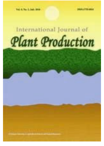

International Journal of Plant Production 11 (1), January 2017
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info
GUASNR

Response of different species of Brassica to water deficit

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Received 20 November 2015; Accepted after revision 17 July 2016; Published online 17 January 2017

Abstract

Development of drought-tolerant cultivars is hampered by a lack of effective selection criteria. In this research, response of 36 genotypes of rapeseed from seven species of Brassica including *B. napus, B. rapa, B. juncea, B. carinata, B. oleracea, B. nigra and B. fruticulosa* was assessed under three moisture levels (no stress, mild stress and intense stress) in the field during 2011-2012. Five drought-tolerance indices were calculated: stress tolerance (TOL), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI). Increasing water stress levels caused significantly more reductions in the seed yield of all species. Species *B. napus* had the highest yields in normal and intense stress conditions while *B. carinata* showed better performance in mild stress condition. Correlation coefficients revealed that among drought tolerance indices, GMP, STI and MP are superior criteria for selecting high-yield genotypes under stress and non-stress conditions. *B. napus* and *B. carinata* were the most drought tolerant while *B. rapa* and *B. oleracea* were the most susceptible species of Brassica based on yield potential. Application of principle component analysis (PCA) for distinguishing drought and susceptible species are discussed.

Keywords: Brassica; Soil moisture; Drought tolerance; Principle component analysis.

Introduction

Rapeseed is an important oilseed crop in the agricultural systems of many arid and semiarid areas regions (Chen et al., 2008; Ahmadi and Bahrani, 2009). It is one of the most important oilseeds in Iran where its yield is often restricted by water deficit and high temperatures during the reproductive growth (Mollasadeghi et al., 2011).

The family Brassicaceae has more than 3,000 species in 370 genera, a number of which have been brought into cultivation. Cultivated brassicas are represented by six interrelated species, three of which are diploids − *Brassica nigra* (2n = 16, genome BB), *B. oleracea* (2n = 18, genome CC) and *B. rapa* (2n = 20, AA) and three amphidiploid derivatives − *B. carinata* (2n = 34, BBCC), *B. juncea* (2n = 36, AABB) and *B. napus* (2n = 38, AACC). The latter three are derived by hybridization and polyploidization of two of the diploid taxa (Warwick, 2010). Over the past few decades, numerous efforts to widen the genetic basis of *B. napus* (AACC, 2n = 38) which is narrow when compared to *B. rapa* and *B. carinata* have been undertaken. It was demonstrated that a hexaploid hybrid (AABBCC), derived from an interspecific cross between *B. carinata* and *B. rapa* (AA, 2n = 20), could be used as a bridge hybrid (Li et al., 2006). By crossing the hexaploid hybrid with *B. napus*, it was possible to create a pentaploid hybrid (AABCC). This pentaploid hybrid was then used to create a variant of *B. napus* whose DNA comprised half the A genome of *B. rapa* and half the C genome of *B. carinata* (Li et al., 2006). The crossability between *B. carinata* and the three basic species of *Brassica* and *B. napus* is very low. Interspecific crosses involving *B. carinata* have been made, which have resulted in the transfer of some desirable traits from *B. carinata* to other *Brassica* species (Tonguc and Griffiths, 2004).

In crop breeding programs, wild relatives have been evaluated as genetic resources to develop new cultivars with biotic and abiotic stress resistance (Kaneko and Bang, 2014). Interspecific hybridization is a potential and useful method for transferring valuable traits between species of commercial interest in Brassica (Malek, 2007). It is also used to elucidate intergenomic relationships to develop synthetic amphidiploids and has been widely applied for improving Brassica (Malek, 2007). Navabi et al. (2010) produced interspecific hybrids between *B. carinata* (BBCC) and *B. napus* (AACC), using an advanced backcross approach to identify and introgress traits of agronomic interest from the *B. carinata* genome and to study the genetic changes that occur during the introgression process. Zhang et al. (2016) reported interspecific hybridization between *B. oleracea* and *B. rapa* using backcross progeny displayed extensive morphological variation, including some individuals that phenocopied subspecies other than their progenitors. Therefore it is possible to improve cultivated species of Brassica through introgression of useful genes from wild relatives.

Drought stress is the most important factor limiting crops production in many areas of the world. Drought is brought about when there is insufficient moisture for maximum or potential growth of crops (Blum, 2012). Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 2012). Loss of yield is the main concern of plant breeder under stress conditions. Thus, drought indices which provide a measure of drought based on loss of yield under drought-conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Khalili et al., 2012; Bahrami et al., 2014).

Fernandez (1992) classified plants (according to their performance in water-stress and stress-free environments) into four groups: genotypes with good performance in both environments (Group A), only in non-stress environments (Group B), in stressful environments (Group C); and genotypes with weak performance in both environments (Group D). Drought susceptibility of genotypes is usually estimated based on yield reduction under stress relative to yield under non-stress conditions (Fernandez, 1991; Blum, 2011). Several selection indices, which provide a measure of drought tolerance based on loss of yield under drought-conditions in comparison with normal conditions, have been suggested for screening drought tolerant genotypes (Clarke et al., 1992; Mitra, 2001). Fischer and Mourer (1978) proposed a stress susceptibility index (SSI) and showed that it is not independent of yield potential. Rosielle and Hamblin (1981) introduced a stress tolerance index (TOL) based on the differences in yields measured under non-stress (Yp) and stress (Ys) conditions. Genotypes with higher SSI and TOL values are considered less drought-tolerant. Rosielle and Hamblin (1981) defined mean productivity index (MP) as the average of Yp and Ys. But MP has an upward bias when there are larger differences between Yp and Ys. The geometric mean productivity (GMP), which is less sensitive to extreme values, is a better indicator than MP for separating superior genotypes in both stress and non-stress environments (Rosielle and Hamblin, 1981). Among stress tolerance indicators, larger values of TOL and SSI represent relatively more sensitivity to stress. Smaller values of TOL and SSI are favored. Selection based on these criteria favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. Genotypes with high STI are superior in performance under both stressed and non-stressed conditions. The geometric mean productivity (GMP) is often used by breeders interested in relative performance, since drought stress can vary in severity in field environments over years (Fernandez, 1992).

Sio-Se-Mardeh et al. (2006) reported that under moderate drought stress conditions, GMP, STI and MP were the most effective indices for identifying cultivars with high yields under both drought stress and non- stress conditions. Zebarjadi et al. (2011) reported correlation analysis in rapeseed genotypes indicated strong association between Yp, Ys, STI, MP and GMP. Also cluster analysis based on STI, MP, GMP and seed yield under both stressed and non-stressed conditions divided the genotypes into four groups. According to the results of principal component analysis, Yarnia et al. (2011) in some winter rapeseed cultivars found that STI and GMP are the two superior indices for identifying drought resistant cultivars. Majidi et al. (2011) in wild and cultivated safflower species revealed that geometric mean productivity (GMP) and stress tolerance index (STI) indices are superior criteria for selecting high-yield genotypes under stress and non-stress conditions. Shirani rad (2012) was observed that in rapeseed, STI is the most appropriate index for drought stress tolerance evaluation due to highest correlation with seed yield in both water and non-water stress conditions. Ebrahimiyan et al. (2012) was found a moderately high relationship between Yp and Ys in a tall fescue population using regression analysis, with a clear relationship in the mild stress condition.

Breeding for drought tolerance by empirical selection for yield is far from optimal, since this is a quantitative trait characterized by low heritability and high genotype × environment interaction. Therefore it is better to evaluate genotypes in different level of drought stress. On the other hand, although various management strategies have been proposed to cope with drought stress, but fast and reproducible screening techniques are rare. Little is known about drought tolerance of different Brassica species and application of drought and susceptibility indices for screening genotypes. Therefore we used 36 Brassica genotypes belonging to seven famous species of Brassica with different genome and ploidy levels to 1) evaluate drought tolerance of them in three different moisture conditions, 2) to assess the efficiency of different drought selection indices under water-stressed field conditions and 3) to identify relationships between indices and possibly identify some drought tolerant genotypes.

Materials and Methods

Experimental site and plant material

The experiment was conducted at the research farm of Isfahan University of Technology, in Lavark, Iran (40 km south-west of Isfahan; 32^0 32′ N, 51^0 23′ E; 1630 m asl), during 2011-2012. The soil was Typic Haplargid (USDA soil classification) (Lakzian, 1989) clay loam with a pH of 7.5. This region has an arid climate with four distinct climatic seasons. Mean annual precipitation was 140 mm and mean annual temperature was 15 °C. The plant materials included cultivars and accessions of *B. napus*, *B. juncea*, *B. carinata*, *B. oleracea*, *B. nigra*, *B. rapa* and *B. fruticulosa* species that were provided from the genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, United States Department of Agriculture (USDA), America and Seed and Plant Improvement Institute (SPII), Iran (Table 1). The genotypes were planted in the field at first of October of each year in three moisture environments. Irrigation treatments were applied after plant establishment.

Table 1. Brassica genotypes evaluated under normal and two drought environment conditions.

Num	Species	Code	Genotype	Origin
1	*Brassica napus*	B.N-1	Modena	Denmark
2	*Brassica napus*	B.N-2	Likord	Hungary
3	*Brassica napus*	B.N-3	RGS	Garmany
4	*Brassica napus*	B.N-4	S.L.M 046	Garmany
5	*Brassica napus*	B.N-5	Hayola-405	Canada
6	*Brassica napus*	B.N-6	Opera	Sweden
7	*Brassica napus*	B.N-7	Okapi	France
8	*Brassica napus*	B.N-8	Ella	France
9	*Brassica napus*	B.N-9	Lilian	France
10	*Brassica rapa.*	B.R.D-10	CR3421	Great Britain
11	*Brassica juncea*	B.J.J-11	CR2692	Soviet Union
12	*Brassica juncea*	B.J.J-12	CR2676	-
13	*Brassica juncea*	B.J.J-13	CR2630	Romania
14	*Brassica juncea.*	B.J-15	CR2496	Italy
15	*Brassica juncea.*	B.J-16	CR2476	Korea
16	*Brassica juncea*	B.J-I-17	CR3470	-
17	*Brassica carinata*	B.C.B-18	BRA927	Ethiopia
18	*Brassica carinata*	B.C.B-19	BRA1196	Ethopia
19	*Brassica carinata*	B.C.B-22	BRA1178	Ethiopia
20	*Brassica nigra*	B.N-27	CR2108	Greece
21	*Brassica nigra*	B.N.N-28	CR2724	-
22	*Brassica nigra*	B.N.N-29	CR2717	Italy
23	*Brassica rapa*	B.R.R-30	BRA2249	Sweden
24	*Brassica rapa*	B.R.O-31	CR2929	Germany
25	*Brassica rapa.*	B.R.C-32	BRA77	China
26	*Brassica fruticulosa*	B.F.F-34	BRA1810	Spain
27	*Brassica rapa*	B.R.C-35	BRA117	China
28	*Brassica juncea*	B.J.J-36	CR2695	Belgium
29	*Brassica olreace*	B.O.V-41		Hungary
30	*Brassica olreace*	B.O.G-44		Iran
31	*Brassica olreace*	B.O.A-45		Thailand
32	*Brassicaolreace*	B.O.C-47		Turkey
33	*Brassica olreace*	B.O.C-52		Iran
34	*Brassica olreace*	B.O.B-58		India
35	*Brassica rapa*	B.R.P-61		China
36	*Brassica rapa*	B.R.P-64		China

Experimental design and irrigation treatments

The 36 genotypes were evaluated under tree levels of irrigation including normal, mild and intense drought conditions (environments) using a randomized complete block design with three replications for each of environments. Under the full irrigation (normal environment), irrigation was non-limiting and supplied when 50% of the total available water was depleted from the root-zone. The deficit irrigation treatment (mild and intense stress environment) was irrigated when 70% and 90% of the total available water was depleted from the root-zone. Therefore, the irrigation interval (number of days between two irrigations) during the growing season was variable because evapotranspiration was variable. Soil samples were taken to determine the gravimetric soil-water content. Three samples were taken per plot at depths of 0–20, 20–40 and 40–60 cm for each water condition treatments. Samples were collected every second day between two irrigations and exactly one day before irrigation. The irrigation depth was determined according to the following equation (Walker and Skogerboe, 1987):

$$I = [(FC-\Theta)/100]\ D\times B \qquad \text{[Eq. 1]}$$

where I is irrigation depth (cm), FC is soil gravimetric moisture percent at field capacity, ϴ is soil gravimetric moisture percentage at irrigating time, D is the root-zone depth (60 cm) and B is the soil bulk density at root-zone (1.3 g cm^{-3}).

Drought and susceptibility indices

Five selection indices (SSI, Fischer and Mourer, 1978; STI, Fernandez, 1992; TOL, Rosielle and Hamblin, 1981; MP, Rosielle and Hamblin, 1981; GMP, Fernandez, 1992) were calculated based on the seed yield (g) under normal-mild and normal- intense stress conditions according to the following formulae:

1. Stress susceptibility index (SSI)
$SSI = (1-(Ysi/Ypi))/SI$ where $SI = 1- (Yms/Ymp)$

2. Stress tolerance index (STI)
$STI = [(Ypi) \times (Ysi) /(Ymp)^2]$

3. Tolerance index (TOL)
$TOL = Ypi-Ysi$

4. Geometric mean productivity (GMP)
$GMP = (Ypi \times Ysi)^{0.5}$

5. Mean productivity (MP)
$MP = (Ypi+Ysi)/2$

where Ysi designates the yield of the i^{th} genotype grown under stress conditions while Ypi designates that of the i^{th} genotype grown under normal conditions, Yms is the yield mean over all genotypes grown under stress conditions and Ymp is the yield mean over all genotypes grown under normal conditions.

Statistical analyses

An analysis of variances for calculated indices was performed for each of the moisture environments using SAS statistical program. The relationship between yield produced under both non-stress and drought-stress environments was calculated by linear regression analysis. The CORR-PROC of SAS was used to estimate correlation between indices. For specifying the drought tolerant genotypes with high yielding potential in both normal and stress environments, a 3-D diagram based on Y_P, Y_S and the best drought tolerance indices was drawn using Sigma plot ver11. Principal component analysis was performed to reduce the multiple dimensions of data space (Johnson and Wichern, 2007) using SAS and the biplot was drawn using Stat Graphics software.

Results

Combined analysis of variance over years and moisture environments (Table 2) indicated no significant difference between years for seed yield and most of seed components. Significant difference was found among the moisture environments for seed yield and seed yield components with the exception of pod length. The effect of genotype was also significant indicating considerable genotypic variation among the studied germplasm. As the difference between years was not significant, the mean data of two year was used for calculating drought tolerance and susceptibility indices and further analysis.

Table 2. Combined analysis of variance for yield and some yield components traits of rapeseed genotypes grown in moisture conditions and two years.

Variation sources	Mean square				
	Seed yield	1000-seed weight	Pods per plant	Branches per plant	Pod length
Year (Y)	558742.539^{ns}	6.783^{ns}	276656.411^{ns}	18.962*	8.569^{ns}
Moisture conditions (M)	3005.321**	0.344*	1729.700*	9.280*	0.5373^{ns}
Y × M	3131.437^{ns}	0.0555^{ns}	6745.411^{ns}	4.425*	0.1152^{ns}
Error 1	1308.021	0.2135	1052.119	1.002	0.2232
Genotype (G)	1255.711*	0.38325^{ns}	2978.626^{ns}	2.8789*	0.4293*
G × Y	1264.836^{ns}	0.2763^{ns}	2932.282^{ns}	2.265*	0.7455**
G × M	4966.180*	0.1104*	5718.845*	1.5741*	0.2413*
G× Y× M	4971.525^{ns}	0.1600*	5267.675^{ns}	1.6079*	0.1811^{ns}
Error 2	1657.1249	0.261186	54784.38	5.40406250	0.34518

* and **: Significant at the 0.05 and 0.01 probability levels, respectively.

Cultivated species, *B. napus* (YP =228.2 g/plant) had the highest yields in normal conditions (Table 3). Under mild moisture conditions, cultivated species of *B. carinata* had the highest yield (158.8 g/plant) while *B. napus* showed better yield performance (122.8) in intense stress (Tables 3 and 4). The species *B. fruticulosa* species accounted for the lowest average yield in the mild stress condition while this species overall destroyed in intense stress condition.

Table 3. Average yields and drought tolerance indices under optimal (YP) and mild stress (YS) conditions in brassica genotypes.

Species	YP(g/m^2)	YS (g/m^2)	TOL	MP	GMP	SSI	STI
B. napus	228.2±19.2	146.5±12.3	82.6	187.5	182.8	1.08	0.98
B. rapa	147.9±10.2	104±10.8	43.2	124.3	121.01	0.87	0.45
B. juncea	190.3±17.3	139.8±14.6	50.5	164.8	163.1	0.79	0.77
B. carinata	209.8±24.7	158.8±19.3	51	184.3	182.5	0.73	0.97
B. oleracea	149.33±11.5	111.1±8.6	38.3	130.1	128.61	0.77	0.48
B. nigra	183.5±22.3	131.1±22.9	52.3	157.3	155.1	0.85	0.70
B. fruticulosa	182±18.6	71±9.6	111	126.5	113.6	1.83	0.37

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index.

Table 4. Average yields and drought tolerance indices under optimal (YP) and intense stress (YS) conditions in brassica genotypes.

Species	YP(g/m^2)	YS (g/m^2)	TOL	MP	GMP	SSI	STI
B. napus	228.2±19.2	122.8±10.8	106	175.8	167.6	0.99	0.82
B. rapa	147.9±10.2	80.5±9.3	67.2	114.1	109.1	0.97	0.34
B. juncea	190.3±17.3	99.6±22.2	90.7	145	137.7	1.02	0.55
B. carinata	209.8±24.7	107.3±18.9	102.5	158.5	150	1.04	0.66
B. oleracea	149.33±11.5	76.8±6.8	72.5	113	107.1	1.03	0.33
B. nigra	183.5±22.3	102.6±11.3	81	143	137.1	0.94	0.55
B. fruticulosa	182±18.6	-*	-	-	-	-	-

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index.

* Under intense drought stress, B. fruticulosa did not have seed yield.

Linear regression analysis (Figure 1) revealed a positive relationship between yield of genotypes under normal conditions and their yield under stress conditions, with a larger coefficient of determination in mild stress ($R^2 = 0.41$) than in intense stress ($R^2 = 0.36$). Correlation coefficients were calculated between Yp, Ys and other indices of drought tolerance for genotypes in both environments (Table 5). There were positive and significant correlations for Yp and Ys with MP, GMP and STI. It can therefore be suggested that MP, GMP and STI were good predictors of yield under both stress and non-stress conditions. The correlation coefficient between SSI and Ys was negatively significant in both moisture stress conditions. Also no correlation was found between Yp and SSI in both environments (Table 5).

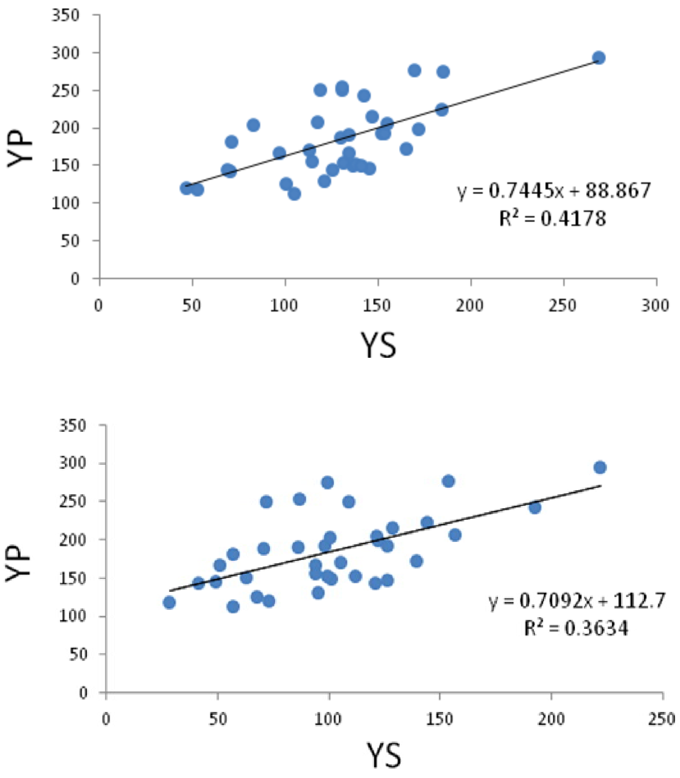


Figure 1. Relationship between yield produced under non-stress and drought stress: (a) mild drought stress; (b) intense drought stress.

Table 5. Correlation coefficients between YP, YS and drought tolerance indices for mild moisture stress (above diameter) and intense moisture stress (below diameter) in brassica genotypes.

Traits	YP	YS	SSI	STI	TOL	GMP	MP
YP	1	0.46*	0.24^{ns}	0.86**	0.42*	0.85**	0.90**
YS	0.56**	1	-0.69**	0.78**	-0.36*	0.82**	0.76**
SSI	0.08^{ns}	-0.74**	1	-0.20^{ns}	0.88**	-0.28^{ns}	-0.17^{ns}
STI	0.82**	0.90**	-0.41*	1	0.17^{ns}	0.98**	0.98**
TOL	0.43*	-0.27^{ns}	0.79**	0.11^{ns}	1	0.12^{ns}	0.24^{ns}
GMP	0.71**	0.84**	-0.46*	0.87**	0.04^{ns}	1	0.99**
MP	0.90**	0.85**	-0.32^{ns}	0.97**	0.25^{ns}	0.87**	1

ns, * and ** not significant and significant at the 5% and 1% levels of probability, respectively.

The best performing genotypes for both stress and non-stress environments were identified in the 3-D plots which were created among Yp (y-axis), Ys (x-axis) and for the best indices (MP, GMP and STI as z-axis). Similar results were obtained for these three indices; therefore, only the results for STI are presented in Figures 2 and 3 for mild and intense drought stress, respectively. Most of the genotypes, in both environment, were located in group A (genotypes that performed highly in both stress and non-stress environments). Based on the 3-D plots on average of species (Figures 4 and 5), it is possible to select the stable species and identify superior (high yielding) species for each environment. For example results showed that under mild stress species

B. napus, *B. carinata* and *B. juncea* were located in group A while under intense stress only the species *B. napus* and *B. carinata* were located in this group. Species *B. nigra* was located in group C under both mild and intense stress conditions (Figures 4 and 5). Among the studied species, *B. friticulosa*, *B. oleracea* and *B. rapa* had the lowest value of STI and were identified as the least stable and productive species (Figures 4 and 5).

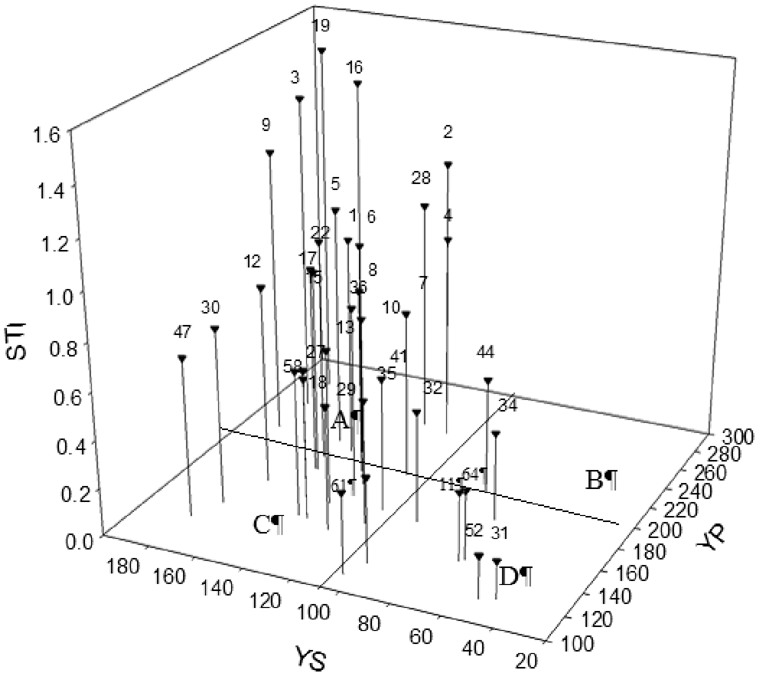


Figure 2. 3-D diagram for specifying the drought tolerance genotypes based on YP, YS and GMP index for mild moisture stress condition. The names of brassica genotypes are according to the code of table 1.

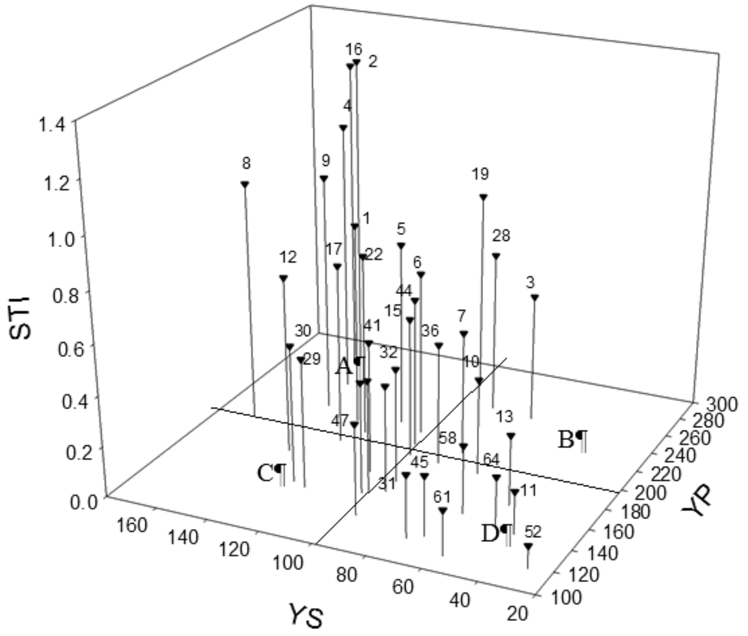


Figure 3. 3-D diagram for specifying the drought tolerance genotypes based on YP, YS and GMP index for intense moisture stress condition. The names of brassica genotypes are according to the code of table 1.

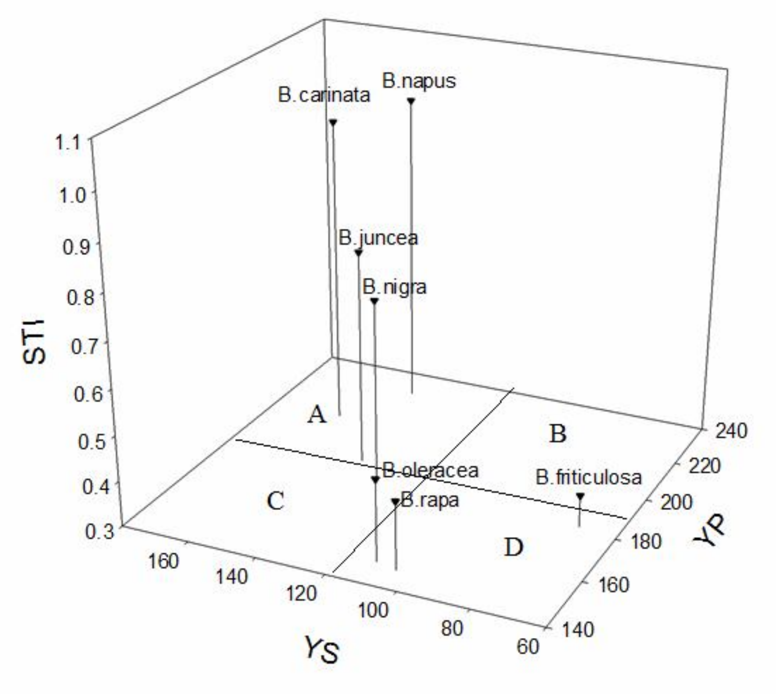


Figure 4. 3-D diagram for specifying the drought tolerance of brassica species based on YP, YS and GMP index for mild moisture stress condition.

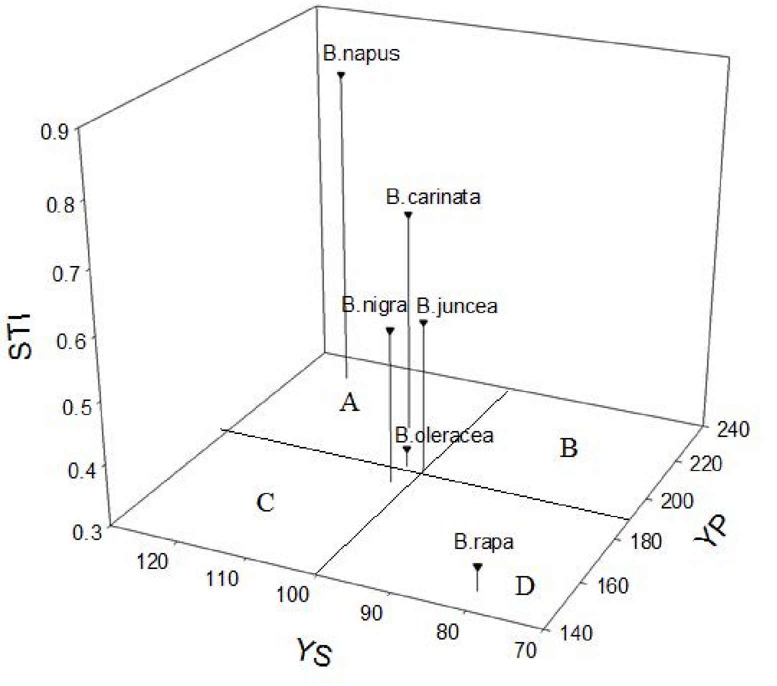


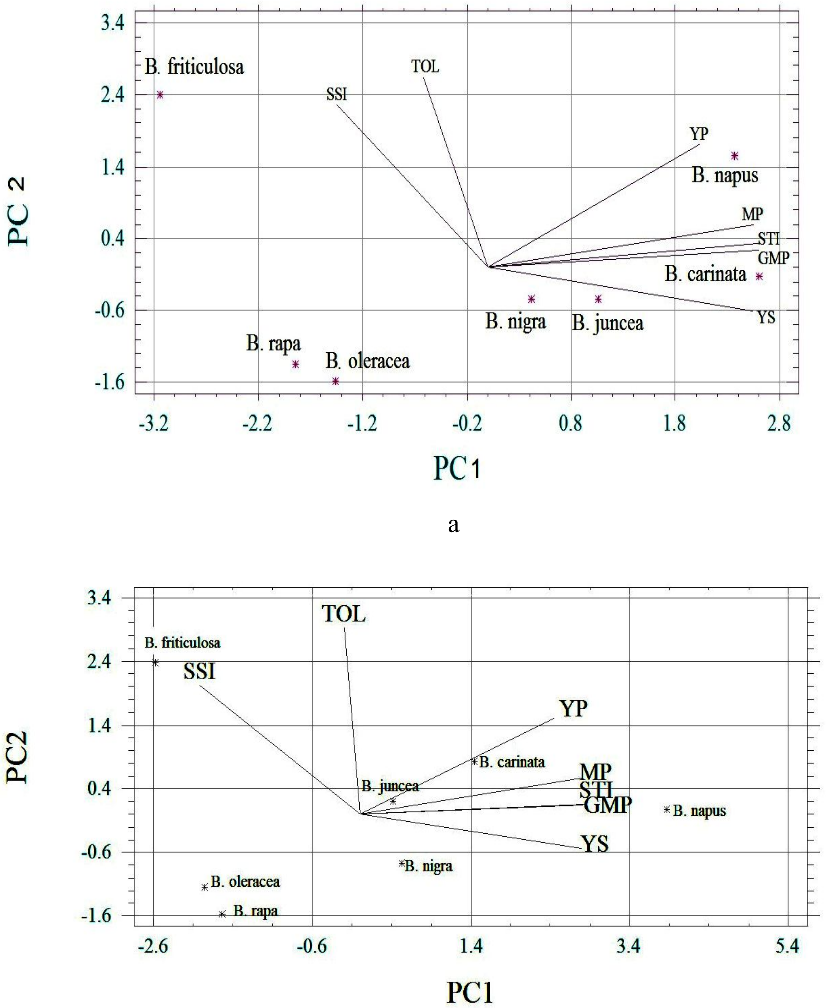
Figure 5. 3-D diagram for specifying the drought tolerance of brassica species based on YP, YS and GMP index for intense moisture stress condition.

Principal component analysis (PCA) was performed on the basis of all measured indices (Table 6) and then genotypes were subjected to biplot analysis for clear determination of species (Figure 6). The first and second principal components (PC1, PC2) explained 99% of the total variability in both environments (Table 6). PC1 had a higher correlation with GMP, MP, Yp, Ys and STI. Thus, the first dimension (PC1) could be named as the yield productivity potential and drought tolerance stress. Considering the high and positive value of this PC on the biplot, selected species will be high yield and tolerance under stress and non-stress environments. The second component (PC2) had positive correlations with TOL and SSI and negative correlations with STI and GMP. Therefore PC2 was named a stress susceptibility dimension and separated the stress-tolerant genotypes from susceptible ones (Table 6). Species with high PC1 (high productivity and tolerance) and low PC2 (low susceptibility) are suitable for both stress and non-stress environments. The species–trait biplots for the mild stress of species (Figure 6a) indicated that species *B. napus* and *B. carinata* had very high PC1 and moderate PC2, therefore were identified as a superior species for both normal and drought-stress conditions. The results of biplot analysis for the intense stress (Figure 6b) indicated that species *B. napus* had high yield and moderate susceptibility. On the other hand species *B. fruticulosa* was recognized as a species with high susceptibility (high PC2) and low productivity (low PC1) in both mild and intense stress. Species *B. oleracea* and *B. rapa* showed very low susceptibility (low PC2) and productivity (low PC1) under mild and intense stress conditions.

Table 6. Principal component loadings for the traits measured on brassica genotypes for mild and intense moisture stress.

Trait	Mild stress		Intense stress	
	PC1	PC2	PC1	PC2
YP	0.49	0.42	0.47	0.46
YS	0.48	-0.67	0.47	-0.27
SSI	-0.04	0.21	-0. 06	0.31
STI	0.46	-0.13	0.50	-0.11
TOL	-0.06	0.58	0.05-	0.83
GMP	0.51	-0.19	0.54	-0.22
MP	0.50	0.13	0.49	0.04
Eigen value	5.04	1.87	5.38	1.57
Cumulate percentage	0.76	0.99	0.81	0.99

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean.



a

b

Figure 6. The species by trait biplots for mild (a) and intense (b) moisture stress in brassica genotypes. The traits are spelled out in capital letters. YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index.

Discussion

Knowledge of genetic diversity in the primary gene pool of crop plants is very important from a plant breeding and genetic point of view (Annisa, 2011). Also insufficient variation, low heritability and lack of effective selection approaches for drought tolerance have limited the development of resistant crop cultivars (Kirigwi et al., 2004). Genetic variation is an essential prerequisite for any crop improvement program. Large genetic variation was observed for drought tolerance among the genotypes and species assessed in three environments used in our research, indicating that selection in this germplasm may be useful. Results of this study indicated that susceptibility indices (SSI and TOL) could not distinguish genotypes in each moisture environments. Therefore, these indices are not suitable to select drought-tolerant genotypes. But the differences among genotypes for tolerance indices (STI, MP and GMP) were highly significant for both environments. Thus, selection based on tolerance indices such as STI and GMP may lead to genotypes with high performance (yield) as well as high drought tolerance. Plants which produce high yield under both stress and non-stress conditions are desirable. This is in agreement with results of Ebrahimiyan et al. (2012) and Majidi et al. (2011) in tall fescue.

Theoretically, if the relationship between yield under stress (Ys) and non-stress (Yp) conditions is unity ($R^2=1$), then the selection indices are not necessary because the high-performing genotypes in normal conditions are also superior in stress conditions. When this correlation is reduced, the necessity of selection based on indices will increase. In this study there was a positive and collinear relationship between Ys and Yp (Figure 1) with a moderate coefficient of determinations ($R^2 = 0.41$ and $R^2 = 0.36$ for mild and intense stress, respectively). Although this positive relationship shows that genotypes with high yield in non-stress conditions generally are superior under stress conditions, selection based on indices is expected to be more efficient due to the moderate R^2 values.

To choose the most desirable indices to select drought-tolerant genotypes, some researchers have used the correlation coefficient of each index with Yp and Ys (Sio-Se- Mardeh et al., 2006; Azizi Chakherchaman et al., 2009; Zebarjadi et al., 2011; Yarnia et al., 2011; Majidi et al., 2011; Ebrahimiyan et al., 2012). The results of our research indicated that in each moisture environment, the indices MP, GMP and STI had high correlation coefficients with yield under non-stress and stress conditions (Table 5), indicating that they can be used for selecting for drought tolerance in Brassica. These three indices (MP, GMP and STI) were also reported as the most desirable drought-tolerance indices by other workers (Farshadfar and Sutka, 2002; Soorninia et al., 2012; Ebrahimiyan et al., 2012; Shirani rad, 2012). Fernandez (1992) reported that MP has an upward bias when there are larger differences between Yp and Ys; therefore, we suggest STI and GMP be considered the most useful in selection for drought tolerance. In order to separate group A genotypes from the other groups, the 3-dimensional plot among YS, YP and one of the best indices was useful. This graphs classified genotypes and species in four groups and showed that there are considerable variations among genotypes and species. This method can be used to effectively distinguish high-yielding genotypes in both stress (mild and intense) and non-stress environments. Most of the genotypes in the present study were located in groups A in mild stress condition while in intense stress condition most of the genotypes were located in groups A and D. Results indicated that species *B. napus*, *B. carinata* and *B. juncea* were the most tolerant species under mild drought stress. Under intense drought stress condition the species *B. napus* and *B. carinata* were identified as the superior species. Among the studied species, *B. friticulosa*, *B. oleracea* and *B. rapa* had the lowest value of STI and were identified as the least stable and productive species.

Principle component analysis (PCA) was performed on the basis of all attributes (Table 6) and species were subjected to biplot analysis for assessing the relationships between all of attributes at once and their comparisons in each stress intensity (Figures 6a and 6b). A similar pattern in both drought environments was observed. PCA revealed that the first component explained 76% and 81% of the variation in mild and intense stress, respectively. The first component (PC1) had higher correlation with Yp, Ys, MP, GMP and STI. Therefore, PC1 was named the 'yield productivity potential and drought tolerance stress.' The second component (PC2) explained 23% and 18% of the total variability in mild and intense stress, respectively and the second component (PC2) had a higher correlation with TOL and SSI in both stress environments and was named the 'susceptibility component.' Based on these two components and according to the distribution of species on biplots, superior species were identified in each stress environment. Results of PCA indicated that species *B. napus* and *B. carinata* are superior species for both normal and mild drought-stress conditions. On the other hand species *B. fruticulosa* was recognized as a species with high susceptibility and low productivity in both mild and intense stress. Species *B. oleracea* and *B. rapa* showed very low susceptibility and productivity under mild and intense stress conditions.

Some species had different behavior from mild stress to intense stress, indicating a significant stress genotype interaction. Biplots may also be used for identifying contrasting genotypes when planning mapping populations for genome studies of drought tolerance in Brassica. Using principle component and biplot for grouping varieties and species have been reported by many researchers (Houshmand et al., 2005; Golabadi et al., 2006; Majidi et al., 2011; Soorninia et al., 2012; Ebrahimiyan et al., 2012).

In conclusion, the results of this study show that large genetic variation exists for drought tolerance among genotypes and species of Brassica. This indicates that selection in this germplasm can be successful. The moderate relationship between yield under stress and non-stress conditions indicated that selection based on indices STI and GMP seems a suitable approach in Brassica. Results indicated that among the wild studied relatives of Brassica, species *B. carinata* and *B. juncea* had high drought tolerance based on drought tolerance and susceptibility indices. Species *B. fruticulosa* was recognized as the most sensitive species. The cultivated species of *B. napus* is an amphiploid originated from two wild relatives *B. rapa* (2n=20, AA) and *B. oleracea* (2n=18, CC) which had the lowest tolerance in both mild and intense moisture conditions. This indicates that interaction between genome CC with AA (resulted to *B. napus*, AACC) and CC with BB (resulted to *B. carinata*, BBCC) caused better performance in yield potential and drought tolerance. Wild Brassica germplasm offers numerous future prospects for agronomic and economic traits and new crops resistant to biotic and abiotic stresses. Some traits can be introduced through artificial hybridization, while traits arising from more distant sources will need to be isolated and introduced through transgenic means (Warwick, 2010). However physiological aspects of drought tolerance is need to clarify the mechanism of drought tolerance in these species.

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