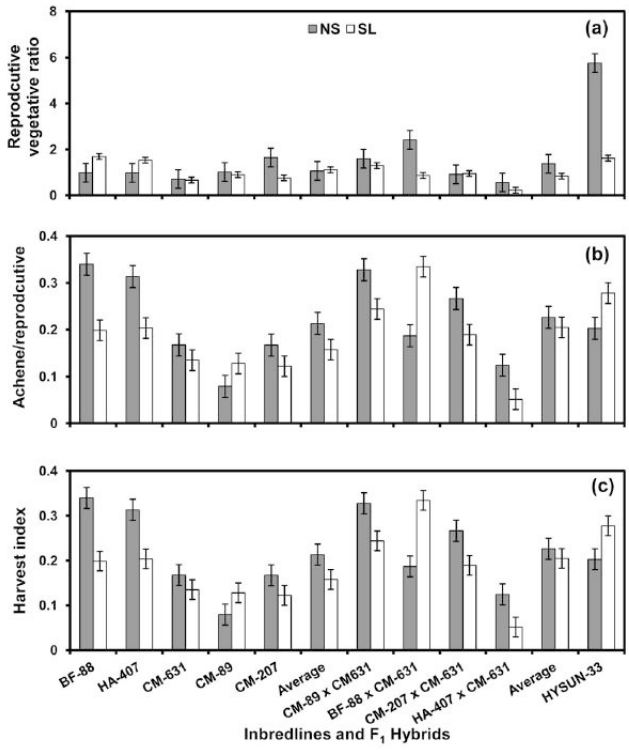

Fig. 8. Relative partitioning of above-ground biomass as indicated from various ratios. Reproductive vegetative ratio (a); achene/reproductive ratio (b); harvest index (c).

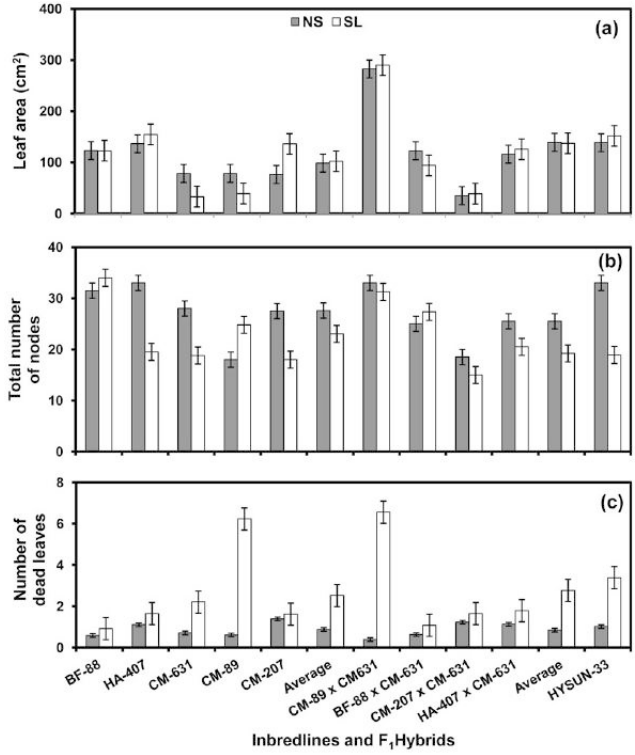

Fig. 9. Effect of salinity on various leaf traits: leaf area (a), total number of nodes (b), number of dead leaves (c).

increase in the reproductive to vegetative ratio (RVR) while hybrids showed a significant decrease in RVR under the saline regime (Fig. 8a). BF-88 and HA-407 showed an increase in RVR under salinity stress while CM-631 and CM-89 showed a non-significant ($P \geq 0.05$) increase and CM-207 showed a significant ($P \leq 0.05$) decrease of RVR. BF-88 showed the highest RVR under salinity regime.

The achene to receptacle ratio (A/R) indicated the preference of genotypes for biomass allocation to achenes over receptacles (Fig. 8b). BF-88 and HA-407 showed the highest A/ R. On average, the A/R of inbred lines was significantly ($P \leq 0.05$) reduced while that of hybrids showed a non-significant decrease under salinity regimes. BF-88 X CM-631 showed the highest and most significant increase in A/R followed by commercial hybrid Hysun-33.

Harvest index (HI) is the preference of a genotype for biomass allocation in achenes over biological yield (Rauf and Sadaqat 2008a) (Fig. 8c). On average, inbred lines showed a significant ($P \leq 0.05$) decrease in HI while hybrid HI losses were non-significant ($P \geq 0.05$) under the salinity regime (Fig. 8c). BF-88 and HA-407 showed the highest HI under salinity stress while hybrid BF-88 X CM-631 showed the highest and most significant increase in HI followed by Hysun-33.

On average, the leaf area (LA) of the inbred line and hybrids was statistically non-significant ($P \geq 0.05$) in the saline regime compared to the non-saline regime (Fig. 9a). BF-88 showed no change, HA-407 showed a non-significant increase and CM-89 showed a non-significant ($P \geq 0.05$) decrease in LA. CM-207 showed a significant ($P \leq 0.05$) increase in leaf area. In contrast, CM-631 showed a significant decrease for this trait.

The total numbers of nodes (TNN) decreased significantly ($P \leq 0.05$) in breeding lines and hybrids in response to salinity (Fig. 9b). BF-88 showed the highest TNN but a non-significant change under salinity stress. CM-89 showed a significant ($P \leq 0.05$) increase under salinity. CM-89 X CM-631 showed the highest and a non-significant decrease in TNN while BF-88 X CM-631 showed a non-significant increase for this trait under salinity. TNN was significantly repressed in all other hybrids under salinity stress.

On average, inbred lines and hybrids showed a significant increase in the number of dead leaves (NDL) under the saline regime (Fig. 9c). Inbred line CM-89 showed the highest NDL followed by CM-631 under salinity while BF-88, CM-207, and HA-407 showed a non-significant increase in NDL under the salinity regime.

Among hybrids, CM-89 X CM-631 showed the highest increase in NDL under the saline regime while BF-88 X CM-631 showed a non-significant change (Fig. 9c). Other hybrids showed a significant increase of NDL under salinity stress.

Analyses of variance for genotypes, crosses, and parents across salinity regimes

Analysis of variance of various morphological characters, which was carried out over non-saline and saline levels, showed significant differences ($P \leq 0.05$) among genotypes for all traits. Variation due to salinity and the interaction between genotypes X salinity level was also significant ($P \leq 0.05$). Genotypes further bifurcated into parents and crosses. Differences among parents and crosses were significant ($P \leq 0.05$) for all traits.

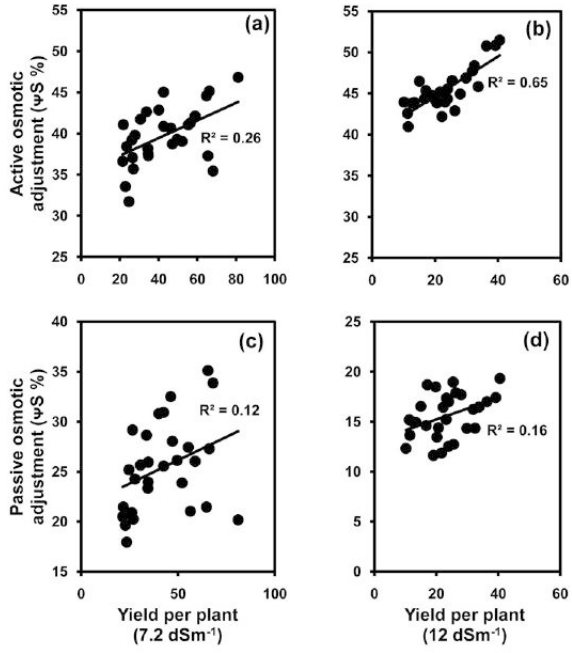

Fig. 10. Relationship of active osmotic adjustment or passive osmotic adjustment to the variation in yield per plant across various salinity stresses (7.2 DSm^{-1}, 12 DSm^{-1}).

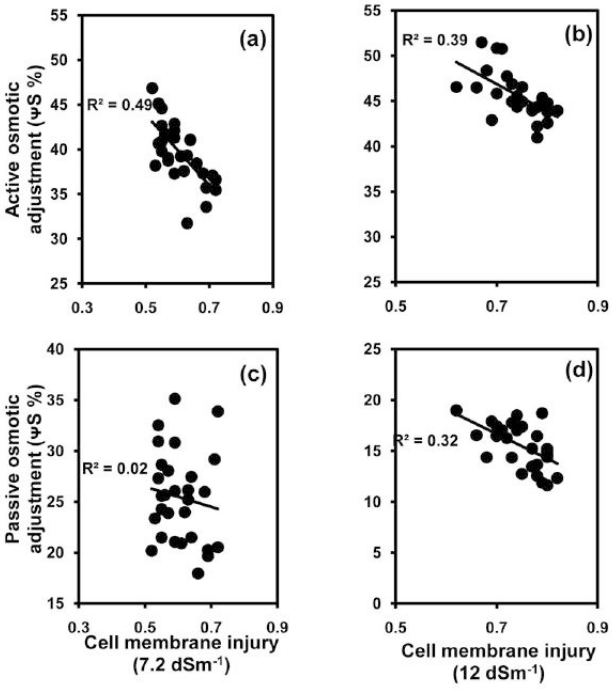

Fig. 11 Relationship of active osmotic adjustment or passive osmotic adjustment to the variation in cell membrane injury under various salinity stresses (7.2 dsm^{-1}, 12 dsm^{-1}).

However, variation in female parents was significant ($P \leq 0.05$) among all traits except for achene yield (AY) while variation among male parents was significant ($P \leq 0.05$) for all traits. Variation due to the interaction between male and female parents was also significant ($P \leq 0.05$) for all traits. Furthermore, variation due to parents versus crosses which was indicative of heterosis was also significant ($P \leq 0.05$).

Interactions such as parents X salinity and crosses X salinity were also significant for all traits ($P \leq 0.05$). The interaction of male X salinity was also significant for all traits except for plant height (PH) ($P \leq 0.05$). The interaction of female with salinity was also significant for all traits except for AY ($P \leq 0.05$).

Table 1. Estimate of genotypic (σ^2 G), additive (σ^2 A), non-additive (σ^2 NA), and heritabilities in broad sense (H) and narrow sense (h) for various morphological traits and biochemical traits and AY (achene yield) over salinity regimes

Traits	σ^2 G	σ^2 A	σ^2 NA	H	h
Achene yield	161.82	46.17	74.16	0.53*	0.15^{NS}
Degree of droopiness	0.02	0.00	0.00	0.73*	0.03^{NS}
Head diameter	3.1	0.33	0.49	0.75*	0.08^{NS}
Plant height	360.35	142.16	14.75	0.69*	0.27*
Number of nodes	7.68	5.16	0.76	0.50*	0.33*
Cell membrane injury	0.0	0.0	0.0	0.18NS	0.09NS
Active osmotic adjustment	1.99	0.71	3.78	0.22*	0.08NS
Passive osmotic adjustment	2.55	0.76	6.94	0.21*	0.06NS

* (significant) when $P \leq 0.05$; ^{NS} = (non-significant) when $P \geq 0.05$

Analysis of variance for biochemical traits such as CMI, active osmotic adjustment (AOA) and passive osmotic adjustment (POA) was carried out, indicating significant ($P \leq 0.05$) differences among genotypes, parents and crosses. Variation among female parents was significant ($P \leq 0.05$) for CMI and non-significant ($P \geq 0.05$) for AOA and POA; similarly, variation among male parents was significant ($P \leq 0.05$) for CMI and non-significant ($P \geq 0.05$) for active and passive osmotic adjustment. On the other hand, variation due to female and male parent interactions was significant ($P \leq 0.05$) for all traits. Salinity level also caused significant variation and the performance of genotypes was significantly affected by saline regimes. This was indicative from significant interactions of genotype X salinity or its components, parents X salinity and crosses X salinity.

σgenotype, σadditive, σdominance and heritabilities

Estimated genotypic variance further bifurcated into additive and dominant variance, which indicated the highest magnitude of dominance variance for traits such as AY, degree of droopiness (DD) and head diameter (HD); therefore, these traits were not suitable for selection of inbred lines and may be exploited for production of high-yielding hybrids on the basis of combining ability of inbreds (Table 1). In the case of PH and TNN, additive variance was higher than dominance variance.

Broad sense heritability was estimated over environment and the highest magnitude of broad sense heritability was estimated for HD and DD (Table 1). The high broad sense heritability was not indicative of narrow sense heritability. Narrow sense heritability over environment was non-significant ($P \geq 0.05$) for traits such as AY, HD while narrow sense heritability for PH and TNN was significant. Therefore these traits may be suitable for selection of inbred lines over contrasting salinity regimes.

Broad sense heritability of biochemical traits was low and non-significant ($P \geq 0.05$) over the environment. Similarly dominance variance was high compared to the additive variance which resulted in an estimate of very low narrow sense heritability over

Table 2. Estimates of genotypic, additive, and non-additive variances, heritabilities in broadsense (H) and narrowsense (h) for head diameter (HD), achene yield per plant (AY), plant height (PH), number of nodes (NN), degree of droopiness (DD), active osmotic adjustment (AOA), passive osmotic adjustment (POA), and cell membrane injury (CMI) within salinity levels of S1 (2.1 dSm^{-1}), S2 (7.2 dSm^{-1}), and S3 (12 dSm^{-1})

Traits	σ^2 Genotypic			σ^2 Additive			σ^2 Non-additive			H			h		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
HD	4.72	3.17	3.86	0.24	0.17	0.31	1.73	1.78	5.98	0.97*	0.93*	0.94*	0.05NS	0.05 NS	0.08 NS
AY	490.95	264.26	66.09	63.91	40.62	18.69	1028.9	514.05	51.88	0.90*	0.84*	0.87*	0.12 NS	0.13 NS	0.25*
PH	471.84	567.36	484.02	58.44	137.3	149.56	199.92	103.15	99.13	0.95	0.98	0.99*	0.12NS	0.24*	0.31*
NN	7.29	12.15	22.92	1.84	3.82	8.45	7.5	3.8	7.66	0.83	0.89	0.93	0.21*	0.28*	0.34*
DD	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.99	0.99	0.99	0.01NS	0.01 NS	0.15
AOA	-	11.8	5.95	-	2.1	1.32	-	25.67	16.5	-	0.90*	0.84*	-	0.16 NS	0.19*
POA	-	19.66	4.74	-	1.08	1.35	-	73.23	9.4	-	0.93*	0.87*	-	0.05 NS	0.25*
CMI	-	0	0	-	0	0	-	0.01	0	-	0.82*	0.81*	-	0.15NS	0.22*

* (significant) when $P \leq 0.05$; ^{NS} = (non-significant) when $P \geq 0.05$

Table 3. General combining ability of inbred lines for morphological traits in salinity level of SL1 (2.1 dSm^{-1}), SL2 (7.2 dSm^{-1}), and SL3 (12 dSm^{-1}).

Parents	Head diameter			Yield			Plant height			Nodes per plant			Degree of droopiness		
	SL1	SL2	SL3	SL1	SL2	SL3	SL1	SL2	SL3	SL1	SL2	SL3	SL1	SL2	SL3
CM-207	-1.34	-1.29	-1.25	-16.48	-6.81	1.75	-10.35	-8.13	-20.80	1.59	-3.22	-4.38	0.04	0.04	-0.04
CM-89	0.04	0.16	-1.10	-6.03	-5.49	-4.49	-15.92	-12.90	1.60	1.09	1.91	-1.58	-0.02	-0.02	0.08
AMES-10103	0.17	0.46	0.88	4.76	2.07	2.13	15.94	25.08	8.86	2.25	3.67	4.92	0.00	0.00	-0.06
BF-88	0.28	-0.07	0.74	6.15	2.57	-4.81	2.61	16.09	28.34	-1.96	0.99	5.38	-0.01	-0.01	0.09
HA-407	0.85	0.72	0.72	11.60	7.66	5.42	7.71	-20.14	-17.99	-2.97	-3.35	-4.34	-0.01	-0.01	-0.07
SE	0.12	0.14	0.14	2.11	2.01	0.89	1.43	1.08	0.63	0.35	0.36	0.38	0.01	0.01	0.00
CM-615	0.42	0.01	0.33	-7.74	-8.24	-0.88	-0.06	-0.18	-0.81	0.45	0.45	0.33	0.05	0.05	0.01
CM-631	-0.51	-0.23	0.49	16.77	8.11	-2.43	-3.29	0.66	-2.08	-0.42	1.00	0.19	-0.04	-0.04	0.02
R-SIN-83	0.17	0.26	-0.23	-9.49	-10.87	-5.30	3.84	1.22	-0.15	0.69	-1.27	-0.92	0.00	0.00	0.04
R-FSS-88	-0.09	-0.03	-0.59	0.46	11.01	8.61	-0.49	-1.70	3.04	-0.72	-0.18	0.40	-0.01	-0.01	-0.07
SE	0.11	0.12	0.13	1.89	1.80	0.80	1.28	0.97	0.57	0.31	0.32	0.34	0.01	0.01	0.00

the environment ($P \geq 0.05$) (Table 1). Low heritability estimates indicate a significant impact of salinity level and genotype X salinity interactions; thus, it was unsuitable to select genotypes on the basis of biochemical traits over the environment.

Analyses of variance within salinity regimes

Analyses of variance was also carried out within environment and thus genotypic variance and its components were estimated by removing the impact of genotype X salinity or saline environments, which gave significantly high estimates of broad sense heritability (Table 2). Generally, additive variance tends to increase with a salinity level. Contrastingly, dominance variance decreased, showing the highest estimates of significant heritability in saline environments having 12 dSm^{-1} for all traits (Table 2). Therefore, an EC level of 12 dSm^{-1} was suitable for selection of the traits and genotypes. The yield component trait i.e., head diameter showed non-significant ($P \geq 0.05$) narrow sense heritability in all salinity levels (Table 2). Yield also showed a non-significant ($P \geq 0.05$) narrow sense heritability in all salinity levels except for 12 dSm^{-1}. Among the morphological traits, the highest significant heritability was estimated for TNN followed by plant height (Table 2). Among biochemical traits the highest significant ($P \leq 0.05$) heritability was available for POA followed by CMI (Table 2).

Combining ability effects of male and female parents

Combining ability effects of male and female parents were estimated within salinity levels which showed significant changes in the ranking of genotypes over the salt environments (Tables 3 and 4). HA-407 and AMES-10103 were good positive general combiners for HD and AY in all environments (Table 3). However, the combining ability effects of HA-407 decreased with salinity level while AMES-10103 increased with salinity level for HD. Among female parents, the highest combining ability effects were estimated for HA-407 under non-saline and saline environments of 7.2 dSm^{-1} for HD while AMES-10103 showed the highest GCA effect under a salinity level of 12 dSm^{-1} (Table 3). Among the male parents, CM-615 was the best combiner in the non-saline environment and R-Sin-83 was the best combiner for head diameter in a saline environment of 7.2 dSm^{-1} while CM-631 was the best combiner for head diameter under a saline environment of 12 dSm^{-1} (Table 3). HA-407 was also exceptional in all environments for yield $plant^{-1}$. Among female parents, HA-407 showed the highest GCA values for all environments while CM-631 was the best male general combiner for AY under non-saline conditions and R-FSS-88 was the best general combiner for yield under saline environments of 7.2 and 12 dSm^{-1} (Table 3). AMES-10103 and BF-88 were positive combiners in all environments for PH. Among females, AMES-10103 was best combiner under the non-saline environment and a saline environment of 7.2 dSm^{-1}, while BF-88 was the best general combiner under a salinity level of 12 dSm^{-1} (Table 3). Among male parents, R-SIN-83 showed the highest general combining ability effects under the non-saline environment and a saline environment of 7.2 dSm^{-1}. R-F-SS-88 was the best male combiner for PH at 12 dSm^{-1} (Table 3).

Ames-10103 and CIM-615 were positive combiners in all environments for number of TNN. General combining effects of

Table 4. General combining ability of inbred lines for active osmotic adjustment (AOA), passive osmotic adjustment (POA), and cell membrane injury (CMI) in salinity levels of SL1 (7.2 dSm^{-1}) and SL2 (12 dSm^{-1}).

Inbred lines	AOA		POA		CMI	
	SL1	SL2	SL1	SL2	SL1	SL2
CM-207	2.05	1.07	3.31	1.15	-0.04	-0.01
CM-89	-3.44	-1.47	-0.12	-1.36	0.05	0.03
AMES-10103	1.15	0.17	-2.46	0.79	0.02	0.00
BF-88	0.88	-1.70	-0.95	-1.58	-0.03	0.02
HA-407	-0.63	1.92	0.22	1.00	0.00	-0.03
SE	0.34	0.30	0.36	0.25	0.01	0.01
CM-615	-0.69	-0.49	-0.43	-0.27	0.03	0.00
CM-631	2.65	-0.67	-0.59	-1.58	-0.05	0.02
R-SIN-83	-1.68	-1.25	-2.14	-0.49	0.02	0.03
R-FSS-88	-0.29	2.41	3.16	2.34	0.00	-0.05
SE	0.30	0.27	0.32	0.22	0.01	0.01

Table 5. Mean values for active osmotic adjustment (AOA), passive osmotic adjustment (POA), and cell membrane injury (CMI) due to salinity levels of SL1 (7.2 dSm^{-1}) and SL2 (12 dSm^{-1}).

	AOA		POA		CMI	
	SL1	SL2	SL1	SL2	SL1	SL2
Female						
CM-207	39.78 ± 0.74	44.36 ± 0.61	24.27 ± 1.16	18.49 ± 1.21	0.55 ± 0.02	0.74 ± 0.01
CM-89	39.22 ± 0.90	45.35 ± 0.51	20.90 ± 0.91	18.71 ± 0.70	0.61 ± 0.01	0.79 ± 0.01
AMES-10103	41.73 ± 1.04	46.49 ± 0.63	25.66 ± 0.80	16.54 ± 0.32	0.56 ± 0.02	0.66 ± 0.03
BF-88	42.64 ± 1.02	46.54 ± 0.70	28.65 ± 1.08	18.98 ± 0.19	0.55 ± 0.02	0.62 ± 0.01
HA-407	38.43 ± 0.59	43.91 ± 0.59	17.95 ± 1.13	14.94 ± 0.32	0.66 ± 0.01	0.80 ± 0.01
Male						
CM-615	36.62 ± 0.72	44.36 ± 0.70	20.51 ± 0.61	14.62 ± 0.29	0.72 ± 0.01	0.80 ± 0.02
CM-631	33.56 ± 0.90	43.82 ± 0.33	19.64 ± 0.84	14.95 ± 0.11	0.69 ± 0.01	0.80 ± 0.01
R-SIN-83	31.73 ± 0.91	42.58 ± 0.49	25.20 ± 0.51	15.20 ± 0.49	0.63 ± 0.01	0.80 ± 0.02
R-FSS-88	41.08 ± 0.27	40.96 ± 0.75	21.48 ± 1.47	13.65 ± 0.42	0.64 ± 0.01	0.78 ± 0.01

AMES-10103 increased with salinity levels. This inbred line was also the best combiner for the non-saline environment and for a saline environment of 7.2 dSm^{-1}, while BF-88 was the best combiner at 12 dSm^{-1} salinity (Table 3). Among male parents, R-SIN-83 was the best combiner for TNN, while CM-631 was the best combiner under salinity of 7.2 dSm^{-1} and RF-SS -88 was the best combiner under salinity of 12 dSm^{-1}. For DD, CM-207, and CM-615 was the best male and female general combiner under non-saline and saline environments (7.2 dSm^{-1}). CM-89 was the best female combiner in EC of 12 dSm^{-1} for DD (Table 3).

CM-207 was a positive combiner under both saline environments (7.2 and 12 dSm^{-1}) for AOA and POA (Table 4). It was also the best female general combiner for these two traits while among male parents, CM-631 was the best combiner at 7.2 dSm^{-1} and R-FSS-88 at 12 dSm^{-1} for active osmotic adjustment (Table 4). R-FSS-88 was the best combiner at both ECs for passive osmotic adjustment.

CM-89 was a good positive female general combiner for cell membrane injury under both saline environments (Table 4). CM-615 was a good positive general combiner in the saline environment having 7.2 dSm^{-1} and R-SIN-83 at 12 dSm^{-1} (Table 4). CM-207 was a negative female general combiner under both saline environments for CMI. CM-631 was a male negative combiner under salinity stress of 7.2 dm^{-1} and R-FSS-88 was a negative combiner under 12 dSm^{-1} (Table 4).

Mean performance of parental inbred lines across saline environments

In the case of physiological traits, AOA and CMI increased with 7.2 and 12 dSm^{-1} salinity levels while passive osmotic adjustment decreased under the same levels (Table 5). AOA and CMI increased by 15.2 and 21.24% when overall mean values under salinity stress of 7.2 and 12 dSm^{-1} were compared. AOA was the sum of three organic osmolytes, i.e. sugars, proline, and free amino acids. Sugars followed by proline constituted the major proportion of AOA and their contribution increased with an increase in salinity level. On the other hand, POA decreased by 28.16% when both salinity levels were compared. Passive osmotic adjustment was the sum of three inorganic osmolytes, i.e. K^+, Ca^{2+}, and Mg^{2+}. K^{2+} made the highest contribution to osmotic adjustment. However, salinity had a repressing effect over the contribution of K^+ with an increase in the salinity level; thus, the overall contribution of passive osmotic adjustment was also reduced under the salinity levels (Table 5). Among the female lines, BF-88 had the highest ability for active, passive osmotic adjustment and the lowest cell membrane injury. Thus, it seems in this genotype that ability to produce highly active osmolytes was not negatively correlated with the passive osmolytes. Among the male inbred lines, R-FSS-88 showed the highest AOA under a salinity level of 7.2 dSm^{-1} while CM-615 showed the highest POA at a salinity level of 12 dSm-1. R-SIN-82 showed the highest POA at both salinity levels (Table 5). The male inbred line R-F-SS-88 showed the lowest CMI in both saline environments.

A constant decrease in various morphological traits was also noted due to salinity stress (Table 6). On average, the highest decrease of 38.57% was observed for AY under salinity stress of 12 dSm^{-1}. A relative decrease in other traits was noted as follows: i.e. HD (6.25 and 25.81%), PH (8.50 and 27.49%), YNN (15.53 and 32.11%) and increase in erectness (9.25%) under salinity stress of 7.2 and 12 dSm^{-1}. Furthermore, relative ranking of inbred lines was also observed across various salinity stresses (Table 6). Among female lines, AMES-10103 showed the highest HD, TNN under non-saline and saline condition of 7.2 dSm^{-1} (Table 6). The same line was the highest yielder under non-stressed condition and had the highest droopiness at 7.2 dSm^{-1}. BF-88 showed the highest HD, AY under salinity regimes (7.2 and 12 dSm^{-1}). CM-207 showed the highest DD under non-saline conditions (Table 6).

Among male lines, R-SIN-82 and CM-615 were the most promising. R-SIN-82 showed the highest PH and TNN under all environments (Table 6). CM-615 showed the highest HD under saline (7.2 dSm^{-1}) and non-saline conditions. Furthermore, CM-631 showed the highest AY in non-stress conditions and saline regime (12 dSm^{-1}). The highest DD was shown by CM-615 under saline regimes (7.2 and 12 dSm^{-1}) (Table 6).

Relationships between biochemical and morphological traits

The relationship between AOA and AY increased with an increase in salinity level of 7.2 and 12 dSm^{-1} (Fig. 10). On the other hand, POA was not-significant ($P \geq 0.05$) R^2 with achene

Table 6. Mean values ± se for various morphological, yield and its component due to salinity levels of SL0, SL1 (7.2 dSm^{-1}), and SL2 (12 dSm^{-1}).

	Head diameter (cm)			Yield (g)			Plant height (cm)			Nodes per plant			Degree of droopiness		
	SL0	SL1	SL2	SL0	SL1	SL2	SL0	SL1	SL2	SL0	SL1	SL2	SL0	SL1	SL2
Female															
CM-207	16.00 ± 0.12	15.30 ± 0.66	12.67 ± 0.53	36.00 ± 2.31	27.73 ± 1.69	19.77 ± 0.78	147.23 ± 5.02	131.59 ± 1.42	88.52 ± 2.12	26.31 ± 1.06	22.27 ± 0.58	14.17 ± 0.53	0.66 ± 0.01	0.74 ± 0.01	0.79 ± 0.00
CM-89	16.37 ± 0.15	14.63 ± 0.26	11.30 ± 0.40	41.87 ± 2.52	26.17 ± 2.50	17.07 ± 1.84	144.96 ± 3.52	137.62 ± 2.02	124.62 ± 2.38	21.77 ± 1.08	22.13 ± 0.59	18.24 ± 0.49	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
AMES-10103	16.47 ± 0.15	15.53 ± 0.23	12.00 ± 0.06	39.20 ± 2.23	30.63 ± 0.30	15.00 ± 1.26	165.58 ± 8.37	162.27 ± 5.85	120.07 ± 2.47	27.88 ± 0.95	27.22 ± 0.51	21.25 ± 0.67	0.59 ± 0.01	0.58 ± 0.02	0.80 ± 0.01
BF-88	15.00 ± 0.06	14.50 ± 0.06	13.30 ± 0.15	32.67 ± 1.33	33.70 ± 1.28	25.40 ± 3.12	172.72 ± 4.80	167.89 ± 1.86	131.93 ± 1.73	26.59 ± 0.92	24.14 ± 0.58	24.88 ± 0.82	0.94 ± 0.02	0.82 ± 0.01	0.78 ± 0.01
HA-407	19.97 ± 0.66	16.43 ± 0.32	10.65 ± 0.28	43.89 ± 1.06	23.50 ± 1.20	13.40 ± 1.04	177.40 ± 3.92	165.26 ± 4.15	97.29 ± 2.05	26.55 ± 0.39	21.53 ± 1.15	15.27 ± 0.60	0.74 ± 0.02	0.85 ± 0.02	0.89 ± 0.01
Male															
CM-615	15.40 ± 0.42	15.60 ± 0.15	11.55 ± 0.53	19.03 ± 1.37	21.43 ± 0.97	16.70 ± 1.33	119.00 ± 1.31	109.58 ± 0.88	81.21 ± 0.56	26.67 ± 0.55	18.38 ± 1.07	13.77 ± 0.25	0.96 ± 0.00	0.67 ± 0.01	0.94 ± 0.00
CM-631	14.87 ± 0.18	13.93 ± 0.09	10.77 ± 0.27	34.87 ± 2.33	22.80 ± 0.25	12.70 ± 1.74	117.67 ± 2.92	89.97 ± 2.85	95.96 ± 0.86	23.66 ± 0.84	17.62 ± 0.57	14.69 ± 0.44	0.95 ± 0.02	1.00 ± 0.00	1.00 ± 0.00
R-SIN-82	14.10 ± 0.06	13.10 ± 0.12	11.80 ± 0.15	21.38 ± 1.43	24.63 ± 2.52	11.27 ± 0.46	143.89 ± 4.15	122.98 ± 2.06	108.67 ± 2.10	26.24 ± 1.15	21.21 ± 0.66	16.34 ± 0.57	0.62 ± 0.01	0.74 ± 0.00	0.95 ± 0.01
R-RSS-88	9.27 ± 0.24	9.80 ± 0.30	7.93 ± 0.31	18.23 ± 1.07	21.77 ± 1.33	11.43 ± 1.18	126.71 ± 2.65	116.27 ± 1.15	105.34 ± 1.53	23.06 ± 0.93	18.71 ± 0.32	16.67 ± 1.17	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

yield showing independence of achene yield of passive osmotic adjustment under salinity stress (Fig. 10). Furthermore, the relationship between active or passive osmotic adjustment and CMI showed a negative relationship between the two traits (Fig. 11). AOA showed a higher and significant relationship with CMI while CMI was only relative with POA under a saline regime of 12 dSm^{-1} (Fig. 11)

Discussion

Biomass partitioning in genotypes with differential salinity resistance

Variation in salinity resistance existed among the sunflower genotypes tested in this study. Previous findings also showed the presence of genetic variation in elite sunflower germplasm for yield components, as well as physiological and biochemical traits (Caterina et al. 2007; Hebbara et al. 2003; Rauf 2008).

Differential responses among the resistant inbred lines to salinity stress were depicted from the study of traits such as MRL, NDL, and HI. Resistant inbred line BF-88 reduced LRD by reducing MRL and LR branches. Contrastingly, CM-89 reduced LRD by increasing MRL while its LR branches were maintained. Furthermore, BF-88 showed a non-significant increase in the NDL under salinity while CM-89 showed a significant increase in the number of dead leaves under salinity stress. Increased NDL under salinity stress is an indication of exceeded amount of salt accumulation in leaves and is used as an ion exclusion mechanism at the above-ground level (Rauf et al. 2010).

A salinity "avoidance" mechanism was indicated in the breeding line BF-88 due to reduction in root characteristics which may have excluded the salts at the root level. This breeding line has accumulated fewer salts in the above-ground plant organs as indicated from the maintained number of nodes, leaf area and number of dead leaves. Munns (2006) also indicated that higher total number of leaves, continuous leaf expansion, and lower number of dead leaves as an indication of less salt toxicity or less accumulation in the leaves. Contrastingly, CM-89 showed a significant increase in main root length and thus exploring the lower soil profile for nutrition in the presence of salts. The genotype excluded salts at the above-ground level as indicated from the higher NDL under saline regime. Increased production of total NDL in the resistant genotype was an indication of recovery mechanism in which toxic salts are excluded by their translocation in older leaves (Rauf et al. 2010). The genotype also increased the total NN in the salinity showing compensation for the lost leaves. However, its LA was reduced under salinity. A "tolerance" mechanism was suggested in this genotype due to increased main root growth under salinity and exclusion of salts at the above-ground level and thus maintaining its yield under salinity. Both resistant inbred lines maintained the root-to-shoot ratio of non-saline conditions in the saline regime and showed lower root-to-shoot ratio as compared to susceptible cultivars. Thus showing that resistant cultivar prioritized above-ground portion as possible sink of photosynthates (Rauf and Sadaqat 2008a).

Contrastingly, susceptible breeding lines showed superior root characteristics and showed a significant increase in the root-to-shoot ratio under salinity stress. Thus it appears that susceptible lines had superior defense against the first impact of salinity, i.e. physiological drought. These salinity susceptible breeding lines may perform well under drought stress. Rauf et al. (2009b, 2010) showed that superior root characteristics of sunflower and maintaining a high root-to-shoot ratio was beneficial and drought tolerant breeding showed significant increase in root length due to translocation of photosynthates and mobilization of food reserves to the roots. However, they were unable to maintain number of nodes under salinity and showed a significant decrease in TNN. Thus, salt susceptible genotypes were characterized by few but large yellowish leaves. Furthermore, they lacked the salt exclusion mechanism at the above-ground level as shown by tolerant breeding line CM-89. A possible introgression of CM-89 X HA-407 or CM-89 X CM-207 may produce trangressive segregants with superior salinity and drought tolerance. Most of the traits were not able to show stabilize inheritance and showed significant change in their mean values in F_1 hybrids. This may be due to positive or negative overdominance associated with the traits (Rauf et al. 2009a). However, traits such as NDL or TNN showed a similar response and mean values were not changed significantly in inbred lines and hybrids. Thus traits may also be used as dominant markers for understanding salinity resistance mechanism. Selected resist-

ant inbred lines produced superior hybrids for yield under salinity stress. Therefore, phenotypic selection on the basis of morphological or biochemical traits is helpful in selecting superior inbred lines for salinity stress.

Genetic variances and selection of breeding material

Analyses of variances were carried out over and within salinity regimes. Genotypes showed significant variation over and within salinity regimes. Genotype X salinity regimes were also significant, thereby indicating relative ranking of sunflower populations across the salinity regimes indicating substantial variation for salinity resistance within sunflower germplasm. Previous studies in sunflower have also indicated substantial genetic variation in sunflower germplasm for salt resistance (Ashraf and Tufail 1995; Maiti et al. 2009). Heritability estimates were low for biochemical and medium for morphological traits across salinity regimes. This showed that genotypes showed more sensitivity for biochemical traits due to salinity regimes than morphological traits. Similarly, narrow-sense heritability estimates were also low for biochemical traits across salinity regimes. Broad-sense heritability estimates were high for biochemical traits within salinity regimes and narrow-sense heritability was low for these traits within salinity regimes. Rauf et al. (2009b) also indicated low genetic variability across environment and high heritability within environment. Thus chances for selection of salt tolerant inbred lines on the basis of biochemical traits in a segregating population were also restricted. On the other hand, biochemical traits carried substantial dominance variance showing suitability for the selection of hybrids with high active osmotic adjustment (Rauf 2008). Good general combiners, i.e. HA-407 and RF-SS-88 or BF-88 and CM-631 for this trait may be used for the development of hybrid. Ashraf and Harris (2004) also reviewed the possibility of exploiting the biochemical indicators for the selection of salt-resistant genotypes. They concluded that biochemical indicators were the potential criteria and may be used in future for the development of salt-resistant genotypes. However, they were unable to show the heritabilities and type of genetic variance associated with these traits. Among various components of genetic variance, the additive part is being exploited by the breeder to select transgressive segregants while non-additive variance was not fixable and may be used for the manipulation of heterosis (Rauf 2008).

Development of hybrids with a higher ability of active osmotic adjustment would bring significant improvement in achene yield under salinity stress as the two traits were significant and positively related to each other. Higher active osmotic adjustment was negatively and significantly related with cell membrane injury. Thus, hybrids develop on active osmotic adjustment would show significant resistance against salinity stress.

Active osmotic adjustment was based on those osmolytes which may also be used as food reserve and perform various function in cell. In our study, it was estimated on the basis of contribution of amino acids, proline, and total soluble sugars to the total osmotic adjustment. The contribution of these osmolytes increased with the concentration of salinity stress. Zheng et al. (2010) also noted that osmotic adjustment ability of sunflower seedling increased after exposure to salinity or drought stress. It was further noted that osmolytes such as K^+, chlorine (Cl^-), amino acid, organic acid, sodium (Na^+), and proline content increased under salinity stress. However, they were not able to differentiate active or passive osmolytes. Hybrids between the good general combining parents HA-407 X RF-SS-88 or BF-88 X CM-631 showed the highest ability for active osmotic adjustment and was also the highest achene yielder in salinity regime of 12 or 7.2 dSm^{-1}. Active osmotic adjustment acted as buffer against the yield reduction in salinity stress. Benefits of osmotic adjustment for yield maintenance have also been observed by some other studies in sunflower under drought stresses (Chimenti et al. 2002; Rauf and Sadaqat 2008a,b).

Agronomic traits showed significant heritabilities over the salinity regime. Narrow sense heritability was also significant over the salinity regimes. TNN $plants^{-1}$ showed the highest magnitude for heritability in narrow sense. This trait may be exploited to carry out selection of salt-resistant inbred lines over or within salinity regimes. A stabilized or genotype with a high mean value under salinity regimes may be considered a criterion for selection of salt resistant inbred lines. Various author(s) have considered possibility of exploiting biochemical or morphological traits as possible selection criteria for salinity resistance breeding (Ashraf and Harris 2004; Flowers and Flowers 2005; Isla et al. 1998). These author(s) concluded that biochemical or physiological traits may be exploited as sole selection criterion for the development of salt-resistance genotypes. Biochemical trait poses some useful features for the plant breeder such as freedom of the stage to measure these traits. They may be measured at any stage of plant development subjected they show positive correlation with yield (Rauf 2008). In addition, they show greater observable variation than morphological. However, these author(s) were not able to differentiate between the environmental variation or genotypic variation. In our studies, a large proportion of this variation was due to environment or genotype X environment showing that biochemical indicators were not stabilized over the range of salinity and may be useful for selection within salinity levels. Moreover, they carried non-additive proportion of genetic variation. On the other hand, morphological traits showed significant heritability over and within salinity level.

Among various saline levels, a salinity level of 12 dSm^{-1} was found the most useful for screening and selection of genotypes with differential salinity tolerance. It showed significant repressing effect over the mean performance of genotypes and increased the magnitude of additive variance which is considered to be useful for fixing the genotype mean performance.

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