

## GENERATION MEAN ANALYSIS OF WATER STRESS TOLERANCE IN OKRA (*ABELMOSCHOUS ESCULENTUS L.*)

ABDUL NAVEED<sup>1</sup>, ASIF ALI KHAN<sup>2\*</sup> AND IFTIKHAR AHMAD KHAN<sup>2</sup>

<sup>1</sup>Directorate of Research, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan.

### Abstract

Field experiments were carried out to assess the genetic potential of okra genotypes for drought tolerance through breeding and selection in 6 generations of 4 crosses between pairs of genotypes with a degree of tolerance to drought. Narrow sense heritability and genetic advance varied across crosses, traits and stress conditions. For fruit yield, narrow sense heritability and genetic advance were high under non-stress condition as compared to drought, which indicated that direct selection of fruit yield would only be feasible under non-stress conditions. Among the agronomic traits, although number of pods per plant had shown good narrow sense heritability and genetic advance under drought, yet leaf water potential appeared to be better indicator for selection criteria owing to higher heritability under drought. Among the crosses, Sanam × Arka Anamika appeared elite in terms of narrow sense heritability and genetic gain compared with other crosses, with highest fruit yield and pod number per plant under both conditions. Thus, chances to find stress tolerant breeding material in segregating populations of this cross are promising.

### Introduction

Okra (*Abelmoschus esculentus* L.) belonging to the family Malvaceae is an important vegetable crop, particularly in Pakistan and India. Like other field crops, okra is also faced with the problem of short supply of irrigation water. Yield is an ultimate objective of any breeding program. However, substantial increase in yield under drought conditions has not been achieved yet in spite of concerted efforts by the plant breeders (Blum, 2005), which is due to the non availability of good selection criteria (Richard, 1996). Several traits being offered as selection criterion eventually failed to bring about the desirable change. Naveed *et al.*, (2008) advocated that the offered traits were either related to the plant survival only, costly or too complex to be measured in large plant population. Furthermore, destructive nature of measuring plant traits at the seedling makes it unsuitable to be used in segregating generations where every plant is characterized by a distinct genotype (Rauf & Sadaqat, 2008; Rauf *et al.*, 2008a) and the lack of success attributable to low heritability and genetic advance under target drought environment (Rauf, 2008).

Leaf hydraulics has been extensively used to study the plant responses to the drought stress (Lu *et al.*, 1998; Bhatt & Rao, 2005). However, very few studies have been carried out to determine their potential under drought stress as a selection criterion. Some studies have shown their positive relationship with yield (Rauf & Sadaqat, 2008; Rauf *et al.*, 2008a). Rauf *et al.*, (2008) reported additive type of gene action associated with leaf hydraulics such as osmotic adjustment and turgor pressure, showing possibility of selection in segregating generation of sunflower under drought stress. Similarly in Pima cotton, increase in stomatal conductance has also showed rapid increase of yield under optimum and heat stress (Lu *et al.*, 1998).

\*E-mail: asifpb@gmail.com

The studies reported here were carried out to determine the potential of morphological and physiological traits for drought tolerance in terms of heritability, genetic advance and type of gene action prevailing in okra using six generation model in four okra crosses.

## Material and Methods

**Development of plant material:** From a preliminary screening experiment four drought tolerant (Sanam, Sabazpari, Ikra 1 and P-1999-31) and four susceptible (Arka Anamika, Chinese Red, Indian Spinell and Superstar) genotypes were selected and used for the development of plant generations to obtain four sets of each generation i.e.,  $F_1$ ,  $F_2$ ,  $BC_1$ ,  $BC_2$ . The plant generations were developed in two phases. In the first season four sets of  $F_1$  generation were developed. In the second season the  $F_1$ s and their parents were used to produce fresh  $F_1$ , and  $F_2$ ,  $BC_1$  and  $BC_2$  generations.

**Development of  $F_0$  seed:** The parental material was sown in the field under optimum conditions. Normal production package and crop husbandry techniques were followed to raise the crop. Using the eight parents, four  $F_1$  cross combinations between a tolerant and susceptible parents were attempted. In the two crosses tolerant parent was used as female while in other two crosses tolerant parent was a male. Crosses were attempted using a hand emasculation and pollination. Floral buds were selected in the evening having candle shape. Candle shape is a peculiar floral phenology that blooms next morning. For emasculation, petal whorl was removed with the help of forceps in order to expose the immature anthers. Immature anthers were also removed with the forceps. Care was taken that all the anthers have been removed and stigma was not injured during the operation. To avoid stray pollen contamination stigma was covered with a soda straw tube. Flowers were tagged with the date of emasculation and pollinated next morning with the selected flower from male parent.

**Development of  $F_1$ ,  $F_2$ ,  $BC_1$ ,  $BC_2$  generation:** In the next cropping season,  $F_0$  seed and all the eight parents were planted in a field. The  $F_0$  seed was used to grow  $F_1$  generation. At maturity  $F_1$  plants were selfed by winding thread around the candle shaped floral buds. This selfed seed was the source of  $F_2$  population. The floral buds on the  $F_1$  plants were also crossed with the first parent (female) of a particular cross to produce  $BC_1$ ; they were also crossed with second parent (male) to produce seed of  $BC_2$ . In this season, parents were again crossed to produce fresh  $F_0$  seed.

**Genetic analysis of morphological and physiological traits:** The experiment was planted in the experimental area of the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad during the year 2005. The experimental material was planted in sandy loam soil with 12% field capacity, 0.96% organic matter, 7.1 pH, 123ppm potassium, 16.8ppm phosphorous and 2.3 EC.

The seeds of all the parents (eight),  $F_1$ 's,  $F_2$ 's,  $BC_1$ 's, and  $BC_2$ 's were planted in split plot design with two factors i.e. generations and water level. Generations consisted of 24 types (8 parents, 4  $F_1$ 's, 4  $F_2$ 's, 4  $BC_1$ 's, and 4  $BC_2$ 's). Two contrasting water levels i.e., normal ( $W_1$ ) and water stressed ( $W_2$ ) were applied to the main plots while generations were allocated to the sub plots. Each sub plots was 6 m  $\times$  6 m. Water levels were devised by irrigating the plots with supplemental water ( $W_1$ ) when ever required while in other plots it was completely held ( $W_2$ ) during the early bud stage to develop water stress

during anthesis. The plots having optimum soil moisture were therefore called as non-stress plots ( $W_2$ ). Water contents of these plots were not allowed to fall below the field capacity. The soil moisture contents were estimated on regular interval i.e., every 10 days. Crop management was uniform following recommended production package.

**Data measurements:** At anthesis plants were analyzed for leaf water potential traits while at maturity the plants were analyzed for morphological and yield traits. Number of plants selected to record the data varied with the generation. The 8 parental and 4  $F_1$  generations were represented by 15 plants within each replication while each segregating generation,  $F_2$ 's,  $BC_1$ 's,  $BC_2$ 's were represented by 100 plants.

At maturity the pods on single plant basis were counted to determine the number of pods per plant. Fruit yield per plant was measured in grams on digital balance. Pressure bomb apparatus was used to determine the leaf water potential of the plants. The apparatus was shifted to field for the measurements. Leaf was excised along with the petiole and inserted in the pressure chamber. The gas was turned on and tip of petiole was carefully observed with the help of lens to observe for a drop of moisture. The gas was immediately turned off after the observation of moisture drop on leaf petiole. The reading was taken on the screen and converted into Mega Pascal (MPa).

**Statistical/biometrical analysis:** Data were analyzed using split plot analysis of variance. There were two factors i.e., generations and water levels. Generations comprised of 24 levels while there were 2 water regimes ( $W_1$  and  $W_2$ ). Variations among the generations were further broken down into parents,  $F_1$ 's,  $F_2$ 's,  $BC_1$ 's and  $BC_2$ 's. Analysis of variance depicted significant variation among generations and generation  $\times$  water level. Therefore, data were subjected to the generation mean analysis to determine the type of genetic variation associated with the traits under study within each water regime. Generation mean analysis was carried out following Mather & Jinks (1982). Joint scaling test and generation mean analyses were computed through computer software developed by Dr. Pooni, University of Birmingham, UK that uses weighted least square method. A weighted least square analysis was performed on the model using parameter 'm' only. Further model of increasing complexity were fitted, where chi square value was significant. The best-fitted model was chosen as the one, which had significant estimates of all parameter along with non-significant chi-square. For each trait the higher value parents was always taken as  $P_1$  model fitting. Additive ( $\sigma^2A$ ) and dominance ( $\sigma^2D$ ) variances and narrow sense heritability ( $h^2$ ) were calculated according to Warner (1952). Environmental variance was calculated as,  $\sigma^2E = (\sigma^2P_1 + \sigma^2P_2 + 2\sigma^2F_1)/4$  (Wright, 1968). Broad sense heritability was estimated as  $H = (\sigma^2F_2 - \sigma^2E)/\sigma^2F_2$ .

## Results

**Fruit yield per plant:** Variation within different generations of four crosses for fruit yield was partitioned into different components i.e. environment, additive and dominance component (Table 1). These components were used to determine broad sense and narrow sense heritability and genetic advance (Table 1). Among the variance components, dominance was the largest in all crosses followed by environment. Additive component decreased under drought stress compared to non-stress condition. Negative estimates of additive components were obtained under drought in all crosses except the cross Super Star  $\times$  P-1999-31. The cross Sanam  $\times$  Arka Anamika showed the highest estimates of additive component under non stress condition while Super Star  $\times$  P-1999-31 showed the

highest estimates under drought stress. This cross also showed the highest dominance component under non-stress condition. Chinese Red  $\times$  Ikra 1 showed the highest dominance component under stress. Broad sense heritability was high and ranged from 0.62-0.98 in both conditions. Broad sense heritability estimates increased for the crosses Sanam  $\times$  Arka Anamika and Chinese Red  $\times$  Ikra 1, while decreased for cross Sabazpari  $\times$  Indian Spineless and remained unchanged in the cross Super Star  $\times$  P-1999-31 under drought stress condition. Narrow sense heritability estimates were low in all the crosses. Due to negative estimates of additive component under drought stress, narrow sense heritability estimates were assumed zero in the crosses Sanam  $\times$  Arka Anamika, Chinese Red  $\times$  Ikra 1 and Sabazpari  $\times$  Indian Spineless. The cross Super Star  $\times$  P-1999-31 showed positive but low estimates of narrow sense heritability under both conditions.

The parents of all crosses showed significant differences in mean performance under both conditions (Table 2). The parents Arka Anamika showed highest yield under non stress condition while Sanam showed highest fruit yield in drought stress condition. Mean performance of  $F_1$  exceed to both parents in all crosses, thereby indicating the presence of heterosis. However,  $F_1$  mean of the crosses Sanam  $\times$  Arka, Anamika and Superstar  $\times$  P-1999-31 showed lower yields than their better parent under non-stress condition. Furthermore, mean performance of  $F_1$  was significant only in non-stress condition for these crosses (Sanam  $\times$  Arka Anamika; Sabazpari  $\times$  Indian Spineless) while it was significant under drought stress condition for the other crosses (Chinese Red  $\times$  Ikra 1; Superstar  $\times$  P-1999-31).  $F_2$  mean reduced in comparison to  $F_1$  signifying the presence of inbreeding depression. However, differences between  $F_1$  and  $F_2$  were non significant in the crosses Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31. In Sanam  $\times$  Arka Anamika cross,  $BC_1$  mean performance was lower than  $BC_2$  under non-stress condition. Conversely,  $BC_1$  mean performance was higher than  $BC_2$  under stress condition. In cross of Sabazpari and Indian Spineless,  $BC_1$  mean performance was higher than  $BC_2$  in both conditions but differences were non-significant in non-stress condition. In Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31 crosses,  $BC_1$  mean performance was higher than  $BC_2$  under non-stress condition. However, under drought stress condition  $BC_2$  mean of Chinese Red  $\times$  Ikra 1 was higher than  $BC_1$  while differences were non significant in Superstar  $\times$  P-1999-31.

Joint scaling was also carried out to further split the genetic variance into additive, dominance and epistatic components (Table 3). The magnitude of additive and dominance estimated through joint scaling test may vary with the variance estimated in Table 3. Narrow sense heritability was estimated by including both additive and additive  $\times$  additive interaction. Scaling test showed the significance of additive, dominance and additive  $\times$  additive interaction in the cross Sanam  $\times$  Arka Anamika under non-stress condition. None of the model was found fit in the crosses Sanam  $\times$  Arka Anamika and Superstar  $\times$  P-1999-31 under stress condition. Similarly none of the model was found fit in the cross Chinese Red  $\times$  Ikra 1 under non-stress condition.

Genetic effects showed preponderance of dominance in all the crosses under the two conditions (Table 3). In the crosses Sabazpari  $\times$  Indian Spineless and Superstar  $\times$  P-1999-31, additive, dominance, additive  $\times$  additive and additive  $\times$  dominance components were significant under normal condition. Additive  $\times$  additive epistasis was absent in Sabazpari  $\times$  Indian Spineless. Dominance tended to increase in the cross Sabazpari  $\times$  Indian Spineless in drought stress when compared with normal condition. In Chinese Red  $\times$  Ikra 1 additive, dominance effects, and additive  $\times$  dominance and dominance  $\times$  dominance interactions were effective (Table 3).



**Pod number per plant:** For pod number per plant additive variance was the largest component of variability within populations under drought stress while preponderance of dominance type of genetic variability was observed under non-stress condition (Table 4). Both dominance and environmental variance tended to decrease under non-stress condition except in the cross Superstar  $\times$  P-1999-31, which showed increased estimates of dominance variance under drought stress. Additive variance increased in stress condition for all crosses and as a result the heritability in broad sense showed an increase in drought stress except in the cross Chinese Red  $\times$  Ikra 1. Narrow sense heritability estimates were assumed zero due to negative direction of additive variance in all the crosses except the cross Superstar  $\times$  P-1999-31 under non-stress condition. Positive additive variance in this cross (Superstar  $\times$  P-1999-31) allowed estimation of narrow sense heritability in both conditions. Narrow sense heritability ranged between 0.20-0.72. Highest narrow sense heritability and genetic gain were observed in the cross Superstar  $\times$  P-1999-31. Genetic gain in this cross was larger in drought stress than that of non-stress condition.

The parents showed significant differences for pod per plant under drought stress conditions (Table 5). Tolerant parents showed significantly higher mean number of pods per plant than the susceptible ones. Under non-stress condition differences between parents were less obvious i.e., parents used in the cross Sanam  $\times$  Arka Anamika showed non-significant differences ( $p \geq 0.05$ ). Similarly parents of the cross Superstar  $\times$  P-1999-31 showed non-significant differences.  $F_1$  generation showed higher number of pods per plant when compared to both parents of each cross under both conditions. However,  $F_1$  of the cross Sanam  $\times$  Arka Anamika was similar to both parents while  $F_1$  of the crosses, Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31, was similar to  $P_1$  under non-stress condition.  $F_2$  means were significantly lower than  $F_1$  in all crosses and conditions. However,  $F_2$  mean of the crosses, Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31 showed non-significant differences in drought stress.  $BC_1$  means of the crosses Sanam  $\times$  Arka Anamika and Sabazpari  $\times$  Indian Spineless were similar to  $BC_2$  generation under non-stress condition. In stress condition it differed with  $BC_2$  for both crosses.  $BC_1$  means were higher than  $BC_2$  for both crosses under drought stress. In the crosses, Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31,  $BC_1$  means were higher than  $BC_2$ . However, under drought stress,  $BC_2$  means were higher than  $BC_1$ .  $BC_1$  of Superstar  $\times$  P-1999-31 showed only differences with  $BC_2$  under non stress while under stress it was similar to  $BC_2$ ,  $F_1$  and  $F_2$ .

Significant genetic component as observed from joint scaling test are given in Table 6. In the cross Sanam  $\times$  Arka Anamika, additive, dominance and additive  $\times$  additive component were significant under non-stress while additive, additive  $\times$  dominance and dominance  $\times$  dominance were significant under stress. Among the components, dominance showed the highest contribution under non-stress. Availability of water changed the direction of components. Additive component were positive under non-stress, which turned negative under stress condition in the cross Sanam  $\times$  Arka Anamika. In the cross Sabazpari  $\times$  Indian Spineless all effects were significant except dominance  $\times$  dominance under non-stress, highest being dominance while additive  $\times$  additive and dominance  $\times$  dominance interactions were absent under drought stress. Additive  $\times$  additive epistasis was the highest under non-stress condition.

None of joint scaling model was fit under drought condition for the crosses Chinese Red  $\times$  Ikra 1 and Superstar  $\times$  P-1999-31. However under non-stress condition, the other two crosses showed significance of all genetic components. However, additive  $\times$  additive component was non significant in Super Star  $\times$  P-1999-31. Dominance was highest in Chinese Red  $\times$  Ikra 1 while dominance  $\times$  dominance interaction was highest in Superstar  $\times$  P-1999-31.





**Leaf water potential:** Among the variances for leaf water potential, additive variance contributed highest to the variance (Table 7). All type of variance estimates increased under drought stress. Broad sense heritability increased under drought as compared to non-stress condition for the crosses Sanam × Arka Anamika and Sabazpari × Indian Spineless while Chinese Red × Ikra 1 and Superstar × P-1999-31 crosses showed decrease in broad sense heritability under drought stress. Narrow sense heritability increased under drought stress. The magnitude of narrow sense heritability was moderate to high in three crosses. The genetic gain was highest under drought stress. Cross Sanam × Arka Anamika showed superior performance in term of narrow sense heritability and genetic gain (Table 7).

Mean performance of generations advanced from four crosses with in water levels are given in Table 8. Parental generation showed significant differences under drought stress in all crosses. In non-stress condition leaf water potential effects were also significant in parental generations of all crosses except the cross Superstar × P-1999-31. In the cross Sanam × Arka Anamika,  $F_1$  generation was superior to both parents while in the cross Sabazpari × Indian Spineless,  $F_1$  generation was higher to parent 1 under non-stress conditions. In the cross Chinese Red × Ikra 1,  $F_1$  was higher than both parents under drought stress.  $F_2$  generation was superior to all generations in all the crosses except in the cross Sabazpari × Indian Spineless in which  $F_2$  generation was similar to  $F_1$  under drought stress.

Joint scaling test showed the preponderance of additive × dominance effects under non-stress condition of the cross Sanam × Arka Anamika and Sabazpari × Indian Spineless (Table 9). The other two crosses, Chinese Red × Ikra 1 and Superstar × P-1999-31, showed highest dominance effects under both condition. In the cross Sabazpari × Indian Spineless additive × additive interaction was highest under drought stress.

## Discussion

The development of okra cultivars having potential to produce optimum pod yields under water stressed conditions is highly desirable in Pakistan where irrigation water is becoming very limited (Wullschleger & Oosterhuis, 1991; Ashraf *et al.*, 2002). For the accomplishment of such a task information on the mode of inheritance of the contributing characters is the first prerequisite (Khan & McNeilly, 1998; Khan *et al.*, 2003; Azhar *et al.*, 2005; Khan & McNeilly, 2005; Azhar *et al.*, 2007; Hussain *et al.*, 2008). From six generations of four crosses, between drought tolerant and susceptible genotypes of okra, environmental, additive and dominance variances were estimated to calculate heritability and genetic advance. In most of the cases narrow sense heritability and genetic advance was zero due to opposite direction of additive and dominance variance. A necessary condition for higher magnitude of narrow sense heritability and genetic advance appeared to be dependent on the direction of additive and dominance effects (Apraku *et al.*, 2004). Estimated narrow sense heritability and genetic advance varied for different crosses, traits and the conditions. None of the trait has shown good estimates of narrow sense heritability and genetic gain under both conditions. For fruit yield, narrow sense heritability and genetic advance were high under non-stress condition as compared to drought where most of the crosses showed zero narrow sense heritability and genetic advance. An increase in error variance under stress conditions has been reported to cause decrease in the heritability estimates (Hulmel *et al.*, 2005). Therefore direct selection of fruit yield would only be feasible under non-stress condition while selection for direct fruit yield under drought stress would yield zero genetic gain in most cases. Among the agronomic traits, although number of pods per plant had shown good narrow sense

heritability and genetic advance under drought but leaf water potential proved better parameter for selection owing to higher heritability under drought. Furthermore, leaf water potential is an important parameter for the assessment of stress tolerance (Khanzada, 2001; Ben-Ahmad *et al.*, 2006) allows early screening of plant genotypes. Rauf & Sadaqat (2008) reported significant positive relationship of physiological traits with yield. Therefore, these traits may be used for selection of drought tolerant genotype and indirect criteria for improving pod yield. Farshadfar *et al.*, (2001) also showed high narrow-sense heritability estimates for excised leaf water losses, relative water content and biomass and concluded that high genetic advance for relative water content and excised leaf water loss may be used for direct selection.

Joint scaling test was carried out to determine the type of significant genetic components. The composition of genetic effects was not similar as indicated by the genetic variances. Since additive variance includes both additive effects and additive  $\times$  additive effects while the joint scaling test separated them. Further more direction of main effects such as additive or dominance and epistatic interaction were also important in determining strength of a particular variance. A negative dominance effects and positive dominance  $\times$  dominance effects resulted in lower overall dominance variance. Joint scaling test indicated the substantial role of epistatic components in most of traits and conditions. Additive components were positive in most cases showing direction towards tolerant parent while dominant effects were negative. Najafabadi *et al.*, (2004) showed that generation mean analysis did not fit an additive-dominance model for any trait with additive  $\times$  additive and dominance  $\times$  dominance epistatic effects predominating in most of physiological traits. Among the crosses, Sanam  $\times$  Arka Anamika was most promising in terms of narrow sense heritability and genetic gain, this cross also showed highest means in both condition. Therefore, the superior parents may be selected on the basis mean performance for recombination.

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