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Assessment of germplasm and development of breeding populations of Okra (*Abelmoschus esculentus*) for drought tolerance

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Summary

Drought susceptibility index was used to screen okra germplasm for drought tolerance. On the basis of fresh pod yield and drought susceptibility index, genotypes were divided into four groups. There was a relative shift in the performance of the genotype across the water levels. Arka Anamika showed the highest fresh pod yield under non-stress condition with high drought susceptibility index while Sanam showed lowest drought susceptibility index with highest yield potential under drought stress condition. Physiological and morphological analyses were carried out to study the mechanisms of drought tolerance. The genotypes showed significant interaction with sampling intervals. However for osmotic adjustment, turgor pressure, relative water contents, harvest index and root/shoot ratio, genotypes' performance were relatively stable over sampling intervals. Among the traits, relative water contents allowed the genotypes to separate into drought tolerant and sensitive groups. Therefore selection for high RWC at 30 days after stress may be useful tool for the selection of drought tolerant genotypes. Furthermore, this trait has also shown very high narrow sense heritability, showing its potential for the selection of drought tolerant breeding material. Segregating generations of the cross Arka Anamika × Sanam was used to establish high yielding progenies.

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable of subtropics, which are characterized by low rainfall, high temperature and strong winds in summer, resulting in very high rate of water loss from plant and soil surface causing drought stress and affect plant productivity. On the other hand, supplemental canal and tube well irrigation water is becoming limited, suggesting the need of breeding plant material for optimum production with low water requirements. Success of a plant breeding program for drought tolerance depends on the usable genetic variability that may exist in the cultivated germplasm. Previous results showed that cultivated germplasm of sunflower had significant genetic variability for drought tolerance (FERERES et al., 1986; RAUF and SADAQAT, 2007). The first step in the drought tolerance-breeding program is the identification of drought tolerance in cultivated germplasm. The wild germplasm is the second option and is used only when existing genetic variability is low since introgression has also been found to associate with linkage drag phenomenon (RAUF, 2008).

Higher plant economical yield is the ultimate objective of any breeding program. However, yield under irrigated and drought conditions have been differentially maximized by yield contributing traits (RAUF, 2008). Therefore, improvement of yield in one environment does not necessary correlate with yield in other environment. Furthermore, direct selection for yield is handicapped by low heritability and genetic advance (BLUM et al., 1979). As a logic consequence, plant breeders shifted their efforts in the selection of traits related to drought tolerance. These traits, i.e. lower leaf transpiration and leaf area, were negatively correlated with yield (RAUF and SADAQAT, 2008). Therefore, fewer breeders have emphasized the need of a screening criterion that shows positive correlation with yield (RAUF et al., 2007b; RAUF and SADAQAT, 2008; RAUF et al., 2008; NAVEED et al., 2009). However, many of the suggested traits were expensive, destructive or laborious for the screening of large plant populations. In addition target trait should also provide selectable genetic variability (additive) in segregating generation.

Okra is one of the important vegetables of Pakistan and its tender pods are consumed fresh as vegetable (ATHAR and BOKHARI, 2006). Present study was undertaken to screen okra germplasm on the basis of drought susceptibility index. Selected okra lines were used to study the physiological and morphological traits to drought tolerance and susceptibility.

Materials and methods

Screening of okra germplasm under water deficit condition

Experimental lay out

Twenty-six genotypes obtained from Vegetable Research Institute, Faisalabad, were sown in two water levels, each having three replications. The soil was sandy loam with low moisture holding capacity. Split plot design was used allocating water levels to main plots, and genotypes were assigned to sub plots. Each sub plot was 4.8 m × 5 m. Optimum level of moisture content was maintained in the non-stressed plots by irrigating the plots at regular intervals. Differential moisture levels were developed by withholding supplemental irrigation in other plots to develop moisture stress conditions during flowering stage. Insect populations were controlled with recommended pesticides before the damage reached beyond economic threshold level while weeds were controlled manually.

Data measurements

Data were recorded for fresh green pods yield as they are consumed as vegetable (ATHAR and BOKHARI, 2006). Two rows within each sub plot and 10 competitive plants per row were used for the measurement. Green pods from each plant were picked when they were tender and 25 pickings were carried out from each plant. At the end all the 25 pickings were added to determine fresh green pod yield per plant.

Morpho-physiological traits of selected okra genotypes under drought stress

Experimental lay out

Eight genotypes belonging to two drought susceptibility index groups were selected to study their physiological and morphological traits. Experimental lay out was Randomized Complete Block design (RCBD) with three replications. The soil was sandy loam with low field capacity i.e. 14% by weight, organic matter 0.96%, phosphorus 32 ppm and potassium 141 ppm. Drought stress was developed by withholding the irrigation water after 30 days of sowing. All the plots were fertilized with equal quantity of nitrogen and phosphorus. Leaf diseases were considered absent while insect populations were controlled before they caused damage beyond economic threshold level and weeds were eradicated manually.

Physiological traits

After withholding irrigation to drought stressed plots, plants were analyzed for different physiological traits. Eight competitive plants within single row of each subplot were used for the measurements. Fresh fully expanded leaf at second node from the top of canopy was taken for the measurement from each plant. Measurements were repeated at every 15 days with the aim to study the effect of gradual increase in the intensity of drought stress on physiological parameter.

Leaf discs of 4 cm^2 were excised from the leaf to determine their fresh weight on analytical balance. Pressure bomb apparatus was used to determine the leaf water potential of the plants while osmotic potential was determined by Cam micro-osmometer. Turgor pressure was calculated on the basis of difference between leaf water potential and osmotic potential. Osmotic adjustment (OA) was determined on the basis of difference between osmotic potential (OP) under non-stress (W_1) condition and osmotic potential under drought stress (W_2) condition at full turgor. In order to determine the osmotic adjustment, leaves were detached from each genotype along with leaf petiole. Only leaf petioles were dipped in the deionized water for eight hour. However, direct contact of leaf blade with water was avoided.
$OA = 100\ OP\ (W_1) - 100\ OP\ (W_2)$

100 OP mean osmotic potential at full turgor.

Stomatal conductance was measured with a delta T-Cam Lab porometer and readings were recorded from the intact leaf blades.

Morphological traits

Plants analyzed for physiological traits were harvested to determine the dry matter partitioning in okra genotypes. Plants were harvested along with their roots every 15 days to determine performance of selected genotypes over sampling interval for dry matter partitioning pattern.

Root length (cm) and root to shoot ratio
The tap roots were traced up to the bottom and the roots were removed, from the soil, packed with mass of soil. These roots were washed under constant pressure to remove the mud. The longest root was measured with a measuring tape in cm. Root to shoot ratio was determined by dividing the height of main plant stem to the root length recorded as the longest root length.

Dry matter partitioning
All the plant organs i.e. root, aerial biomass (leaves + main stem) and pods were put into kraft paper bags and dried in an oven at 70°C until constant weight was achieved. Aerial biomass was cut into small pieces to facilitate the packing and drying. All the dried material was measured on an electric balance. Dry root weight was considered as root biomass, while biomass comprising of dried leaves and main stem gave aerial vegetative biomass. Dried pods formulated the pod yield. Total biomass consisted of the total weight of roots + aerial vegetative biomass + pods.

Genetics of relative water contents
The selected eight genotypes were used for the development of four sets of plant generations i.e. F_1, F_2, BC_1, BC_2. The method for the development of generations is described earlier (NAVEED et al., 2009). The experiment was planted in the vegetable area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad during the year 2005. The seed of all the parents (eight) and their F_1's, F_2's, BC_1's, and BC_2's was planted in a field under split plot design. Two contrasting water levels were applied to the main plots while generations, 24 types (8 parents, 4 F_1's, 4 F_2's, 4 BC_1's, and 4 BC_2's) were allocated to the sub plots. Each sub plots was 4.8 m × 5 m. Contrasting water levels were devised by irrigating the plots with supplemental water while in other plots supplemental irrigational water was completely withheld during the early bud stage to impose water stress during anthesis. Thus the plots having optimum soil moisture were called as non-stress plots. Water contents of these plots were not allowed to fall below the field capacity. The soil moisture contents were estimated at regular interval of 10 days. Crop husbandry practices were similar in all plots and followed according to the recommended production package in the province of Punjab. Relative water contents were determined from leaf discs as described in an earlier section of material and method.

Statistical analysis
Analysis of variance was carried out with two factors genotypes and sampling intervals under split plot arrangement using computer based statistical programme Minitab for windows. Drought susceptibility index (S) was calculated following FISCHER and MAURER, (1978) formula, which is based on the difference of yield under non stress and stress conditions.

$$S = \frac{1 - Y/Y_p}{1 - X_d / X_p}$$

Where Y is the green pod yield per plant of a given genotype under drought, Y_p is the green yield per plant of the same genotype under irrigation, X_d is the mean green yield of all genotypes within group under drought, and X_p is the green pod yield per plant of all genotypes within group under irrigation. A genotype with higher susceptibility index was considered as susceptible while genotype with low index was considered tolerant. Genetics analysis of the six generation was also carried out according to the methods given by MATHER and JINKS (1982) and as described by NAVEED et al. (2009).

Results

Evaluation of okra germplasm using drought susceptibility index

There were significant differences ($P \leq 0.01$) among genotypes and the performance of the genotypes was affected by the supply of water. Significant interaction between the two factors showed that performance of genotypes was inconsistent across the water levels.
The fresh pod yield of non-stress and drought conditions was plotted against the drought susceptibility index (Fig. 1). Relationship between pod yield and drought susceptibility index was only significant under drought stress condition. The genotypes showed a reduction of 45% for fresh pod yield under drought stress.
The data plotted were subdivided into 4 quadrants, allowing separation of genotypes on the basis of fresh pod yield and drought susceptibility index (Fig. 1). Dotted lines shown on the X and Y axis of both graphs are averages of drought susceptibility index and fresh pod yield expressed by all genotypes (Fig. 1). The genotypes Sanam, Sabazpari, P-1999-31, Ikra 1 having lower susceptibility index and high yield fell in quadrant I. Sanam falling in this quadrant showed the highest fresh pod yield under drought stress. Quadrant II was characterized by the genotypes with high yield and high drought susceptibility index. Arka Anamika, Chinese Red, IN-97, Indian Spineless and Superstar fell in this quadrant. The genotype, Arka Anamika was the highest fresh pod yielder under non-stress condition. The genotypes MS-04, MS-02, Green Wonder and OS-31 showing low yield and low drought susceptibility index were included in quadrant III. MS-04 was the lowest yielder under non-stress condition. Quadrant IV was the most populated including genotypes with low yield and high drought susceptibility index. The genotype Diksha belonging to this quadrant showed lowest yield under drought stress and highest value for drought susceptibility index.

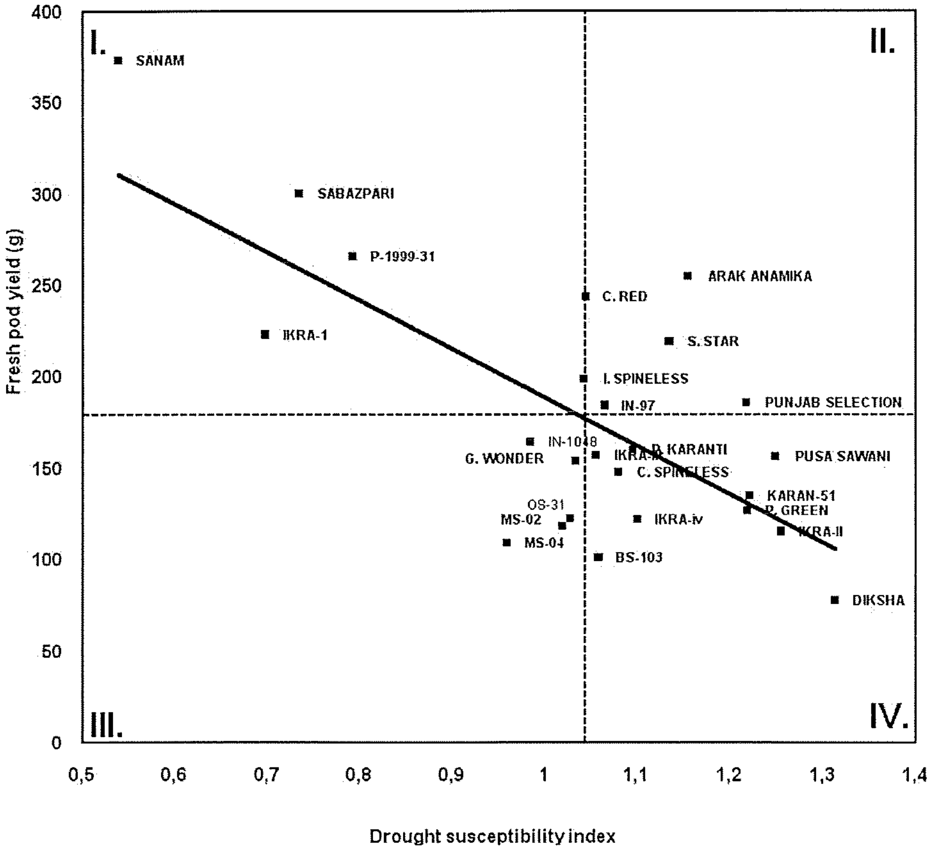

Fig. 1: The response of drought susceptibility index (DSI) to the variation in fresh pod yield (FPY) under drought stress condition.

Morpho-physiological traits of selected okra genotypes under drought stress

Analysis of variance showed significant variability due to genotypes and sampling intervals for all traits (Tab. 1 and 2; $P \leq 0.01$). Similarly, interaction component was also significant ($P \leq 0.01$) for all dry matter partitioning and water relation traits (Tab. 1, 2). These results showed that the genotypes had changed their relative performance over sampling intervals. However, interaction was not significant ($P > 0.05$) for traits related to dry matter partitioning such as harvest index (HI) and root to shoot ratio (R/S) (Tab. 1). Similarly among water relations, osmotic adjustment, turgor pressure and relative water contents showed non-significant interaction of genotypes with sampling interval ($P \geq 0.05$). This is a good note since the absence of interaction with sampling interval would allow the traits to be evaluated at any stage during drought stress.

The leaf water potential decreased with the sampling interval. Genotypes showed maximum genetic variability at 90 days after stress (Fig. 2). At this point, Sabazpari showed the highest leaf water potential, while Chinese Red showed lowest leaf water potential. The genotypes intersected at various points (Fig. 2). This may be due to inconsistent performance of genotypes over sampling interval (Tab. 1). Generally drought tolerant genotypes showed higher values for leaf water potential (Fig. 2). At 90 days after stress, genotypes split up into two drought tolerant and drought sensitive groups. However within groups, genotypes showed almost non-significant differences for leaf water potential.

Tab. 1: Analysis of variance for traits related to dry matter partitioning i.e. aerial vegetative biomass (AVB), fruit yield (FY), root biomass (RB), root length (RL), root to shoot ratio (R:S) and harvest index (HI).

S.O.V.		Mean Squares					
	d.f.	AVB	FY	RB	RL	R:S	HI
Replication	2	12.78*	2.16^{NS}	0.52^{NS}	7.66^{NS}	0.00^{NS}	0.00^{NS}
Parents (P)	7	6659.41**	2566.71**	637.95**	1070.78**	0.08**	0.02**
Sampling interval (SI)	6	8306.12**	2703.06**	628.04**	2436.58**	0.01**	0.00**
P × SI	42	1186.59**	386.15**	89.72**	348.08**	0.00^{NS}	0.00^{NS}
Error	110	3.58	1.55	0.98	3.12	0.00	0.00

** = highly significant at $P \leq 0.01$; NS = Not significant at $P > 0.05$

Tab. 2: Analysis of variance for water relations i.e. osmotic adjustment (OA), turgor pressure (TP), relative water contents (RWC), leaf water potential (LWP), osmotic potential (OP), stomatal conductance (SC) and stomatal resistance (SR).

S.O.V.		Mean Squares						
	d.f.	OA	TP	RWC	LWP	OP	SC	SR
Replication	2	0.00**	0.01NS	0.00**	0.00NS	0.02NS	0.01NS	0.15NS
Parents (P)	7	0.30**	0.34**	0.10**	0.02**	0.32**	0.14**	3.31**
Sampling interval (SI)	6	0.10**	0.44**	0.15**	2.83**	1.17**	1.81**	13.68**
P × SI	42	0.00NS	0.00NS	0.00NS	0.40**	0.17**	0.26**	1.95**
Error	100	0.01	0.01	0.01	0.00	0.01	0.03	0.02

**= highly significant at $P \leq 0.01$; NS = Not significant at $P > 0.05$

Similar to leaf water potential, osmotic potential tended to decrease with the sampling interval (Fig. 2b). All genotypes showed significant genetic variability at two sampling intervals i.e. 15 days after stress and 90 days after stress. However, genotypes showed maximum range in the mean performance (-2.16 to -2.64) at 90 days after stress. The genotypes separated into equal distinct groups. These groups were major source of genetic variability. The difference between the groups was greater than within groups at 90 days after stress. The general trend for drought sensitive group was to show higher value of osmotic potential while drought tolerant genotypes showed lower osmotic potential. At 90 days after stress, Superstar showed highest osmotic potential while Sanam showed lowest osmotic potential.

Turgor pressure (TP) also tended to decreased with the sampling interval (Fig. 2c). There was no intersection of genotypes between the groups at thirty days after stress. Drought tolerant genotypes showed higher mean performance for turgor pressure as compared to drought sensitive genotypes. The maximum range in mean was observed at 90 days after stress. Drought tolerant genotypes showed significant differences in TP at 90 days after stress, and differences between drought sensitive genotypes were non significant ($P \geq 0.05$).

For relative water contents, genotypes were divided into two significant half at 30 days after stress (Fig. 2d). In addition, genotypes also showed minimum intersection at sampling interval for this trait. Significant differences ($P \leq 0.05$) between all genotypes were observed at 30 days after stress and 90 days after stress. The major part of the variation was due to between the groups while within groups genotypes tended to remain close to each other. Mean performance range expanded with stress duration. Maximum mean performance for drought tolerant genotypes was observed for Sanam followed by Sabazpari, Ikra 1 and P-1999-31 respectively while within drought sensitive groups the highest mean performance was observed for Arka Anamika while lowest was shown by Indian Spineless.

Stomatal conductance decreased with the sampling interval (Fig. 3a). However, drought tolerant genotypes maintained higher stomatal conductance than drought sensitive genotypes. After 30 days of stress, the genotypes were separated into three groups on the basis of stomatal conductance (Fig. 3a). Group one (Ikra 1 and P-1999-31) maintained the highest stomatal conductance at 45 days after stress and onward, while group two comprised of Sanam and Sabazpari, which initially showed higher stomatal conductance and than maintained medium stomatal conductance. Third group was formed by drought sensitive genotypes, which showed low stomatal conductance through out sampling interval.

The genotypes of group 1 (Ikra-1 and P-1999-31) showed the lowest stomatal resistance while Sanam and Sabazpari showed medium stomatal resistance (Fig. 3b). However, all the drought sensitive genotypes maintained high stomatal resistance. After 45 days of stress genotype were divided into three group i.e. low medium and high stomatal resistance groups.

Changes in osmotic adjustment over sampling interval are shown in Fig. 3c. Osmotic adjustment increased with sampling interval (Fig. 3c). The genotypes started to move into their respective groups after 45 days of stress. Three genotypes i.e. Ikra 1, Sabazpari and P-1999-31 did not show any intersection at any sampling interval. Root length also increased with sampling interval (Fig. 3d). Differences between all the genotypes were significant at 60 days of stress. The genotype Sanam showed maximum root length while Indian Spineless showed minimum root length (Fig. 3d).

Fruit biomass increased with sampling interval and showed peak at 60 days after sowing while drought sensitive genotypes showed peak at 75 days after stress. Differences between drought sensitive genotypes and drought tolerant genotypes started to become distinct after 30 days of stress. At 60 days of stress, Sanam showed the highest fruit biomass while Indian Spineless showed lowest fruit biomass (Fig. 4a).

At 30 days after stress and onward, differences for root biomass appeared between drought tolerant and drought sensitive genotypes (Fig. 4b). After this sampling interval genotypes were separated into two groups on the basis of drought tolerance. The peak root biomass accumulation was achieved at 60 days after stress for drought sensitive genotypes and 75 days after stress for drought tolerant genotypes. At 60 days after stress, Sanam showed maximum root biomass followed by Sabazpari, Ikra-1 and P-1999-31.

For harvest index, genotypes were separated into two groups on the basis of tolerance at 30 days after stress and subsequently (Fig. 4c). The genotype Sanam remained distinct from all genotypes due to the highest harvest index at all sampling intervals. As a result this genotype did not show intersection at any point. Differences between all genotypes were significant at 45 days after stress.

Differences among genotypes were highest at 15 days after stress for root/shoot ratio (Fig. 4d). Afterwards root/shoot ratio decreased with sampling interval. Differences between the drought sensitive and tolerant genotypes were less obvious. Ikra-1 followed by P-1999-31 showed the highest mean root/shoot ratio at 15 days after stress as compared to other genotypes ($P \leq 0.05$).

Genetic analysis of relative water contents

All components of variance increased under drought stress (Tab. 3). The additive genetic variance was highest. Broad sense heritability showed an increase in its magnitude under drought stress for all crosses except cross 2 (Sabazpari × Indian Spineless). The narrow sense heritability was also higher under drought stress. Cross 1 (Sanam × Arka Anamika) showed the highest estimates of narrow sense heritability. Magnitude of narrow sense heritability was high in all crosses under drought stress except cross 3 (Chinese Red × Ikra 1), which gave zero heritability. Genetic advance was also high under drought stress as compared to non-stress condition.

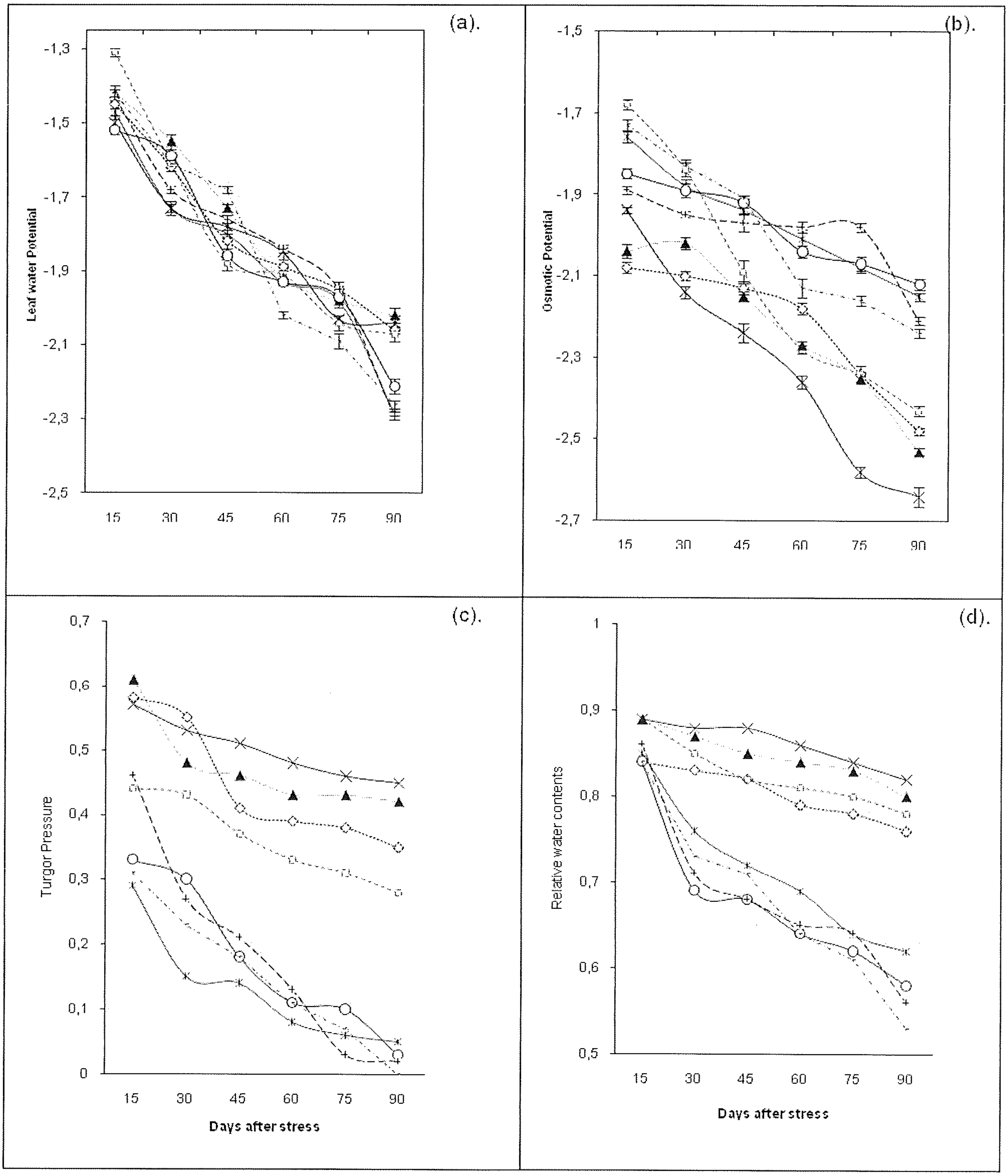

Fig. 2: Change in (a) leaf water potential (b) osmotic potential (c) turgor pressure (d) relative water contents measured over time under drought stress condition in okra cultivars i.e P-1999-31 (---◇---), Ikra-1 (- - □ - -), Sabazpari (····▲····), Sanam (—✕—), Arka Anamika (—✱—), Superstar (—○—), Chinese Red (— + —) and Indian Spineless (··-✱-··)

Six generations in four crosses showed non-significant differences under non-stress condition (Tab. 4). The parents, however, showed significant differences for mean values under drought stress. Increasing value of relative water content showed resistance of genotypes under drought stress. F_1 generation was higher than susceptible parent while lower than tolerant parents. F_2 values were lower than F_1, however they were statistically similar to F_1 in cross 1 (Sanam × Arka Anamika), 3 (Chinese Red × Ikra 1) and 4 (P-1999-31 × Superstar). BC_1 showed lower estimates than both parent in cross 1 (Sanam × Arka Anamika) while it was higher than susceptible parent and lower than resistant parent under drought stress. In crosses1 (Sanam × Arka Anamika) and 2 (Indian Spineless × Sabazpari), BC_1 mean was high-

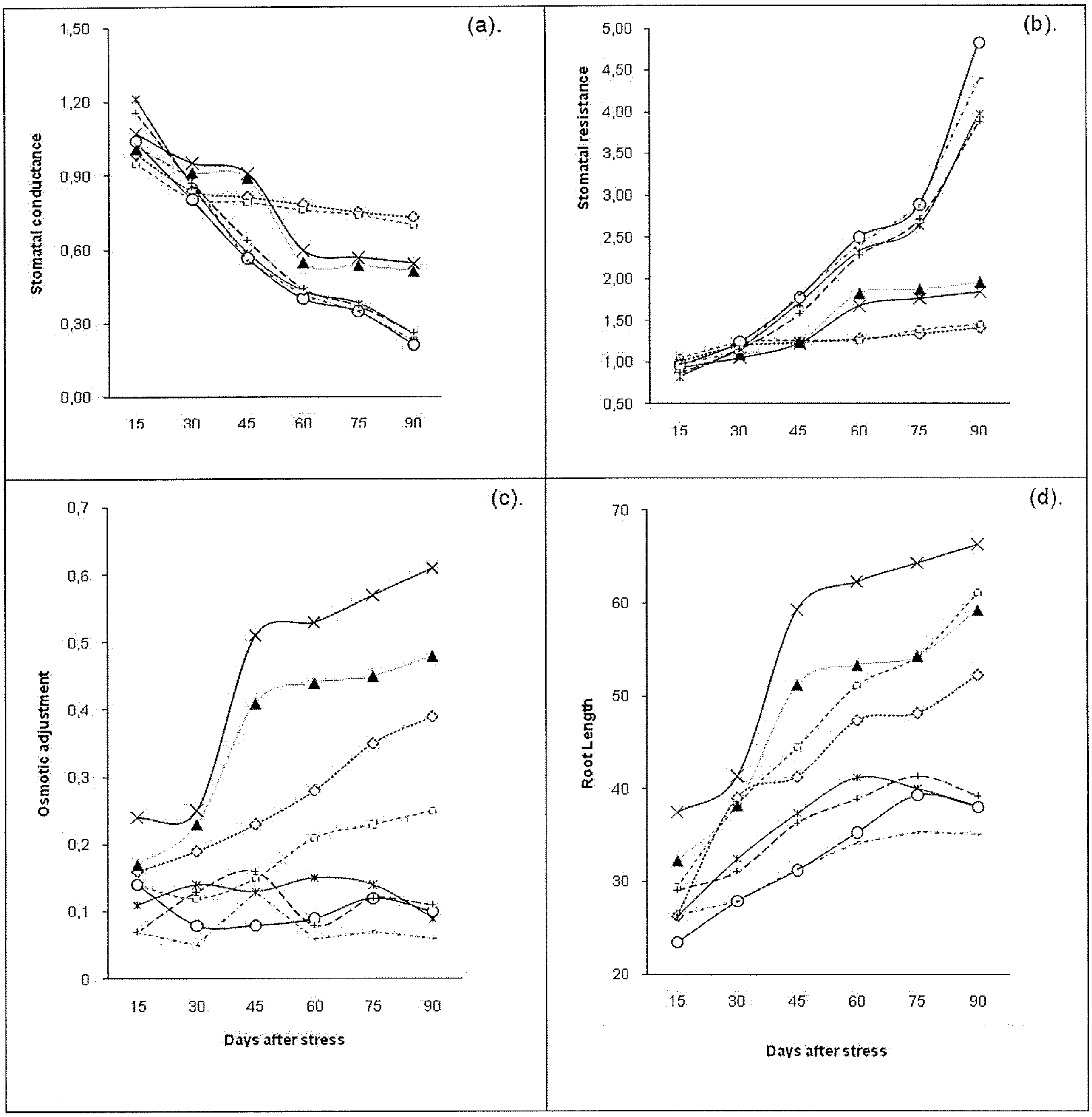

Fig. 3: Change in (a) stomatal conductance (b) stomatal resistence (c) osmotic adjustment (d) root length measured over time under drought stress condition in okra cultivars i.e P-1999-31 (---◇---), Ikra-1 (- - □- -), Sabazpari (····▲····), Sanam (—×—), Arka Anamika (—✳—), Superstar (—○—), Chinese Red (— + —) and Indian Spineless (- · - · -)

er than BC2 while it was lower than BC_2 in crosses 3 (Chinese Red × Ikra 1) and 4 (P-1999-31 × Superstar) under drought stress.

Joint scaling test showed the significance of additive, dominance, additive × additive and dominance × dominance under drought stress in cross 1 (Sanam × Arka Anamika) (Tab. 5). The dominance component was negative while dominance × dominance was positive. In cross 2 (Sabazpari × Indian Spineless), additive, dominance, additive × dominance and dominance × dominance were significant under non-stress condition. The magnitude of additive × dominance and dominance × dominance were highest under this condition. Under drought stress additive × dominance effects were highest. In cross 3 (Chinese Red × Ikra 1) and 4 (P-1999-31 × Superstar) dominance effects were highest under non stress condition while additive effects showed highest effects under stress condition.

Minimum number of effective factors increased under drought for all crosses except in crosses 2 (Sabazpari × Indian Spineless) (Tab. 6). Effective factors were more than one in crosses 1 (Sanam × Arka Anamika) and 2 (Sabazpari × Indian Spineless) while they were greater more than two under drought stress. Under non-stress condition, effective factors were only greater than two in cross 2 (Sabazpari × Indian Spineless) while in all other crosses it was less than unity in all crosses.

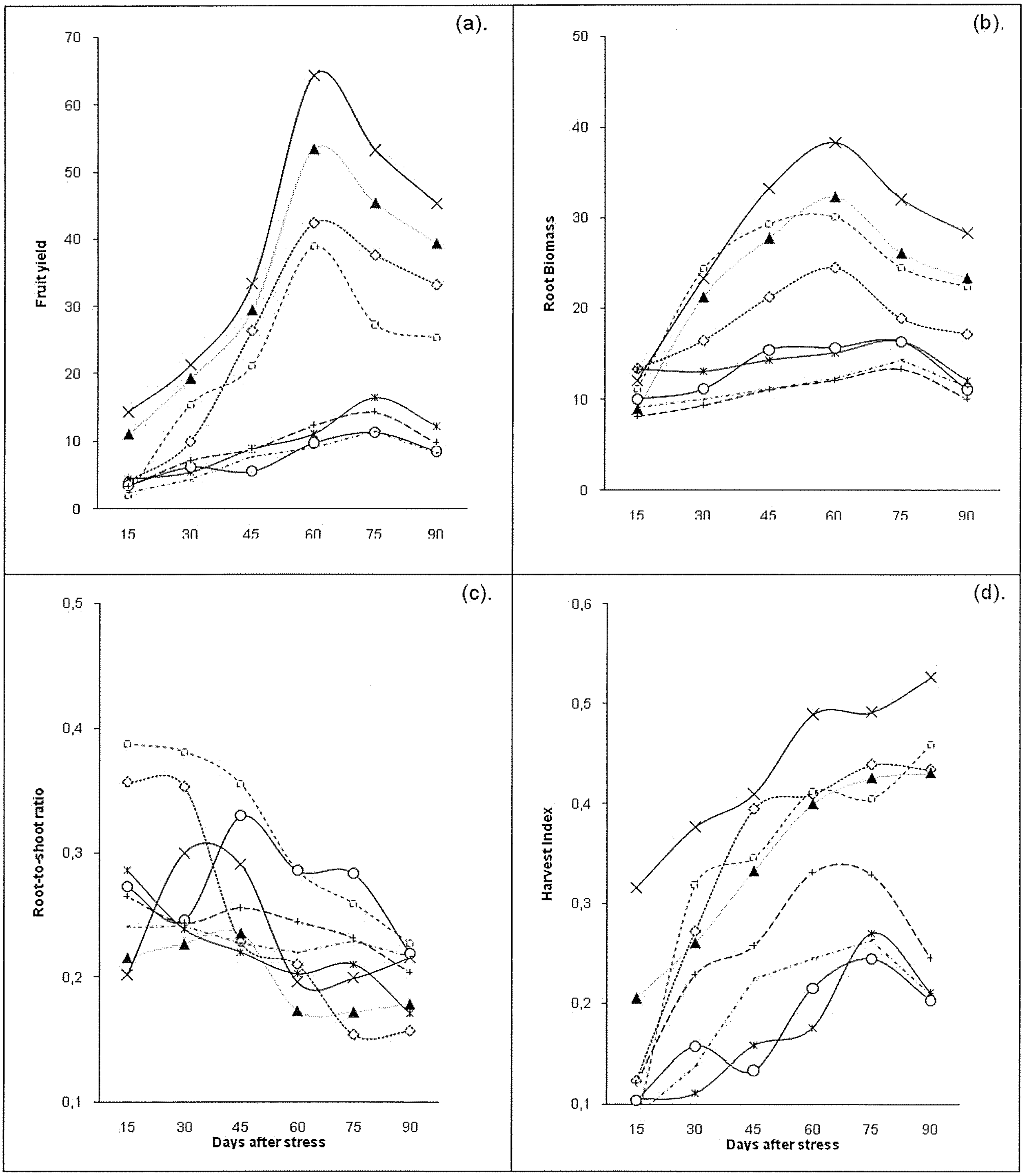

Fig. 4: Change in (a) fruit yield (b) root biomass (c) root-to-shoot ratio (d) harvest index measured over time under drought stress condition in okra cultivars i.e. P-1999-31 (----◇----), Ikra-1 (- - □- -), Sabazpari (·····▲·····), Sanam (——×——), Arka Anamika (——✳——), Superstar (——○——), Chinese Red (— —+— —) and Indian Spineless (- · - - - ·)

Discussion

Drought tolerance is a complex phenomenon that does not always solely depend on single plant trait. Drought susceptibility index (DSI) represents drought tolerance at whole plant level regardless of drought tolerance mechanism in operation (GRZESIAK et al., 1996; RAMIREZ-VALLEJO and KELLY, 1998). The selected plant for lower drought susceptibility index may have diverse tolerance mechanisms rather than based on single drought tolerant traits. Therefore, such type of population may successfully cope with the varying degree of drought.

Significant negative relationship (R^2=0.50) was obtained between drought susceptibility index and fruit yield per plant under drought stress. However, this type of relationship was absent between drought susceptibility index and fresh pod yield of non-stress condition.

Tab. 5: Genetic studies of four crosses for relative water contents (%) under normal (W1) and drought (W2) conditions

	Sanam × *Arka Anamka*		*Sabazpari* × *Indian-Spineless*		*Chinese Red* × *Ikra 1*		*Superstar* × *P-1999-31*	
	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
m	0.89± 0.00	0.81± 0.04	0.91± 0.00	0.75± 0.01	0.88± 0.00	0.72± 0.00	0.83± 0.00	0.69± 0.01
d	-0.02± 0.00	-0.10± 0.00	-0.02± 0.00	-0.05± 0.00	0.01± 0.00	-0.07± 0.00	-0.01± 0.00	-0.09± 0.00
h	0.01± 0.00	-0.26± 0.08	-0.03± 0.00	0.06± 0.00	0.05± 0.00		0.09± 0.00	
i		-0.09± 0.04		0.04± 0.01	0.03± 0.00	-0.04± 0.00	0.05± 0.00	-0.03± 0.01
j			0.04± 0.00	0.11± 0.00	0.01± 0.00	0.02± 0.01	-0.01± 0.00	-0.02± 0.01
l		0.18± 0.05	0.04± 0.00					0.03± 0.01
$\chi 2$	3.96	0.07	0.02	0.01	0.54	2.91	0.13	2.92
df	3	1	1	1	1	2	1	1

Where m = mean, d= additive, h = dominance, i = additive × additive, j = additive × dominance, l = dominance × dominance

Tab. 6: Estimates of number of genes or effective factor controlling relative water contents in four tolerant by susceptible crosses

Crosses	Non Stress					Average	Drought stress					Average
	E1	E2	E3	E4	E5		E1	E2	E3	E4	E5	
Cross 1	0.4810	0.7625	0.4531	0.3813	0.5582	0.5272	1.4642	2.9505	1.4479	1.4753	1.4215	1.7519
Cross 2	0.7398	-7.8965	0.6583	-3.9483	0.3038	-2.0286	0.8275	3.2981	0.7595	1.6491	0.4934	1.4055
Cross 3	0.7205	-2.9436	0.3183	-1.4718	0.1436	-0.6466	3.3687	-13.4562	2.8194	-6.7281	1.1655	-2.5661
Cross 4	2.3429	-0.2345	0.2311	-0.1172	0.0582	0.4561	2.0873	3.7877	1.8003	1.8938	1.7155	2.2569

Where cross 1 include parents (Sanam, Arka Anamika), and subsequent F_1, F_2, BC_1, BC_2 from these parents; Cross 2 = Indian spineless , Sabazpari and subsequent F_1, F_2, BC_1, BC_2 from these parents; Cross 3= Parents (Chinese Red, Ikra) and subsequent F_1, F_2, BC_1, BC_2 from these parents; Cross 4= (P-1999-31, Superstar) and subsequent F_1. F_2, BC_1, BC_2.

Thus, selection for lower drought susceptibility index may improve fresh pod yield under drought stress and drought susceptibility index was independent of fresh pod yield under non-stress condition. This showed that drought susceptibility index and fresh pod yield were independent of each other and presence or absence of drought will establish their relationship. In addition, fresh pod yield of non-stress and drought condition should be considered as separate resultant traits that were not always contributed by the same independent traits (FALCONER, 1990). Similar type of relationship has also been obtained in other crops (FRERES et al., 1986; RAUF and SADAQAT, 2007a, b).

On the basis of fresh pod yield and drought susceptibility index, genotypes were divided into four groups. Majority of the genotypes tended to produce low yield and high drought susceptibility index while least number of genotype showed high yield and lower drought susceptibility index. This showed that genotypes have been selected and evolved to perform well under optimum conditions and therefore breeding for drought tolerance is required. The quadrant with low susceptibility index and high yield was almost occupied by the indigenous genotypes. It may be due to their evolution under local condition that these genotypes were able to perform better than exotic genotypes under drought stress (BECK et al., 1997; VASAL et al., 1997; RAUF and SADAQAT, 2007a, b). There was a relative shift in the performance of the genotype across the water levels. Arka Anamika produced the highest fresh pod yield under non-stress condition with high drought susceptibility index, while Sanam showed lowest drought susceptibility index with highest yield under drought stress condition. In the region with limited irrigational water, plant breeders are always keen to develop and select a genotype, which could produce maximum yield with minimum available irrigation.

Tab. 3: Estimates of additive (σ^2A), dominance (σ^2D) and environmental (σ^2E) variances, broad (H) and narrow (h^2) sense heritability and genetic gain through selection (Gs) for relative water contents

Crosses	σ^2E		σ^2A		σ^2D		H		h^2		Gs	
	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2
Cross1	0.000	0.000	0.000	0.003	0.00	0.000	0.830	0.974	0.79	0.856	0.001	0.006
Cross 2	0.000	0.000	0.000	0.001	0.00	0.001	0.940	0.892	0.00	0.411	0.000	0.001
Cross 3	0.000	0.000	0.000	0.000	0.00	0.001	0.950	0.962	0.000	0.00	0.000	0.000
Cross 4	0.000	0.000	0.000	0.002	0.00	0.000	0.920	0.968	0.000	0.810	0.000	0.004

Where cross 1 include parents (Sanam , Arka Anamika), and subsequent F_1, F_2, BC_1,BC_2 from these parents; Cross 2 = (Indian Spineless , Sabazpari) and subsequent F_1, F_2, BC_1,BC_2 from these parents; Cross 3= Parents (Chinese Red, Ikra) and subsequent F_1, F_2, BC_1,BC_2 from these parents; Cross 4= (P-1999-31, Superstar) and subsequent F_1, F_2, BC_1,BC_2 from these parent.

Tab. 4: Mean performance of the generations advanced from four crosses over water levels for relative water contents.

	Sanam × *Arka Anamika*		*Sabazpari* × *Indian Spineless*		*Chinese Red* × *Ikra*		*Superstar* × *P-1999-31*	
	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
P_1	0.87 a	0.81 a	0.89 a	0.79 a	0.92 a	0.61 e	0.87 a	0.57 d
P_2	0.89 a	0.62 e	0.92 a	0.69 e	0.90 a	0.75 a	0.89 a	0.75 a
F_1	0.92 a	0.73 c	0.92 a	0.76 b	0.93 a	0.72 c	0.91 a	0.71 b
F_2	0.88 a	0.72 c	0.90 a	0.73 c	0.90 a	0.73 bc	0.88 a	0.70 b
BC_1	0.88 a	0.75 b	0.91 a	0.77 b	0.92 a	0.68 d	0.88 a	0.63 c
BC_2	0.90 a	0.65 d	0.89 a	0.71 d	0.91 a	0.74 ab	0.90 a	0.74 a

Population sharing letter a common are statistically non-significant ($P<0.05$).
Where P_1= female parent, P_2=male parent; F_1= ($P_1 \times P_2$); F_2= Selfed F_1;
BC_1= $P_1 \times (P_1 \times P_2)$; BC_2= $P_2 \times (P_1 \times P_2)$.
W_1= Non-stress condition while W_2= drought stress condition

Therefore, crosses between drought tolerant and high yielding line such as cross between Sanam × Arka Anamika would be useful in generating useful breeding material for the selection of drought tolerant okra lines with high fresh pod yield.

The genotypes changed their relative performance over sampling intervals. This may be due to differential response of okra genotypes to changing soil moisture contents since drought intensity increased with sampling intervals (SIDDIQUE et al., 2000; ATTEYA et al., 2003). For osmotic adjustment, turgor pressure, relative water contents, harvest index and root/shoot rato, the performance of genotypes was relatively stable over sampling interval. Therefore, these traits may be useful in evaluating genotypes during the earlier growth stages under drought stress. However, suitability of traits for evaluation in segregating population has also been shown to be dependent on some other factors such as ease and speed to measure and non-destructiveness (EL-JAAFARI, 2000). Among the traits, harvest index and root/shoot ratio has been shown to be unsuitable for evaluating large number of plants due to laborious and destructive nature of the traits.

The change in the relative performance of genotypes was also apparent from the figures in which genotypes intersect each other at various points. Within the traits drought tolerant genotypes were more stable than drought sensitive genotypes.

All physiological traits except stomatal resistance and root / shoot ratio decreased with drought stress at all sampling intervals. ATTEYA et al. (2003) also indicated that exposure of plants to drought led to noticeable decreases in leaf water potential, relative water content and osmotic potential. Means of morphological traits increased with sampling interval. However, rate of dry matter accumulation was much lower in drought sensitive genotypes as compared to the drought tolerant genotypes. This may be due to higher accumulation of cytokinins under drought stress (RAUF and SADAQAT, 2007a, b).

With an increase in sampling intervals or drought intensity, the genotypes separated into two distinct groups i.e. tolerance and sensitive. This trend was observed for all traits except root to shoot ratio. However, in stomatal resistance and stomatal conductance, a third group appeared within drought tolerant genotype, at 45 days after stress. This group of Sanam and Sabazpari showed medium stomatal conductance and stomatal resistance.

Among physiological traits, differences between drought tolerant genotypes and drought sensitive genotypes for leaf water potential appeared very late. In addition, differences within drought sensitive genotypes and drought tolerant genotypes were non-significant (ATTEYA et al., 2003).

Initially variation appeared as a result of differences between the genotypes but later as the intensity of drought increased variation appeared as a result of differences between the drought tolerant and sensitive groups. Within group genotypes differences were narrow. After separation of genotypes into two distinct groups there was intersection between the genotype within groups but intersection did not take place between groups. Therefore, selection in earlier stresses phases may at least help to select drought tolerant genotypes. Among the traits, relative water contents allowed the genotypes to separate into two distinct groups at earliest drought stress. Selection for high relative water contents at 30 days after stress may be useful for the selection of drought tolerant genotypes. In addition, relative performance of drought tolerant genotypes was same at this point (30 days after stress) as was at the last sampling interval. Maintenance of high relative water contents has been useful for determining a resistance of any genotypes, and it is a consequence of adaptive characteristics such as osmotic adjustment (GRASHOFF and VERVERKE, 1991).
The narrow sense heritability and genetic advance varied for different crosses and conditions. Narrow sense heritability estimates was high for the cross Sanam × Arka Anamika and its subsequent generations, therefore, this cross seemed more promising for the selection of drought tolerant material under drought stress regime. F_2 population of this cross has also shown significant relationship with fruit yield ($r=0.68$; $P<0.01$) of this cross under drought stress, as has been observed in sunflower (RAUF and SADAQAT, 2008). Therefore, this trait 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 and genetic advance for excised leaf water losses, relative water contents of biomass and suggested their use for direct selection for improving yield.

Conclusions

The studies presented were carried out to obtain information on the highlighted issues. The conclusions drawn during answering the issues are given here with recommendations.

1. Whether sizeable genetic variability existed for drought tolerance among okra genotype?

The results of the various experiments indicated that the okra genotypes showed substantial genetic variability for drought related traits under local condition of drought. The performance of the genotypes for drought tolerance was nearly consistent in all experiments. There may be some insignificant fluctuations in performance but, in general, a genotype considered tolerant in one experiment did not change its group or converted to susceptible in other experiments.

2. Relative importance of physiological and morphological trait in a drought tolerance breeding program

Value of a trait in drought breeding programme was found to depend on the:

i. Genotypes sensitivity and stability for a particular trait.
The assessed okra genotypes showed relative sensitivity to drought stress for different traits. The relative water contents allowed earlier grouping of genotypes into tolerant or susceptible groups, and was, therefore, considered ideal for screening okra genotypes for drought tolerance.

ii. Narrow sense heritability and genetic gains
Relative water contents showed high narrow sense heritability under drought stress. However, fruit yield and its components could not show the promise for selection under drought stress except number of pods per plant. Therefore, genotypes may be selected for physiological traits such as relative water contents under drought stress.

iii. Association of drought related traits with yield
Positive association of drought related traits with yield is equally important, since negative association will improve drought tolerance at the expense of yield. Positive association of relative water contents with yield was obtained among F_2 plant of all crosses.

iv. Cost and labour
Some traits such as root length and traits related to dry matter partitioning are labour intensive and destructive in nature, while other such as osmotic adjustment are costly. Therefore root length and dry matter partitioning may be used for preliminary screening and physiological traits may be used for confirmation and final selection of parents for a drought tolerance breeding programme.

3. Genetic basis of drought tolerance in okra

Considerable genetic variability has been obtained as a result of new recombination between tolerant and susceptible crosses. The difference in the degree of drought tolerance in various okra genotypes appeared to have substantial proportion of heritable (additive) variation, as the estimates of narrow sense heritability obtained from the six generations of the four crosses between drought tolerant and susceptible were high.

4. Breeding for drought tolerance in okra

Based on the higher additive variance and consequently heritability for the drought related traits, it would be safe to recommend that simple progeny to row selection in early segregating generations would be effective for the genetic improvements of okra genotypes for physiological traits under drought stress.

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