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Morphological evaluation of advanced breeding lines of rapeseed-mustard for salinity tolerance using multivariate and genetic analyses

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ABSTRACT

Salinity is a barrier of crop production while salinity affected areas are expanding in Bangladesh. To increase oilseed production in saline soils, a useful tactic could be developing salinity tolerant mustard varieties. This study screened advanced breeding lines of rapeseed-mustard along with a check variety for salinity tolerance. An experiment was conducted for multivariate and genetic assessments following a split plot design with six genotypes (BD-6950, BD-7104, BD-10115, JUN-536, BJDH-12 and BARI Sarisha-14 as a check) and five salinity treatments (0, 6, 8, 10 and 12 dSm^{-1}). A total of 16 morphological characters were recorded. Significant variations were accounted among the genotypes and treatments for all the characters. A significant and positive correlation was found among seed yield $plant^{-1}$ and 1000-seed weight, number of seeds $siliquae^{-1}$, length of siliqua, length of primary branches. All morphological characters except number of secondary branches and length of siliqua showed high heritability, from 62% to 98%, which indicated that these characters can be selected for further improvement. Genetic analyses revealed high heritability with genetic advance in percentage of mean for number of seeds $siliquae^{-1}$, number of siliqua in primary axis, number of siliqua in secondary axis, total siliqua $plant^{-1}$ and seed yield $plant^{-1}$ signifying their suitability as indicators for salinity tolerance. Principal component analysis revealed that days to first flowering and days to maturity were contrasting with other variables for salinity stress tolerance in the genotypes BD-7104 and BD-10115 under 8 and 10 dSm^{-1} salinity levels. Based on the multivariate analyses, the genotype BD-7104 followed by BD-10115 were revealed as the most suitable salt tolerant genotypes which can greatly contribute to oilseed production in saline prone areas. Further researches emphasizing QTL mapping of salt tolerant traits in order to develop new, improved and climate smart salt tolerant variety of oilseed *Brassica* are suggested.

Introduction

The genus *Brassica* is the world's third most important sources of edible vegetable oils after soybean and palm (Hussain *et al.,* 2016). The Oleiferous Brassicaceae is commonly referred to the diploid species *Brassica rapa* L. (turnip rape) and the amphidiploid species *Brassica juncea* L. czern. (Indian mustard) together termed as rapeseed-mustard in Bangladesh. Rapeseed or mustard is a major oil plant in the world (Rameeh, 2016). Bangladesh has a total cultivable area of 2.86 million ha and total production of oil seeds occurs at 8.71% of the total cultivable area (BBS, 2017). So, more and more lands are needed to be brought under the cultivation of food crops each year in order to cope with increasing demand for food due to an ever-increasing population. Total population of Bangladesh will cross 170 million in 2021 if population rises at a rate of 1.32% (BBS, 2017). Based on the current consumption pattern (9.70 kg $year^{-1}$ or 26.57 g day^{-1}), the total oil requirement for a population of 160 million becomes 0.15 million Metric Tons (MTs) (Quaiyum *et al.,* 2015). Because domestic production of edible oil is an average of only 0.02 million MTs per year, the country needs to import about 0.13 million MTs of edible oil from outside (BBS, 2017). Actually, domestic production can only provide about 21% of the total demand and rest (79%) are imported every year (BBS, 2017). Unless action is taken to increase domestic production of edible oil from domestic seeds or rice bran, foreign currency drainage will increase to meet the growing demand in 2021 (Quaiyum *et al.,* 2015).

Agriculture is a major economic sector of Bangladesh. Over 30% cultivable land of Bangladesh is in the coastal area. Approximately, 1.1 million ha of arable lands from 2.86 million ha is affected by varying degrees of salinity in coastal and offshore lands (SRDI, 2010). In the wet season, farmers mainly cultivate low yielding, conventional rice varieties while in the dry season (January-May), most of the field remains fallow due to soil salinity as well as lack of good irrigation water and poor drainage system (SRDI, 2010). According to the survey by SRDI, the total saline area has increased from

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0.83 million ha to about 1.06 million ha in the last four decades (SRDI, 2010). In the recent past, ordinary crop production is becoming very difficult due to the changing degree of salinity in some areas and further infiltration of saline water.

Soil salinity is one of the major abiotic stress factors affecting production and quality of food crops worldwide that limiting growth and development as well as yield potential of crop plants (Bray *et al.,* 2000; Tester and Davenport, 2003). Salinity creates adverse environmental and hydrological conditions that limits the regular crop production. Higher level of soil salinity affects elementary physiological and metabolic processes, hence seed germination, seedling establishment, vegetative and reproductive growth are hampered, leading to reduced economic yield associated with poor quality of produce (Ahmad *et al*., 2013). Salinity induced osmotic stress may cause a reduction in leaf area and photosynthetic rate (Kauser *et al*., 2006; Shah, 2007) and alteration of the light phase of photosynthesis (Qiu *et al*., 2003). Salinity causes either a nutritional imbalance due to ions and low soil water potential in both uptake and translocation processes or a toxic effect due to the accumulation of Na^+ and Cl^- ions in the cytoplasm (Villalta *et al*., 2008). Ion toxicity which inhibits the enzymatic function of key biological processes (Zhang and Shi, 2013). Salinity significantly reduces the growth, seed yield and oil production of *Brassica* and also reported to show great magnitude of interspecific variation for salinity tolerance (Ashraf and McNeilly, 2004). The most common adverse effects of salinity on oilseed crops are the reduction of seed germination, seedling growth, seedling height, number and size of leaves, seed number, seed weight, seed yield as well as deterioration of qualitative value (Bybordi, 2010; Flagella *et al.,* 2004; Houle *et al.,* 2001; Kumar, 1995).

Crop improvement is greatly dependent on genetic diversity of crop plant. It is estimated that not even 15 percent of the potential diversity has been utilized. Thousands of important allelic variants of economic value features remain unused. These can be explored and used successfully to face the country's current and evolving oilseed development challenges. The country needs to diversifying desirable crops in a specific area with increasing population. As such, the production of suitable varieties with urgent need is necessary. To meet future oil requirements successfully, continued genetic erosion must be protected and the issues of genetic conservation and optimum utilization of what remains of the genetic diversity of important crops such as oil seeds must be addressed (FAO, 2003). There is a great opportunity of adopting the coastal belt with salt-tolerant varieties of rapeseed and mustard. Screening of tolerant genotypes of rapeseed-mustard in medium to strong saline soil will provide us a tremendous opportunity to increase the area of oil seed production without curtailing the present area of other crops. Therefore, it is necessary to elucidate the salt tolerance potentiality of rapeseed-mustard genotypes and the quality of seed produced in the saline area.

This study was planned to identify the most suitable saline tolerant genotype from the existing advanced breeding lines of rapeseed-mustard. The focus of the experiment was to evaluate variation in morphological, genetic characters and yield of advanced breeding lines of rapeseed-mustard grown under salinity stress.

Materials and Methods

Plant culture and management

Salinity is a dynamic and complex trait which is very difficult to maintain different levels of salinity in the pots. It was tried best to make artificial salinity conditions in the pots. The experiment was conducted in the pot at the field laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh in the year 2018-19 under open field condition. The experiment was performed with six rapeseed-mustard genotypes (BD-6950, BD-7104, BD-10115, JUN-536, BJDH-12 and BARI Sarisha-14 as check) under five treatments: 0 (control), 6, 8, 10 and 12 dSm^{-1}. Seeds of 6 genotypes were collected from Oil Seed Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. Topsoil was collected from the experimental farm of Oilseed Research Centre, Bangladesh Agricultural Research Institute, Gazipur. Pulverization and drying were done in the field laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. After drying thoroughly, the dried soil was evenly mixed with well rotten cow dung at the ratio of 3:1 (by volume). Then the air dried soil was mixed with respective amount of salt to make the respective levels of salinity. The salinity was checked by EC meter to ensure respective salinity treatments. Plastic pots having 24 cm diameter at the top and 22 cm at the bottom were filled with 15 kg air dried soil. Before filling the pot, the soil were mixed with chemical fertilizer as per recommendation of fertilizer recommendation guide-2012. Seeds were sown by making holes and keeping more or less equal distances. At fifteen days after germination, five seedlings of each genotype in each pot were allowed to grow till maturity. Breaking of earth crust and watering were applied periodically to maintain favorable environment.

Data collection

A total of sixteen morphological traits were studied in this experiment**.** Data on days to first flowering (DFF), plant height (PH), leaf area (LA), chlorophyll content (ChlC), days to maturity (DMa), number of primary branches (NPB), number of secondary branches (NSB), length of primary branches (LPB), number of siliqua in main axis (NSiM), number of siliqua in primary branches (NSiP), number of siliqua in secondary branches (NSiS), total siliqua $plant^{-1}$ (TSiP), siliqua length (SiL), number of seeds $siliquae^{-1}$ (NSSi), 1000-seed weight (TSW), seed yield $plant^{-1}$ (SYP) were recorded.

Estimation of genetic parameters

Estimation of genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2b), genetic advance (GA) and genetic advance in percentage of mean (GA%) were done according to the given formula from the published articles. GV and PV were estimated by Johnson *et al.* (1955). GA (%) was calculated by the formula of Comstock and Robinson (1952). Heritability in broad sense (h^2b) was classified as low (below 30%), medium (30-60%) and high (above 60%) as suggested by Johnson *et al.* (1955). GA (%) was categorized as low (0-10%), moderate (10-20%) and high (≥20%) as given by Johnson *et al*. (1955). GCV and PCV values were estimated according to the formula given by Burton (1952) and Singh and Chaudhury (1979). GCV and PCV values were categorized as low (<10%), moderate (10-20%) and high (>20%) (Sivasubramanian and Madhavamenon, 1973).

Statistical analysis

Data were analyzed using MINITAB 17 statistical software packages (Minitab Inc., State College, Pennsylvania, USA). A two-way Analysis of Variance (ANOVA) was executed for different morphological traits following the General Linear Model (GLM). Principal Component Analysis (PCA) of the morphological traits was carried out to investigate the associations between different morphological traits under different salinity treatments. The principal component (PC) scores were stored and ANOVA of the PC scores was performed using the one way ANOVA to explore the statistical significnce of between treatment and genotype. PCs with eigenvalues more than one were discussed as per the suggestion given by Brejda *et al*. (2000), which determines as a minimum 10% of the variation. Superior eigenvalues were considered as the best attributes in principal components. A Pearson correlation analysis was carried out to explore relationship among the traits.

Results

Effect of salt treatment

Salt treatment significantly affected different morphological traits of the genotypes of rapeseed-mustard ($P < 0.001$, $P < 0.01$, $P < 0.05$) (Table 1, Fig. 1). The increasing trends were observed among different treatments with increase in salinity days to first flowering, days to maturity (Table 2). The value of days to first flowering (control= 33.3d, 6 dSm^{-1}=40.4d, 8 dSm^{-1}=48.4d, 10 dSm^{-1}=51.1d, 12 dSm^{-1}= 52.1d) and days to maturity (control= 97.2d, 6 dSm^{-1}=99.2d, 8 dSm^{-1}=103.1d, 10 dSm^{-1}=105.5d, 12 dSm^{-1}=108.2 d) were increased with higher levels of salinity (Table 2). On the other hand, the decreasing trends were observed among different treatments with increase in salinity levels in rest of the all traits (Table 2). Higher level of salinity triggered/elicited/prompted severe reduction in morphological traits. The traits plant height (control=78.2cm, 6 dSm^{-1}=69.5cm, 8 dSm^{-1}=65.9cm, 10 dSm^{-1}=59.8cm, 12 dSm^{-1}=49.9cm), number of primary branches (control=6.7, 6 dSm^{-1}=5.8, 8 dSm^{-1}=5.4, 10 dSm^{-1}=5.1, 12 dSm^{-1}=4.4), seed yield $plant^{-1}$ (control=10.4g, 6 dSm^{-1}=6.6g, 8 dSm^{-1}=6.3g, 10 dSm^{-1}=5.4g, 12 dSm^{-1}=3.8g) exhibited decreasing trend with increase in salinity levels (Table 2).

Genotypic differences

Significant genotypic differences were observed in all genotypes of rapeseed-mustard ($P < 0.001$, $P < 0.01$, $P < 0.05$) (Table 1). All the advanced breeding lines revealed as better performer over check varieties (Table 3). The genotypes JUN-536 and BD-7104 accounted for greater siliqua length (4.3cm, 4.2cm respectively), seed yield $plant^{-1}$ (7.7g, 7.2g respectively) whereas BARI Sarisha-14 performed lower siliqua length (3.7cm), seed yield $plant^{-1}$ etc. (4.4g) (Table 3).

Treatment × Genotype differences

For treatment × genotype interactions, some interesting results were found. Morphological traits like days to first flowering ($P < 0.001$), plant height ($P < 0.05$), number of primary branches ($P < 0.001$), number of siliqua in primary branches ($P < 0.05$), siliqua length ($P < 0.01$), number of seeds $siliquae^{-1}$ ($P < 0.001$), seed yield $plant^{-1}$ ($P < 0.01$) showed significantly different salinity treatment × genotype interactions (Table 1). Traits like leaf area ($P > 0.05$), length of primary branches ($P > 0.05$) total siliqua $plant^{-1}$ ($P > 0.05$) 1000-seed weight ($P > 0.05$) showed nonsignificant different salinity treatment × genotype interactions (Table 1).

Principal Component Analysis (PCA)

The most apposite combination of the studied traits was obtained from the PCA where the vector length on biplot exhibited the magnitude of variation explained by respective trait and treatment × genotype combinations in the PCA. PC1 and PC2 showed significant difference for treatment ($P < 0.001$) and genotype ($P < 0.001$) (Table 4). PC3 and PC4 showed significant difference for genotype ($P < 0.001$) (Table 4). The first four principal components (PC) explained about 76.5% of the total data variation for the effect of salinity stress on different morphological traits/variables. PC1, PC2, PC3 and PC4 explained 49.7%, 13.5%, 7.3%, and 6% variation respectively (Table 4). PC1 accounted for significant treatment and genotypic variations for higher coefficients of plant height, number of secondary branches, length of primary branches, number of siliqua per plant and yield per plant (Table 4). PC1 and PC2 accounted for a greater separation of the genotype BD-7104 and BD-10115 for morphological traits like days to first flowering, days to maturity under higher salinity treatments (8, 10 and 12 dSm^{-1}) (Fig. 2).

Table 1. Mean squares of the respective sources of variances with significance levels under salinity stress for morphological traits

SOV	*df*	Traits				
		DFF	PH	LA	ChlC	DMa
Treatment (T)	4	998.4***	1654.4***	211.9***	345*	297.8***
Variety (V)	5	129.8***	2177.9***	104.3***	294.7*	118.8***
T × V	20	97.7***	140.2*	27.9	298**	33.8**
E	44	22.2	73.3	17.9	119.4	14.2
		NPB	LPB	NSiM	NSiP	NSiS
Treatment (T)	4	11.2***	375.9***	202.6***	2758.6***	16683***
Variety (V)	5	4.1***	645.3***	273.4***	11099.2***	6007**
T × V	20	3.5***	56.7	41.9***	773*	2051
E	44	0.59	33.5	9.7	418	1698
		TSiP	SiL	NSSi	TSW	SYP
Treatment (T)	4	36862***	0.40***	20.3***	3.1***	91.9***
Variety (V)	5	38243***	0.54***	325.4***	0.58**	20.1***
T × V	20	4360	0.14**	12***	0.22	6.4**
E	44	3350	0.05	1.8	0.2	2.3

*, ** & *** represent level of significance @ 5%, 1% & 0.1% respectively; DFF= Days to first flowering, PH= Plant height, LA= leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Fig. 1. Effect of five salinity treatments on growth and flowering of a rapeseed genotype Jun-536. Salinity levels 10 and 12 dSm^{-1} severely affected growth and hampered flowering

Table 2. Mean performances of different morphological traits against five salinity treatments

Items	DFF	LA	ChlC	DMa	PH	NPB	NSB	LPB
Control	33.3 c	25.8 a	63.9 a	97.2 c	78.2 a	6.7 a	13.8 a	39.9 a
6 dSm^{-1}	40.4 b	19.6 b	67.1a	99.2 c	69.5 b	5.8 b	11.9 a	38.7 a
8 dSm^{-1}	48.4 a	19.5 b	66.6 a	103.1 b	65.9 bc	5.4 bc	10.9 a	35.5 ab
10 dSm^{-1}	51.1 a	17.6 b	55.8 a	105.5 ab	59.8 c	5.1 b	10.6 a	31.1 bc
12 dSm^{-1}	52.1 a	16.5 b	58.1 a	108.2 a	49.9 d	4.4 c	10.3 a	27.7 c
Level of significance	***	***	*	***	***	***	*	***
Items	NSiM	NSiP	NSiS	TSi	SiL	NSSi	TSW	SYP
Control	32.6 a	104 a	131 a	268 a	4.3 a	15.2 b	3.9 a	10.4 a
6 dSm^{-1}	27.4 b	97.1 a	86.2 b	209 b	4.2 ab	16.3 ab	3.2 b	6.6 b
8 dSm^{-1}	25.7 bc	86.2 ab	76.4 b	190 bc	4.1 a-c	16.8 a	3.1 bc	6.3 b
10 dSm^{-1}	26.9 b	86.1 ab	58.0 b	169 bc	4.0 bc	16.3 ab	2.8 c	5.4 bc
12 dSm^{-1}	22.6 c	68.6 b	47.1 b	138 c	3.8 c	13.8 c	2.7 c	3.8 c
Level of significance	***	***	***	***	***	***	***	***

*, ** & *** represent level of significance @ 5%, 1% & 0.1% respectively; DFF= Days to first flowering, PH= Plant height, LA= leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Table 3. Mean performances of different morphological traits against six genotypes

Items	DFF	LA	ChlC	DMa	PH	NPB	NSB	LPB
BD-6950	46.9 a-c	23.2 a	60.79ab	107 a	68.8 b	5.2 ab	15.1 a	40.8 a
BD-7104	42.0 bc	18.9 ab	61.2 ab	101 b	60.9 b	4.5 b	10.3 bc	36.3 ab
BD-10115	40.1 c	19.8 ab	66.65 ab	102 ab	67.9 b	6.0 a	13.0 ab	33.8 b
JUN-536	48.3 a	22.2 a	62.4 ab	103 ab	83.1 a	5.8 a	12.9 ab	41.6 a
BJDH-12	45.5 a-c	20.0 ab	68.6 a	102 ab	65.3 b	5.6 a	11.3 ab	33.4 b
BARI Sarisha-14	47.5 ab	14.8 b	54.4 b	97.4 c	41.9 c	8.8 a	6.5 c	21.5 c
Level of significance	***	***	*	***	***	***	***	***
Items	NSiM	NSiP	NSiS	TSiP	SiL	NSSi	TSW	SYP
BD-6950	29.1 ab	100.9ab	108.5 a	240.5a	4.23 a	13.06 b	3.17 ab	7.71 a
BD-7104	28.2 b	81.5 b	83.2 ab	192.9 a	4.27 a	13.98 b	3.11 ab	7.26 a
BD-10115	28.4 ab	102.3 a	88.6 ab	217.4 a	4.16 a	13.3 b	3.18 ab	5.97 ab
JUN-536	32.2 a	122.5 a	91.0 ab	246.4 a	4.3 a	14.04 b	3.12 ab	7.74 a
BJDH-12	26.2 b	90.3 b	64.1 ab	180.6 a	4.02 ab	13.58 b	3.57 a	6.19 ab
BARI Sarisha-14	18.2 c	33.8 c	43.4 b	93.7 b	3.75 b	26.12 a	2.88 b	4.42 b
Level of significance	***	***	***	***	***	***	**	***

*, ** & *** represent level of significance @ 5%, 1% & 0.1% respectively; DFF= Days to first flowering, PH= Plant height, LA= leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Table 4. Principal component analysis (PCA) of selected morphological traits of rapeseed-mustard under different salinity treatments

Variables	PC1	PC2	PC3	PC4
Days to first flowering	-0.17	-0.42	-0.02	0.10
Plant height (cm)	0.32	-0.07	0.11	-0.01
Leaf area (cm^2)	0.22	0.10	0.34	-0.13
Chlorophyll content	0.10	-0.01	0.70	0.39
Days to maturity	-0.01	-0.57	-0.10	0.15
No. of primary branches	0.15	0.33	-0.02	0.62
No. of secondary branches	0.28	-0.20	-0.11	0.25
Length of primary branches (cm)	0.32	-0.12	-0.11	-0.16
No. of siliqua in main axis	0.30	-0.10	0.18	-0.08
No. of siliqua in primary branches	0.31	-0.18	-0.04	0.10
No. of siliqua in secondary branches	0.29	0.04	-0.33	0.16
Total no. of siliqua plant^{-1}	0.34	-0.06	-0.21	0.14
Length of siliqua (cm)	0.25	0.06	-0.04	-0.36
No. seeds sliquae^{-1}	-0.17	0.37	-0.30	0.27
1000-seed weight (g)	0.20	0.33	0.15	-0.26
Seed yield plant^{-1}	0.30	0.15	-0.22	-0.05
P (treatment)	<0.001	<0.001	0.696	0.899
P (genotype)	<0.001	<0.001	0.025	0.003
P (treatment × genotype)	0.116	0.002	0.045	0.025
Eigenvalue	7.95	2.16	1.16	0.97
% variation explained	49.7	13.5	7.3	6

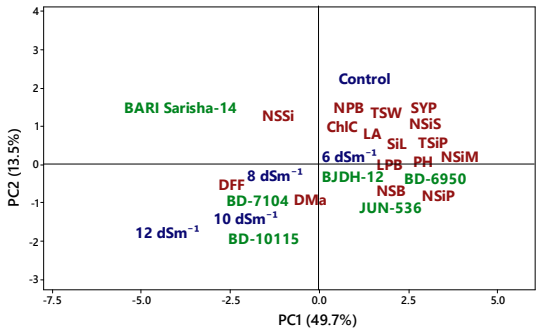

Fig. 2. Biplot of morphological traits along with treatments and genotypes. DFF= Days to first flowering, PH= Plant height, LA= Leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Correlation among morphological traits

Relationships among the sixteen morphological traits were studied through correlation analysis. Correlation coefficients were presented in the Table 5. In the present study out of 120 associations, 96 associations were significant but 24 associations were non-significant. Among the 96 associations, 62 associations were highly significant and the rest of the associations were significant. Plant height had highly significant associations with 11 traits whereas chlorophyll content and number of seeds siliquae^{-1} had no significant associations with other traits. There were 10 associations were highly significant but negatively correlated and rest of them were positively correlated (Table 5). And out of 34 significant associations, 10 associations were negatively correlated and surprisingly number of seeds siliquae^{-1} had the significant and negative association with most of the traits. Total number of siliqua plant^{-1} had strong and positive association with plant height, number of secondary branches, length of primary branches, number of siliqua in main axis, number of siliqua in primary branches and number of siliqua in secondary branches (Table 5). It was found that strong and positive correlation with highly significant association found in between seed yield plant^{-1} and plant height, length of primary branches, number of siliqua in main axis, number of siliqua in primary branches, number of siliqua in secondary branches, total number of siliqua plant^{-1} and siliqua length (Table 5).

Estimation of genetic parameters

Genotypic variance (GA), phenotypic variance (PV), heritability (h^2b), genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV), genetic advance (GA) and genetic advance as percentage of mean, GA (%) for all sixteen morphological traits were presented in Table 6.

Variability Parameters

A wide range of variation was observed among the five genotypes of rapeseed- mustard along with a check for sixteen morphological traits. The perusal of data showed that variances had inherent genetic difference among the genotypes. Significant variation in various components traits exhibited by the genotypes indicated that this genotype might be potential. Coefficient of variation indicated that phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits indicating that they all interacted with the environment to some extent. Among all the traits, number of siliqua in primary branches exhibited high estimates of genotypic coefficient of variation (GCV, 66.5%) and phenotypic coefficient of variation (PCV, 70.3%) followed by the number of seeds in secondary branches (PCV, 66.5%) and number of seed siliquae^{-1} (GCV, 65.4% and PCV, 66%). On the other hand, days to maturity (5.7% and 6.8%) and siliqua length (9.6% and 11.1%) showed low genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) (Table 6).

Heritability

The traits studied in the present investigation expressed to high heritability estimates ranging from 61.5% to 98.3% except 32.8% for chlorophyll content and 45.5% for 1000-seed weight (Table 6). Among the traits, number of seeds siliquae^{-1} showed high heritability (98.3%) whereas the lowest heritability was observed for chlorophyll content (32.8%) (Table 6).

Genetic advance

In the present study genetic advance (GA) was the highest for total number of siliqua plant^{-1} (195) and was the lowest for 1000-seed weight (0.52) (Table 6). The genetic advance as percentage of mean (GA %) was the highest for number of seeds siliquae^{-1} (133%) followed by number of siliqua in primary branches (129%) and was the lowest for days to maturity (10.1%) (Table 6).

Table 5. Correlation coefficient of sixteen morphological traits of six genotypes of rapeseed-mustard under different salinity treatments

Traits	DFF	PH	LA	ChlC	DMa	NPB	NSB	LPB	NSiM	NSiP	NSiS	TSi	LSi	SSi	TSW
PH	-0.29	1													
LA	-0.26	0.55	1												
ChlC	-0.08	0.34	0.27	1											
DMa	0.48	0.00	-0.14	-0.03	1										
NPB	-0.43	0.34	0.27	0.16	-0.27	1									
NSB	-0.23	0.67	0.43	0.25	0.27	0.28	1								
LPB	-0.29	0.84	0.46	0.18	0.08	0.17	0.74	1							
NSiM	-0.30	0.87	0.55	0.35	0.03	0.22	0.62	0.75	1						
NSiP	-0.26	0.87	0.45	0.20	0.22	0.38	0.78	0.81	0.77	1					
NSiS	-0.40	0.65	0.39	0.12	-0.07	0.32	0.74	0.71	0.59	0.62	1				
TSi	-0.37	0.83	0.48	0.20	0.07	0.36	0.84	0.85	0.77	0.87	0.93	1			
LSi	-0.33	0.66	0.40	0.13	-0.12	0.19	0.42	0.72	0.61	0.60	0.46	0.59	1		
SSi	0.12	-0.49	-0.32	-0.22	-0.36	0.14	-0.50	-0.48	-0.55	-0.58	-0.22	-0.44	-0.25	1	
TSW	-0.56	0.43	0.51	0.16	-0.29	0.32	0.30	0.40	0.35	0.35	0.36	0.40	0.41	-0.17	1
SYP	-0.43	0.73	0.51	0.12	-0.15	0.35	0.58	0.72	0.67	0.65	0.84	0.84	0.60	-0.21	0.58

Blue, orange and black colours respectively represent 1% level of significance, 5% level of significance and non-significance; DFF= Days to first flowering, PH= Plant height, LA= leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Table 6. Estimation of genetic parameters for morphological traits of six genotypes of rapeseed-mustard under different salinity treatments

Traits	PV (δ^2p)	GV (δ^2g)	PCV (%)	GCV (%)	h^2b (%)	GA	GA (%)
DFF	35.8	58.1	17.3	13.6	61.7	9.68	22.1
PH	701	774	42.3	40.3	90.5	51.8	78.9
LA	28.7	46.7	33.8	26.5	61.5	8.66	42.8
ChlC	58.4	177	21.1	12.1	32.8	9.03	14.3
DMa	34.8	49.1	6.8	5.7	71.0	10.3	10.1
NPB	1.17	1.76	23.6	19.2	66.3	1.82	32.3
NSB	31.3	43.5	56.5	48.0	71.9	9.77	83.9
LPB	203	237	44.0	40.7	85.8	27.2	77.8
NSiM	87.8	97.6	36.0	34.2	90.0	18.3	66.9
NSiP	3560	3978	70.3	66.5	89.4	116	129
NSiS	1436	3134	66.5	45.0	45.8	52.8	62.7
TSiP	11631	14981	61.1	53.8	77.6	195	97.7
SiL	0.16	0.22	11.1	9.65	74.6	0.71	17.0
NSSi	107.8	109	66.0	65.4	98.3	21.2	133
TSW	0.14	0.30	17.1	11.5	45.5	0.52	16.1
SYP	5.93	8.26	42.1	35.7	71.7	4.24	62.4

PV= Phenotypic variance, GV= Genotypic variance, PCV= Phenotypic coefficient of variation, GCV= Genotypic coefficient of variation, h^2b= Heritability in broad sense, GA= Genetic advance, GA (%)= Genetic advance in percentage of mean, DFF= Days to first flowering, PH= Plant height, LA= leaf area, ChlC= Chlorophyll content, DMa= Days to maturity, NPB= Number of primary branches, NSB= Number of secondary branches, LPB= Length of primary branches, NSiM= Number of siliqua in main axis, NSiP= Number of siliqua in primary branches, NSiS= Number of siliqua in secondary branches, TSiP= Total siliqua plant^{-1}, SiL= Siliqua length, NSSi= Number of seeds siliquae^{-1}, TSW= 1000-seed weight, SYP= Seed yield plant^{-1}

Discussion

Salinity effect on morphological traits

Five different salinity treatments (0 dSm^{-1}, 6 dSm$^{-1,}$ 8 dSm^{-1}, 10 dSm^{-1} and 12 dSm^{-1}) with five genotypes of rapeseed-mustard (BD-7104, BD-7104, BD-10115, JUN-536, BJDH-12) along with a check (BARI Sarisha-14) were used to evaluate the salinity effect on morphological traits. All the genotypes displayed considerable amount of difference in their mean performance with respect to all the mean sum squares for these characters, which indicated that, the genotypes under study were genetically diverse. An increasing trend was observed for days to first flowering and days to maturity but decreasing trend was observed for the rest of the traits except chlorophyll content and number of seeds siliquae^{-1} with the increase salinity levels (Table 2). It was obvious that salinity induced osmotic stress which adversely affect in the growth and development of the plant. Salinity stress caused prevention of water uptake, reduced the ability of plant cell to expand, reduced turgor pressure, decreased cell division that caused drastic effect on the growth and development of the plant especially in shoot morphology (Bybordi, 2010). Similar trends were reported by others (Maurya *et al.,* 2012; Bybordi, 2010; Ashraf, 1994).

Genotypic differences

A significant genotypic difference was found in all the traits in this experiment. Each of the traits showed significant difference with the morphological traits. The genotype like JUN-536 was accounted for higher mean in terms of days to first flowering, plant height, length of primary branches, number of siliqua in primary branches and seed yield plant^{-1} whereas the genotype BD-7104 had the better performance for days to first flowering, days to maturity, number of siliqua in main axis, seed yield plant^{-1} compared to rest of the genotypes (Table 3). The possible reason might be genotypic inherent properties influenced the morphological traits. Similar trends were found by others with different genotypes or varieties (Maurya *et al.*, 2012; Ashraf, 1994).

Trait associations

PC1 and PC2 collectively separated the genotype BD-7104 and BD-10115 for shorter days to maturity and days to first flowering at higher salt doses from 8 to 12 dSm^{-1} (Fig. 2). Correlation studies provided the information on the nature and extent of association between any two pairs of metric characters. Plant height had the most significant associations with all the traits except days to maturity and negative association with number of seeds siliquae^{-1}. Similar results were observed by Halder *et al.,* 2016; Singh *et al.,* 2014; Uddin *et al.*, 2013; Ara, 2010. Positive change in traits like plant height, length of primary branches, total siliqua plant^{-1} positively influenced the seed yield plant^{-1}. When traits having direct bearing on yield are selected, their associations with other traits were to be considered simultaneously as this would indirectly affect the yield.

Estimation of genetic parameters

Variability parameters

The genotypes showed wide range of variation which provides ample scope for selection of superior and desired genotypes by the plant breeder for further improvement. This suggested that there was inherent genetic differences among the genotypes (Table 6). Significant genetic variation in various traits exhibited by the genotypes indicated these traits might be effective for selection of suitable genotype for salinity tolerance. Phenotypic variances (PV) were higher than the genotypic variances (GV) for all the traits thus indicated the influences of environmental factor on these traits. Coefficient of variation indicated that the estimates of

phenotypic coefficient of variation (PCV) were higher than the corresponding genotypic coefficient of variation (GCV) for all the traits (Table 6) indicating that they all interacted with the environment to some extent. Low values of genotypic coefficient of variation indicated the need to create variability either by hybridization or mutation followed by selection. Similar results were reported by other researchers for example, Sikarwar *et al.,* 2017; Mekonnen *et al.,* 2014; Khan *et al.,* 2013; Jahan, 2008. Higher value of PCV and GCV for these traits might be suitable for selection of superior genotypes against salinity tolerance.

Heritability

The coefficient of variation does not offer the full scope of heritable variation. It can be determined with greater degree of accuracy when heritability in conjunction with genetic advance is studied. The estimates of heritability act as predictive instrument in expressing the reliability of phenotypic value. Therefore, high heritability helps in effective selection for a particular trait. All the traits studied in the present experiment expressed high heritability estimates ranging from 61.5 to 98.4% (Table 6) except for chlorophyll content and 1000-seed weight (g). High heritability value indicated that the traits under the study are less influenced by the environment in their expression and have greater possibility of genetic improvement by selection. Other researchers found similar results in their study but they did not found high heritability for all traits (Sikarwar *et al.,* 2017; Hussain *et al.,* 2016; Mekonnen *et al.,* 2014; Jahan, 2008; Mahak *et al.,* 2004). There might be difference in the traits for different treatment or environmental influences. But having similar result exhibited that these traits can be suitable and effective for the selection of particular traits that will be helpful in selecting superior genotype for salinity tolerance.

Genetic advance

The genetic advance (GA) is a useful indicator of the progress that can be expected as a result of exercising selection on the pertinent population and heritability in conjunction with genetic advance would give a more reliable index of selection value (Sikarwar *et al.,* 2017). The highest genetic advance (GA) was found in total siliqua plant^{-1} (195), number of siliqua in primary branches (116) whereas the lowest genetic advance was found for 1000-seed weight (0.52) (Table 6). The genetic advance in percentage of mean (GA %) was the highest for number of seeds siliquae^{-1} (133%) and the lowest for days to maturity (10.1%) (Table 6). Alam (2010) observed similar results like plant height, total siliqua plant^{-1} had high genetic advance and high genetic advance in percent of mean. Sikarwar *et al.* (2017) was also found similar result in *Brassica rapa*. Experiment conducted by Khan *et al.* (2013) with 32 genotypes in *Brassica rapa* including two commercially cultivated varieties as check suggested low genetic advance in percentage of mean for 1000-seed weight, number of secondary branches plant^{-1}, number of seeds siliqua^{-1} and siliqua length. Field experiment was conducted by Ghosh and Gulati (2001) with 36 genotypes of Indian mustard revealed that high genetic advance for oil content, harvest index, number of primary branches plant^{-1}, number of siliqua on main axis, main axis length and number of seeds pod^{-1}. Some other studies also reported similar results that justify these traits might be suitable for selection. The information on genetic variation, heritability and genetic advance helps to predict the genetic gain that could be obtained in later generation, if selection is made for improving the particular trait under study. In general the traits that show high heritability with high genetic advance are controlled additive gene action and can be improved through simple method. Selection for the traits having high heritability coupled with high genetic advance like number of seeds siliquae^{-1}, number of siliqua in primary axis, number of siliqua in secondary axis, total siliqua plant^{-1} and seed yield plant^{-1} are likely to accumulate more additive genes leading to further improvements for salinity tolerance.

Conclusion

Rapeseed-mustard is the most important oilseed crop in Bangladesh but its yield is much lower than the potential yield capacity. Salinity is one of the main reasons among the abiotic stresses for this. The genotype BD-7104 followed by BD-10115 found most suitable as salt tolerant genotypes that can be cultivated in saline prone areas to increase cultivable area in coastal zone. Genetic diversity that attributed these phenotypic diversities might be discovered through further intensive experimentation. The genotypes BD-7104 and BD-10115 can be utilized in breeding program for exploring salt tolerant QTL for developing sustainable and improved salt tolerant variety.

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