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PRINCIPAL COMPONENT ANALYSIS AND ASSESSMENT OF *BRASSICA NAPUS* L. ACCESSIONS FOR SALT TOLERANCE USING STRESS TOLERANCE INDICES

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Abstract

Due to genetic variations, the crop plants show different responses on exposure to salinity stress, enable plant biologists to find salinity tolerant types of crop plants. Many plant breeders have been successful in improving the salinity tolerance in various crops using plant vigor or seed yield as the main criteria for selection. Selection becomes more useful and feasible if the crop possesses distinguishing indicators of salt tolerance at the cellular, tissue or whole plant level. Soil salinity occurs in patches and is more heterogeneous in field conditions. Therefore screening plants in greenhouse conditions where saline conditions are reasonably uniform is effective and credible. Hydroponic culture technique is commonly used for studying the effects of salinity on crop plants as it helps in observing the effects of elemental deficiencies and toxicities. In the present study 60 accessions of *Brassica napus* were evaluated for salinity tolerance using hydroponic technique. Data were recorded on various seedling traits (root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and Na^{+}/K^{+} ratio) and salinity stress indices were calculated. Ten salt tolerant (G-96, ZNR-1, ZM-M-5, 23627, R-3, ZMR-10, BLBN, RBJ-8007, ZMR-2, B-56 and six sensitive (Legend, Laclone, Faisal, Shiralee, Long and ZMR-5) accessions were selected on the basis of computed indices through principal component analysis (PCA). This study may be helpful for the comparison of salinity indices in a controlled experimental assay and for the identification of salinity tolerant brassica types to be used in further breeding programs.

Key words: Salinity stress; *Brassica napus*; Principal component analysis; Hydroponics

Introduction

To ensure food security, a major task of producing 70% more food crops for an additional 2.3 billion people has to be achieved by 2050 (Anon., 2009). Salinity is one of the major abiotic stresses for crop plants that cause significant yield losses in irrigated as well as rainfed areas. This hampered yield due to salt affected soils can be increased by inducing salt tolerance in crop plants. Leaching of the salts from soil or cultivation of salinity tolerant crops can be useful in reclaiming the saline soil. *B. napus* is also considered to grow potentially good on salt affected areas (Ulfat *et al*., 2007) but sufficient subject matter on genetic variability of *B. napus* for salinity tolerance is still not clear and needs to be reconnoitered. Upward movement of soil solutions followed by evaporation results in high concentration of salts at soil surface which affects brassicas germination adversely and also causes salts accumulation in leaves leading to leaf death before maturity (Shirazi *et al*., 2011). Salinity decreases root, shoot lengths, fresh weights, leaf emergence and first node formation in Brassica species (Shirazi *et al*., 2011; Tarinejad *et al*., 2013). This may be due to retarded cell division and inhibitory effect of salinity on cell elongation due to limited water and nutrients. Salinity negatively affects vegetative as well as reproductive growth in brassicas due to over utilization of energy resources in maintenance of plants rather than in growth and development (Gul & Ahmad, 2004). Negative influence of salinity in yield attributes results in reduction in dry matter, increased root/shoot ratio, accumulation of more sodium ions and restriction of the availability of potassium required in many metabolic processes leading to the possible way of reducing yield (Wani *et al*., 2013). Accumulation of Na^{+} ions occurs more quickly in sensitive cultivars than the tolerant ones causing cell death ultimately leading to plant death in *Brassica napus*. Plants tolerate the salinity by osmotic adjustment and through maintenance of Na^{+}/K^{+} ion ratio by regulating the uptake of K^{+} and restricting Na^{+} ions from entering the cell (Ashraf & McNilley, 2004).

Substantial inter and intraspecific variation present within brassica genome can be utilized through selection and breeding for salinity tolerance in plants (Sadiq *et al.*, 2002; Mahmood *et al.*, 2007). Amphidiploids are more salt tolerant and it is suggested that they have acquired salt tolerance from *B. napus* and *oleraceae*. Two species i.e. *B. napus* and *B. campestris* are considered as salinity tolerant (Maas & Hoffman, 1977). Even though both species have high thresholds for salinity tolerance, the decrease in crop production beyond the threshold is much more than most other crops in salt tolerant group (Mass, 1990). Plant breeders try to search for accessions from available brassica species which are salinity tolerant. The present research work was aimed at estimation of genetic variability and development of selection criteria based on of salinity tolerance indices.

Material and Methods

Collection of germplasm: Germplasm comprising of 60 *Brassica napus* accessions and two check varieties was collected from Oilseed Research Group of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

Experimental conditions: Research experiment for screening of germplasm for salinity tolerance at seedling stage was conducted in the wire house of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

Screening of germplasm for salinity tolerance: The experiment was carried out in a triplicate completely randomized design with factorial structured treatments. Seeds of 60 *Brassica napus* accessions and two check varieties were sown in sand filled polythene bags (22.9cm×7.6cm). Six seeds of each accession per bag were sown and four plants per bag were maintained for fifteen days. On alternate days 250ml water was applied to each bag.

Table 1. Recipe of hoagland nutrient solution.

Reagents	X1000	g/L	Reagents	X1000	g/L
KH_2PO_4	68	136	$MnCl_2.4H_2O$	0.905	1.81
KNO_3	252.5	101	$ZnSO_4.7H_2O$	0.11	0.22
$Ca(NO_3)_2H_2O$	590	236	$CuSO_4.5H_2O$	0.04	0.08
$MgSO_4.7H_2O$	246	246	$H_2MoO_4.H_2O$	0.01	0.02
H_3BO_3	1.43	2.86	FeEDTA	18.665	37.33

Three tubs each of 200 L capacity were used for preparing Hoagland nutrient solution using the recipe presented in Table 1 (Hogland & Arnon, 1938). Three seedlings of each accession per replication were transplanted to hydroponic solution after 15 days of sowing (Fig. 1a, b). Three replications for each accession under one treatment were maintained in the same tub. Three salinity treatments i.e. T1= 0mM (control), T2= 120mM, T3 = 150mM were developed using NaCl salt. After seven days of transplantation salt solutions of 40mM and 50mM were prepared and added to Hogland nutrient solution. Salt levels of 120mM and 150mM were developed in aliquots of 40mM and 50mM respectively on alternate days. Portable EC meter (HI-99300) was used for measuring electrical conductivity of the solution and pH of solution was maintained at 7 using NaOH and HCl. Two plants of each accession per replication of each treatment were uprooted after twenty-one days of exposure to salinity and data were recorded on the following traits.

Root and shoot lengths (cm): Uprooted seedlings were separated in roots and shoots and their lengths were recorded in cm with measuring scale. Average root and shoot lengths were calculated.

Fresh and dry weights of roots and shoots (g): Fresh weights of roots and shoots of uprooted seedlings were measured using electronic balance (Setra BL-410S). These roots and shoots were placed in paper bags separately and dried at 65°C for 48 hours Tanveer-ul-Haq *et al*., 2014) and reweighed.

Determination of Na^+ and K^+ in plant tissues: Na^+ and K^+ ions were measured following the protocol used by Wolf (1982). The oven dried crushed leaves (0.1g) of each sample were placed in different digestion flasks and 2.5ml of H_2SO_4 was added to each flask followed by overnight incubation at room temperature. Added 1ml of H_2O_2 (35%) in flask and it was heated on hot plate until the production of fumes at 350°C. Then digestion flasks were removed from hotplate for cooling and placed back on hot plate after adding 1ml of H_2O_2. This practice was repeated until the cooled sample became colorless. Distilled water was added to the sample to maintain the volume of extract up to 50ml in volumetric flasks. The extracted material was filtered with Whatman filter paper and then used for the determination of Na^+ and K^+ ions. Single channel flame photometer (Spectronic Camspec Ltd, Model Jenway, PFP-7, UK) was used for analyzing the K^+ and Na^+ ions with extra pure A grade series of standards (5 to 60 mg L^{-1}).

Fig. 1. (a) and (b) Seedlings of *Brassica napus* in hydroponic solution.

The recorded data were used to calculate different indices by using the following formulae (Ashraf *et al*., 2006; Ahmad *et al*., 2009).

RLSI= (RL of stressed plant / RL of control plant) × 100
SLSI = (SL stressed plant / SL of control plant) × 100
FRWSI = (FRW of stressed plant / FRW of control plant) × 100
FSWSI = FRWof stressed plant / FRW of control plant) × 100
DRWSI= (DRW of stressed plant / DRW of control plant) × 100
DSWSI = (DSW of stressed plant / DSW of control plant) × 100
Na^+/K^+SI= (Na^+/K^+ of stressed plant / Na^+/K^+ of control plant) × 100

Biometrical approaches: The recorded data were subjected to analysis of variance following Steel *et al.*, (1997). Ten salinity tolerant and six sensitive accessions were selected using principal component analysis (PCA) on recorded seedling parameters (Gabriel, 1981; Yan & Kang, 2011).

Results and Discussions

Genetic variability: The accessions differed significantly for all the stress indices except that of fresh shoot weight and dry root weight (Table 2). Significant differences were present among the treatments for all the stress indices except fresh root weight stress index. Accessions × Treatments interaction was significant for Na^+/K^+ stress index only. All the stress indices of T3 were less than that of T2 stress indices except root length, fresh root weight and Na^+/K^+ stress indices (Fig. 2). Mean values of T2 and T3 stress indices are presented in Table 3. Root length stress index ranged from 45.9% to 135.8% for T2 and 40.1% to 204.3% for T3; shoot length stress index from 52.9% to 110.9% for T2 and 41.2% to 145.3% for T3; fresh root weight stress index from 15.0% to 4335.1% for T2 and 15.8% to 2169.3% for T3 and fresh shoot weight stress index from 15.4% to 483.7% for T2 and 56.9% to 6130.7% for T3; dry root weight stress index 10.1% to 114.8% for T2 and 0.3% to 28.3% for T3; dry shoot weight stress index from 37.6% to 2760.4% for T2 and 22.2% to 1705.9% for T3 and Na^+/K^+ stress index from 142.9% to 360.7% for T2 and 219.4% to 531.7% for T3.

Principal component analysis: Biplots for T2 and T3 i.e. PCA1 for T2 and PCA2 for T3 are presented in Fig. 3 and 4 respectively. In PCA1, accessions 49 (BLBN), 27 (G-96), 50 (RBJ-8007), 53 (ZMR-1), 60 (ZMR-2), 28 (23627), 18 (B-56), 46 (ZNR-1), 17 (ZMM-6), 54(R-3), 26 (ZM-12), 48 (ZMM-5) were present in quadrant. I where all the indices had positive response towards salinity tolerance for most of the traits. Accessions 56 (RGS), 29 (Ames-6102), 14 (Shiralee), 20 (Legend), 9 (Long), 52 (ZMR-11), 22 (ZMR-8), 33 (ZMR-10), 21 (Faisal), 16 (ZMM-8), 59 (64A), 12 (Toria), 36 (Laclone) were present in quadrant III where all the indices had negative response towards salinity hence, were salinity sensitive. From the PCA 2, the accessions 44 (ZM-21), 49 (BLBN), 50 (RBJ-8007), 27 (G-96), 53(ZMR-1), 45 (NIFA-7), 18 (B-56), 28 (23627), 54 (R-3) , 5 (KN-258), 48 (ZMM-5), 29 (Ames-6102), 16 (Excel), 52(KN-256), 46 (ZNR-1), 60 (ZMR-2) in quadrant I were considered as salinity tolerant and accessions 24 (ZMM-3), 21 (Faisal), 36 (Laclone), 20 (Legend), 42 (R-5), 2 (Punjab Sarsoon), 4 (ZMR-3), 14 (Shiralee), 19 (FSB), 10 (Chakwal), 55 (RBN), 9 (Long), 4 (ZMR-3), 36 (Laclone), 37 (G-46), 1 (AUB-2000), 3 (ZRM-12) in quadrant IV were considered salinity sensitive. Salinity tolerant and sensitive lines were selected on the basis of results obtained from principal component analysis. Salt tolerant accessions were selected from quadrant I. Sensitive accessions were selected from quadrant IV for PCA1 and PCA2. Accessions B-56 (18), BLBN (49), G-96 (27), R-3 (54), RBJ-8007 (50), ZMR-1 (53), ZMR-2 (60), ZNR-1 (46), ZMM-5 (48) and 23627 (28) were selected as salinity tolerant and Faisal (21), Shiralee (14), Laclone (36), Long (9), Legend (20) and ZMR-3 (4) as salinity sensitive.

Table 2. Mean squares for stress indices of seedling traits in *Brassica napus* L. accessions under normal and salinity stress conditions.

SOV	DF	RLSI	SLSI	FRWSI	FSWSI	DRWSI	DSWSI	Na^+/K^+ SI
Accessions (A)	59	3791.4*	1045.86*	1312901*	2989115	456.9	885101*	14904*
Treatments (T)	1	2719860*	5990.46*	104046	9.515*	22960.2 *	3367661*	1952790*
A × T	59	1456.1	433.99	299257	2719860	455	198240	2857*
Error	238	1723.3	420.35	642774	3567839	0.0034	30.49	0.006

*=Significant at 5% probability level

SOV= Sources of variation, **DF**= Degrees of freedom, **RLSI** = Root length stress index, **SLSI**= Shoot length stress index, **FRWSI**= Fresh root weigh stress index, **FSWSI** = Fresh shoot weight stress index, **DRWSI**= Dry root weight stress index, **DSWSI** = Dry shoot weight stress index, **Na^+/K^+ SI**= Na^+/K^+ stress index

Table 3. Ranges of mean values of salinity stress indices (%) in *Brassica napus* L.

Stress indices	T2 (120mM)				T3 (150mM)			
	Maximum		Minimum		Maximum		Minimum	
	(%)	Accessions	(%)	Accessions	(%)	Accessions	(%)	Accessions
RLSI	135.83	Bulbul	45.88	ZM-21	204.28	ZMR-1	40.11	ZM-21
SLSI	110.91	ZM-21	52.93	B-56	145.34	ZMM-9	41.21	RBN
FRWSI	4335.1	RBJ-8007	15.04	AUB-2000	2169.26	RBJ-8007	15.75	ZMM-5
FSWSI	483.74	FH-10	15.40	E-18	6130.67	ZMR-1	56.97	ZMR-11
DRWSI	114.82	ZM-8	10.06	ZMM-5	28.33	ZNR-1	0.33	Faisal
DSWSI	2760.39	E-18	37.65	DGL	1705.98	ZMR-11	22.21	AC-Excel
Na/KSI	360.74	ZRM-12	142.98	ZMR-6	531.67	ZM-21	219.35	ZMR-6

RLSI= Root length stress index, **SLSI**= Shoot length stress index, **FRWSI**= Fresh root weigh stress index, **FSWSI=**Fresh shoot weight stress index, **DRWSI**= Dry root weight stress index**, DSWSI**= Dry shoot weight stress index, **Na^+/K^+ SI**= Na^+/K^+stress index

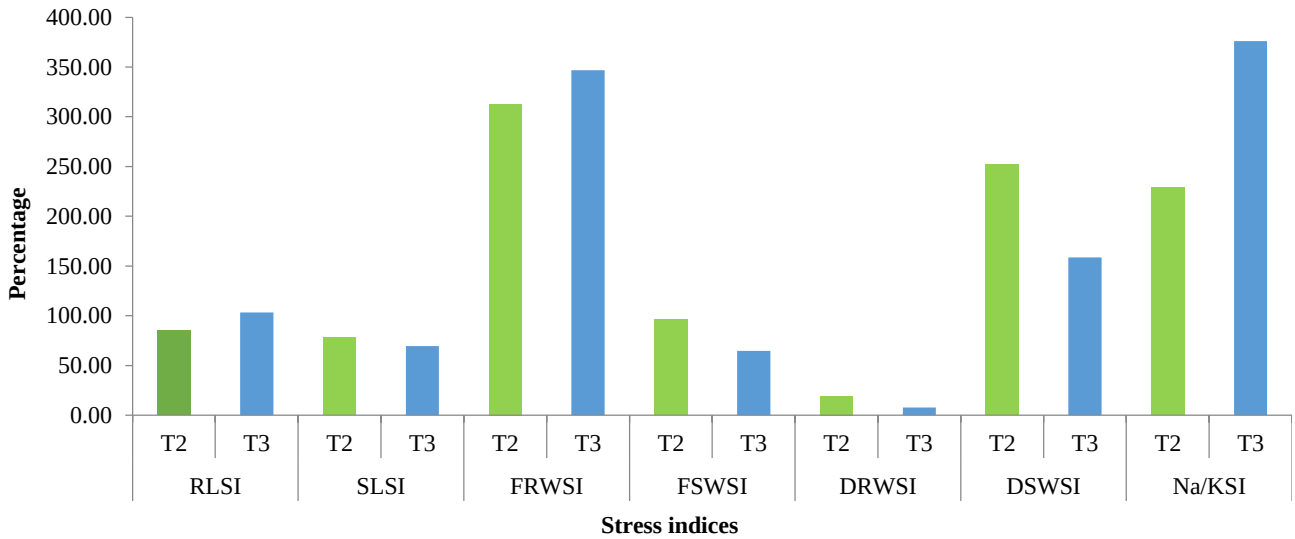

Fig. 2. Comparison of salinity stress indices of T2 and T3 in *Brassica napus* L. accessions.
RLSI = Root length stress index, **SLSI**= Shoot length stress index, **FRWSI**= Fresh root weigh stress index, **FSWSI**= Fresh shoot weight stress index, **DRWSI**= Dry root weight stress index**, DSWSI** = Dry shoot weight stress index, **Na^+/K^+ SI**= Na^+/K^+ stress index

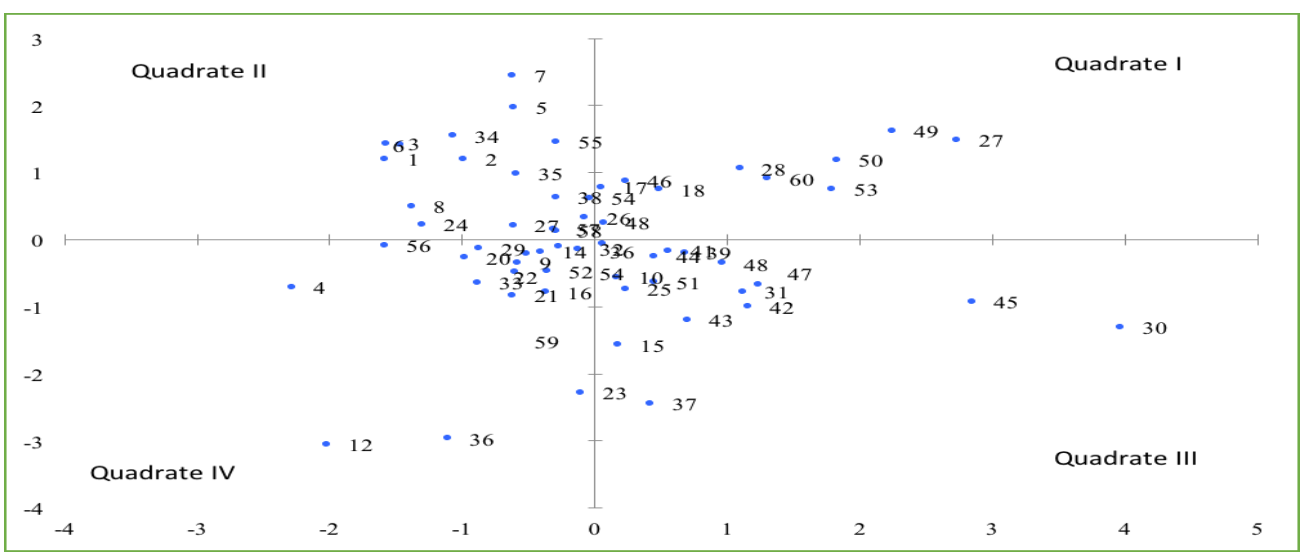

Fig. 3. PCA1 of stress indices of T2 (120mM) in *Brassica napus*.

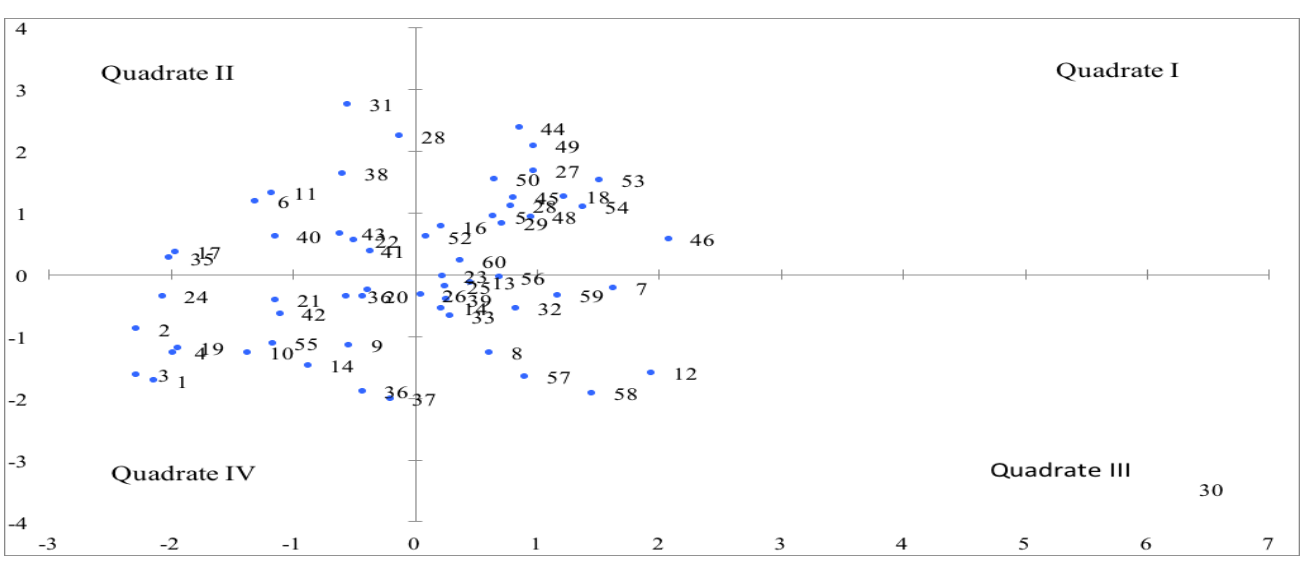

Fig. 4. PCA2 of stress indices of T3 (150mM) in *Brassica napus*.

Discussion

Salinity threatens crop production and increasing levels of salinity negatively influence vegetative and reproductive growths of rapeseed (Zadeh & Naeni, 2007). Two lines of action may be adopted to tackle the salinity problem: a) reclamation of saline soils through chemical amendments and b) development of salinity tolerant cultivars. There are situations where good quality water is unavailable or where saline soils cannot be reclaimed due to restricted availability of natural and economic resources. Under saline conditions intraspecific genetic variability can be exploited by screening of a large number of genotypes using yield and salinity tolerance related traits as selection criteria (Ashraf, 2004; Ashraf & Harris, 2004). Selection of the genotypes for salt tolerance under greenhouse conditions or under field conditions after full growing season gives the same results. Genotypes were selected under greenhouse conditions using hydroponic technique as it was well demonstrated that genotypes tested under greenhouse condition showed tolerance to salinity in field conditions as well (Sammons *et al*., 1978). Furthermore, salinity in field conditions occurs in patches thus making it difficult to screen out the salt tolerant types in such heterogeneous fields, so it is more reliable to evaluate plants in greenhouse conditions where saline conditions are practically uniform (Munns & James, 2003). Hydroponic culture technique is commonly used for studying the effects of salinity on crop plants as it helps in observing the effects of elemental deficiencies and toxicities. It also helps in observing the effects of salinity on development of plants at different levels. It is difficult to score salinity tolerance of crop plants in field; hence salinity tolerant lines may be selected by growing them under different levels of salinity using hydroponic technique (Ashraf & Ali, 2008; Ulfat *et al*., 2007).

Vast genetic variability in brassica cultivars for salinity tolerance may be exploited in breeding for salinity tolerance (Toorchi *et al*., 2011). This suggested that selection in this material may be effective to identify salinity tolerant and sensitive genotypes. It often can be difficult to identify which traits are more important contributing to salinity tolerance. To ease this difficulty salinity stress indices may be calculated to identify the contribution of important traits to salinity tolerance. Stress indices include both overall production of biomass under control conditions and ability to maintain under stress conditions favoring the selection of genotypes that perform good under both controlled and stress conditions.

Choosing the efficient method for improving the proficiency of selection in breeding programs is a major challenge. Principal component analysis assists identification of genotypes when dealing with a large number of genotypes and traits (Gabriel, 1981 and Yan & Kang, 2011). Based on the results obtained from principal component analysis, the accessions BLBN, B-56, G-96, R-3, RBJ-8007, ZNR-1, ZMR-1, ZMR-2, 23627, ZMM-5 were selected as salinity tolerant and Faisal, Shiralee, Laclone, Long, Legend and ZMR-3 were salinity sensitive.

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