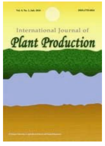

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Physiological traits related to drought tolerance in *Brassica*

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Abstract

Physiological basis of genetic variability in drought response and its association with yield and related indices is not clear in *Brassica*. In this study 36 accessions belonged to seven species of *Brassica* were evaluated under normal, moderate and severe stress environments. Physiological traits along with seed yield, seed yield components, oil content and two selection indices (stress tolerance index, STI and drought susceptibility index, DSI) were studied. Moderate and intense stress caused reduction in seed yield and the most studied traits. Based on the STI, *B. carinata* and *B. juncea* were identified as the superior species in moderate stress condition while *B. oleracea* was the most tolerant under intense stress. Moderate drought stress significantly increased the ratio of chlorophyll a to chlorophyll b (Chla/Chlb) while severe stress decreased it. Although relative water content (RWC) had positive correlation with STI, its heritability was low. Chlorophyll content (TChl) was associated with STI and had moderate heritability. Positive correlation was found between proline content and DSI under both stress conditions. Results showed large variation is among studied species for drought tolerance and related traits indicating that selection in this germplasm would be useful. Changes in chlorophyll content can be recognized as a key component affecting drought tolerance in *Brassica*.

Keywords: *Brassica*; Moisture stress; Chlorophyll content; Leaf praline; Genetic association.

Introduction

The family *Brassicaceae*, are considered as one of the ten most economically important plant families (Warwick et al., 2006). Amongst crops, the genus *Brassica* is contains some 100 species such as rapeseed (*Brassica napus* L.), mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.) and turnip rape (*Brassica rapa* L.) that are mainly grown for oil, condiments, vegetables or fodder (Ashraf and McNeilly, 2004; Hosaini et al., 2009). There are also many wild relatives that possess number of useful agronomic traits such as male sterility, resistance to disease and pests, tolerance for cold, salty and drought conditions which could be incorporated into breeding programs (Warwick, 1993). Generally, *Brassica* species has been developed in the areas with high rainfall and performs poorly in the areas with low rainfall (Resketo and Szabo, 1992;

Richards, 1978). Growth and seed yield production of *Brassica* species have greatly decreased owing to drought conditions. This situation can be alleviated by an approach combining water storage and irrigation, crop management and plant breeding. There is great interest in breeding stress-tolerant varieties, since significant inter- and intraspecific variation for drought and salinity tolerance exists within *Brassica*, which needs to be exploited through selection and breeding.

Drought tolerance is a complex trait controlled by numerous genes (Blum, 2005; Pinto et al., 2010). Also, plant responses to water deficit stress are confounded by several factors such as time, intensity, duration and frequency of stress as well as by plant, soil and climate interactions (Reynolds and Tuberosa, 2008). In addition, the difficulty to establish well-defined and repeatable water stress conditions makes screening of drought tolerant genotypes more complex (Ramirez and Kelly, 1998). Therefore different indicators should be used for the phenotyping of drought tolerance (Tuberosa, 2012). Presently, a number of selection indicators, such as stress tolerance index (STI), water-use efficiency (WUE), drought susceptibility index (DSI), relative vigor index (RVI) and leaf wilting index (LWI), are widely used in research and breeding practices for identification of genotypes which produce high yield under both stress and non-stress conditions. However plants respond and adapt to drought stress by the induction of various morphological and physiological responses (Wang and Huang, 2004). Many physiological factors could be involved in the drought stress injury (Jiang and Huang, 2001) which may promise for characterizing drought resistance in screening studies. For example, water stress can be caused increase of proline content, stomata close and photosynthesis inhibit. Also, water stress induced a significant decrease and increase in chlorophyll contents and accumulation of proline in *Brassica* crops, respectively (Gibon et al., 2000). All these abnormalities as a result overall ultimately are decreased production of crop. Maliwal et al. (1998) have reported reduced yield in *Brassicas* in response to water stress. Kumar et al. (1984) and Singh et al. (1985) have reported close associations between osmotic adjustment and both stomatal conductance and canopy temperature in many *Brassica* species. Keles and Oncel (2004) suggested that the high relative water content is closely related to drought resistance. Result of a study shows that with increasing drought stress, amount of relative water content is reduced (Sepehri and Golparvar, 2011). Din et al. (2011) reported significant differences among the various canola genotypes for leaf chlorophyll a, chlorophyll b and proline accumulation. Previous studies reported that STI, MP, GMP and MSTI are useful indices for screening drought tolerant rapeseed genotypes under stress condition (Malekshahi et al., 2009; Shirani-rad and Abbasian, 2011; Yarnia et al., 2011; Khalili et al., 2012), however the association of these indices with physiological and morphological traits was not assessed.

Knowledge of genetic association between selection indices, yield and morpho-physiological traits can be useful to improve the efficiency of breeding programs in deficit irrigation conditions. The objectives of this study were to measure the genetic variability of specific agronomic and physiologic traits of *Brassica* species under non-stress, moderate and intense water stress conditions and to evaluate the association of these traits with drought tolerance and susceptibility indices (STI and DSI).

Material and Methods

Experimental site and plant material

The field experiment was carried out at research farm of Isfahan University of Technology, Najafabad, Iran (40 km south west of Isfahan, 32° 32' N and 51° 23' E, 1630 m asl) during 2011 and 2012 growing seasons. This location has a typic Haplargid clay loam soil with the average bulk density of 1.48 g/cm^3 in the top 60 cm soil surface. The mean annual precipitation and mean annual temperature were 140 mm and 15 °C, respectively. Summers are dry and there is usually no rain from end of May to mid of October.

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).

Table 1. Name and country of origin of the *Brassica* genotypes used in the experiment.

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

Experimental design and irrigation treatments

Plant materials were evaluated in a randomized complete block design following two factorial arrangements (genotype and irrigation) with three replications in 2011 and 2012. Genotypes were sown in early October in each year. The two factors used were the accessions and irrigation treatments including a full irrigation (normal irrigation condition) and two levels of deficit irrigation (moderate and sever stress conditions). Each plot consisted of 6 rows, 2 m long with 30 cm apart. Plants in normal, moderate and severe experiments were irrigated when 50%, 70% and 90% of the total available water was depleted from the root zone, respectively. The irrigation intervals during the growing season and between the three irrigation treatments were variable upon weather conditions. Soil moisture was measured based on standard gravimetric methods (Clarke et al., 2008) at depths of 0-20, 20-40 and 40-60 cm. Soil 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 (Keller and Blienser, 1990).

$$I = [(FC-\Theta)/100]\ D\times B$$

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}).

Measurements

Agro-morphological traits including number of branches per plant (BP), number of pods per plant (PP), 1000-seed weight (TSW), seed yield per plant (SY) (g/plant) and oil content (%) were measured at the harvest maturity stage of the crops. The measurements were done using 20 randomly selected plants per plot. For oil content, five grams of powdered seeds were extracted for oil in the Soxhlet apparatus, using petroleum ether as solvent for 6 h according to the AOCS method (AOCS, 1993) and then oil content percentage was calculated for each sample.

Total chlorophylls (TChl), chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoids (Car) were determined spectrophotometrically using 80% acetone as a solvent (Lichtenthaler and Buschmann, 2001). Then the ratio of chlorophyll a to chlorophyll b (Chla/b) and ratio of total chlorophyll to carotenoids (Tchl/Car) were calculated. Proline was determined by the ninhydrin method described by Bates et al. (1973). Leaf water status was determined by measuring relative water content (RWC). RWC was obtained by the method of Weatherley (1950). Fresh leaves were taken from each genotype and weighted immediately to record fresh weight (FW). They were floated in distilled water for four hour and then weighted again to record turgid weight (TW). The leaves were dried in the oven at 60 °C for 24 hour and then dry weights (DW) obtained. Later, the fresh weight (FW), TW and DW were used to calculate RWC using the following equation:

$$RWC = [(FW–DW) / (TW–DW)] \times 100$$

Selection indices of stress tolerance index (STI) and drought susceptibility index (DSI) were calculated based on the seed yield under stress and normal conditions using the following relationships (Fernandez, 1992; Dencic et al., 2000).

STI = (Ys × Yp) / Ymp

DSI = [1 – (Ys / Yp)] / [1–(Yms / Ymp)

In the above formulas, Y_s, Y_p, Y_{ms} and Y_{mp} are the seed yield of the i^{th} genotype in the stress condition, the seed yield of the i^{th} genotype in the normal condition, the seed yield mean over all genotypes in the stress condition and the seed yield mean over all genotypes in the normal condition, respectively.

Statistical analysis

Analysis of variance was carried out to determine differences among treatments and genotypes for each variable using the general linear model (GLM) of SAS (SAS Institute 2001). The means comparisons were conducted using least significant difference (LSD) test. Broad sense heritability estimates on an entry mean basis were calculated (Hallauer and Miranda 2010). Simple correlation coefficients between seed yield and studied traits were estimated to determine the association between traits using proc COR of SAS software. The genetic correlations between traits were calculated from the variance and covariance components using following equations:

$$r_{g(xy)} = S_{g(xy)} / (S_{g(x)} S_{g(y)})$$

where $r_{g(xy)}$, $S_{g(xy)}$, $S_{g(x)}$ and $S_{g(y)}$ are the genotypic correlation between traits X and Y, the genotypic covariance between traits X and Y, root of genotypic variance of trait X and root of genotypic variance of trait Y, respectively.

Stepwise multiple linear regressions was used according to Montgomery (2006) to determine the variables accounting for the majority of total seed yield variability using SPSS software (SPSS Inc 2001). Principal component was carried out to reduce the multiple dimensions of data space using SAS packages (Johnson and Wichern, 2007) and the biplot was drawn using StatGraphics (Statgraphics, 2007).

Results

Combined analysis of variance over years and environments (different moisture conditions) indicated no significant difference between years (Data not shown) therefore the mean data of two years was used for further analysis. Significant difference was found among the moisture environments for all of the traits. The effect of genotype was also significant indicating significant genotypic variation among the studied germplasm (Data not shown). Moderate and severe stress significantly reduced SY in studied species (Table 2). Moderate stress significantly decreased TSW compared to non-stress condition especially in the species of *B. napus*, *B. rapa* and *B. oleracea* species. TSW of all *Brassica* species was significantly decreased under severe stress compared to non-stress condition. Moderate and severe stress caused a significant decrease in PP of all studied species compared to non-stress condition. Moderate stress decreased BP of *B. rapa*, *B. carinata*, *B. oleracea* and *B. fruticulosa* while it did not affect BP of *B. napus*, *B. juncea* and *B. nigra*. BP significantly was reduced under severe stress compared to non-stress condition. Drought stress increased proline contents in leaves of all *Brassica* species except *B. carinata* species under moderate stress condition (Table 2). Chla content decreased in all *Brassica* species expect *B. carinata* and *B. oleracea* under moderate stress condition while it reduced in all *Brassica* species under severe stress condition. Moderate stress caused decreases in Chlb content of all *Brassica* species expect *B. oleracea*. Chlb of *B. oleracea*, *B. carinata* and *B. nigra* were decreased under severe stress condition but non-significant different was observed for other studied species. Moderate stress significantly decreased accumulation of Car content in *B. napus*, *B. juncea* and *B. fruticulosa*, while accumulation of Car content significantly reduced in all studied species under severe stress condition. TChl content significantly reduced in all species under both moderate and intense stress conditions. The ratio of Chla/Chlb declined remarkably in *B. juncea* and *B. fruticulosa* under moderate and severe stress conditions while non-significant different was found in the raito of TChl /Car under moderate and stress conditions. Oil content of all studied species except *B. rapa* decreased under both stress conditions (Table 2).

To compare the variation among various traits genetic coefficient of variation (GCV), phenotypic coefficients of variation (PCV) and broad sense heritability (h^2) were calculated for normal, moderate and severe stress conditions (Table 3). The highest GCV and PCV were obtained for BP in non-stress, moderate and severe stress conditions. Also, the lowest GCV and PCV were observed for Car content. TSW (84%) and RWC (7%) had the highest and lowest broad sense heritability, respectively. Since TSW has high heritability, selection might be effective for breeding this trait. However genetic variation for RWC was low and direct selection may not be useful for improving it.

Correlation coefficients between different traits with drought susceptibility index (DSI) and stress tolerance index (STI) are presented in Table 4. There was no significant correlation between STI and DSI. STI had significant positive correlation with SY, but drought susceptibility index (DSI) was not significantly correlated with SY under moderate and severe drought stress conditions. Positive correlation were found between most photosynthetic pigments (Chlb, TChl and Car content) and RWC with STI under intensive stress conditions whereas under moderate stress condition only TChl and RWC were correlated with STI. Positive correlation was found between proline content and DSI under both stress conditions (Table 4).

Correlation coefficients of different traits under different moisture conditions are presented in tables 5 and 6. Seed yield had significantly positive correlation with TSW, PP, PB and TChl whereas its correlation with oil content was negative. Under moderate stress condition, SY was positively correlated with TSW, PP, PB, TChl content, Proline content, Car content, RWC and ratio of TChl/car but it had significantly negative correlation with oil content. Under severe stress condition, significantly positive correlation was observed between SY with TSW, proline content, TChl content and RWC. There was no significant correlation between RWC and proline content.

The relationships between TChl content and STI under moderate and severe drought stress were presented in Figure 1a and 1b, respectively. Results showed that no relationship was found between TChl content and STI under moderate stress condition while negative relationship was observed under severe stress condition. This result indicates that genotypes with high yield production (biomass yield) under severe drought stress had more reduction in total chlorophyll.

Table 2. Means of seed yield, yield components and physiological trait under drought stress and non-stress treatments in *Brassica* species.

Environment	Trait	Species						
		B. napus	*B. rapa*	*B. juncea*	*B. carinata*	*B. oleracea*	*B. nigra*	*B. fruticulosa*
Non-stress	SY	228.2±26.5	147.9±40.5	190.3±6.15	209.8±61.9	149.3±57.01	183.5±62.2	182
	TSW	4.23±0.44	3.43±0.55	2.8±0.92	3.6±1.1	4.06±0.83	2.16±0.64	3.2
	PP	944.7±52.9	641.7±297.1	545±151.5	614.5±248.5	492.4±88.3	733.4±229.6	505
	BP	6.1±0.86	8.7±1.9	7.6±2.2	10.5±2.6	8.2±2.5	8.5±1.15	8
	Proline (µmol/g)	0.30±0.09	0.27±0.20	0.25±0.07	0.25±0.05	0.22±0.04	0.22±0.12	0.35
	Chla (mg/g)	9.05±3.6	10.7±6.03	10.7±3.6	11.5±2.1	10.3±3.7	12.4±1.9	4.91
	Chlb (mg/g)	4.26±2.12	5.01±2.9	5.5±2.3	4.09±0.69	4.3±2.5	4.8±1.5	6.75
	Car (mg/g)	851±320.9	1031.1±501.5	965.9±268	977.4±254.7	995.5±337	1209.3±93.9	555.1
	RWC	91.9±7.8	88.1±6.3	92.1±6.6	92±7.8	91.7±7.5	93.9±6.4	88.1
	TChl (mg/g)	13.3±4.7	15.7±8.1	16.2±3.6	15.6±2.5	14.6±5.8	24.2±3.06	11.7
	Chla/Chb	2.61±1.39	2.6±1.3	2.4±1.4	2.8±0.49	2.8±1.08	2.5±0.67	0.73
	TChl/Car	0.016±0.006	0.015±0.005	0.017±0.002	0.016±0.001	0.014±0.004	0.014±0.001	0.021
	Oil content (%)	34.2±0.18	27.4±4.03	22.8±2.01	21.8±1.5	31.8±2.2	24.2±2.1	28.8
Moderate stress	SY	146.5±37.01	104.1±61.1	139.8±45.1	158.8±28.4	111.1±54.9	131.1±14.2	71
	TSW	4.04±0.43	2.99±0.5	2.7±0.99	4.1±1.1	3.7±0.6	2.04±0.1	3.1
	PP	656.3±102.7	461.9±213	378.2±243.9	488.8±172.6	354.4±153.6	656.7±226.6	376.6
	BP	5.7±3.1	4.6±2.07	6.9±3.7	8.3±4.1	5.8±3.3	7.4±2.1	6.3
	Proline (µmol/g)	0.38±0.11	0.33±0.05	0.36±0.05	0.26±0.02	0.25±0.05	0.28±0.18	0.38
	Chla (mg/g)	10.3±2.2	10.5±1.7	9.1±3.2	11.01±2.1	10.5±1.6	9.4±1.9	12.37
	Chlb (mg/g)	3.7±0.79	3.8±0.7	3.1±1.9	3.7±0.83	4.4±1.6	3.3±1.1	4.41
	Car (mg/g)	985.2±211.9	1023.6±162.3	855.4±191.6	991.2±192.3	1058.3±265.6	957.16 ±93.9	1250.2
	RWC	81.7±6.7	85.3±5.2	86.2±5.04	90.1±3.3	87.7±6.5	88.9±6.4	81.8
	TChl (mg/g)	14.1±3.04	14.3±2.4	12.2±4.5	14.7±2.9	15.05±2.8	12.2±3.06	16.8
	Chla/Chb	2.8±0.17	2.7±0.10	3.4±1.8	2.9±0.09	2.5±0.57	2.6±0.67	2.8
	TChl/Car	0.014±0.002	0.013±0.008	0.014±0.002	0.015±0.008	0.014±0.002	0.013±0.001	0.013±0.006
	Oil content (%)	29.3±4.2	27.7±4.4	27.2±2.75	24	26.5±2.4	15.8±1.3	26.30
Level of significance (moderate with normal)	SY	**	**	**	**	*	*	**
	TSW	*	**	ns	ns	*	ns	ns
	PP	**	**	**	*	**	*	**
	BP	ns	*	ns	*	*	ns	*
	Proline (µmol/g)	*	*	**	ns	*	*	*
	Chla (mg/g)	*	*	*	ns	ns	*	**
	Chlb (mg/g)	*	**	**	*	ns	*	**
	Car (mg/g)	**	ns	*	ns	ns	ns	**
	RWC	*	*	*	ns	*	*	**
	TChl (mg/g)	*	*	**	*	*	**	**
	Chla/Chb	ns	ns	*	ns	ns	ns	**
	TChl/Car	ns	ns	ns	ns	ns	ns	*
	Oil content	**	ns	**	*	**	**	*

Continue Table 2.

Environment	Trait	Species						
		B. napus	*B. rapa*	*B. juncea*	*B. carinata*	*B. oleracea*	*B. nigra*	*B. fruticulosa*
Severe stress	SY	122.8±18.2	80.5±46.6	99.6±48.2	107.3±14.43	76.8±36.7	102.1±20.16	-
	TSW	3.7±0.28	2.4±0.72	2.1±0.38	2.9±0.36	3.3±0.72	1.8±0.14	-
	PP	540.6.4±73.7	320.1±92.7	285.4±209.8	331.8±66.9	307.7±115.3	540.6±115.4	-
	BP	3.5±1.2	4.06±3.08	4.7±2.8	4.5±3.14	4.8±2.6	5.5±0.88	-
	Proline (µmol/g)	0.50±0.09	0.52±0.08	0.43±0.07	0.45±0.26	0.32±0.06	0.51±0.25	-
	Chla (mg/g)	10.9±5.15	11.9±3.4	10.5±2.5	13.3±3.3	13.1±4.4	13.2±2.41	-
	Chlb (mg/g)	5.06±1.02	4.6±1.2	3.9±0.92	5.6±2.4	6.6±4.3	4.7±0.61	-
	Car (mg/g)	1194.2±281.9	1186.6±309.1	1064.9±262.2	1307.1±358.4	1303.8±459.2	1327.2±121.9	-
	RWC	70.07±13	82.12±6.6	82.9±4.4	87.5±4.7	81.5±2.2	80.08±8.1	-
	TChl (mg/g)	16.02±5.7	16.5±4.6	14.5±3.4	19±5.6	19.8±8.5	18.9±2.9	-
	Chla/Chb	2.16±0.9	2.5±0.19	2.7±0.25	2.47±0.50	2.2±0.61	2.4±0.20	-
	TChl/Car	0.013±0.002	0.013±0.001	0.013±0.002	0.014±0.001	0.014±0.001	0.013±0.001	-
	Oil content (%)	30.6±2.81	33.8±4.9	25.8±5.1	29.4±2.1	28.4±1.4	22.8±1.2	-
Level of significance (severe with normal)	SY	**	**	*	**	*	*	-
	TSW	*	*	*	**	*	*	-
	PP	*	**	**	**	*	*	-
	BP	**	ns	*	*	*	*	-
	Proline (µmol/g)	**	**	*	**	*	**	-
	Chla (mg/g)	*	*	*	**	**	**	-
	Chlb (mg/g)	ns	ns	ns	*	**	*	-
	Car (mg/g)	**	*	*	**	**	*	-
	RWC	*	*	*	*	*	*	-
	TChl (mg/g)	**	**	**	**	**	*	-
	Chla/Chb	ns	ns	*	ns	ns	ns	-
	TChl/Car	ns	ns	ns	ns	ns	ns	-
	Oil content (%)	*	**	*	**	*	*	-

*: Significant at 5% level of probability, **: Significant at 1% level of probability, ns: non-Significant. SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car.

Table 3. Genetic coefficient of variation, heritability and expected gain from selection of Brassica species under stress and non-stress treatments.

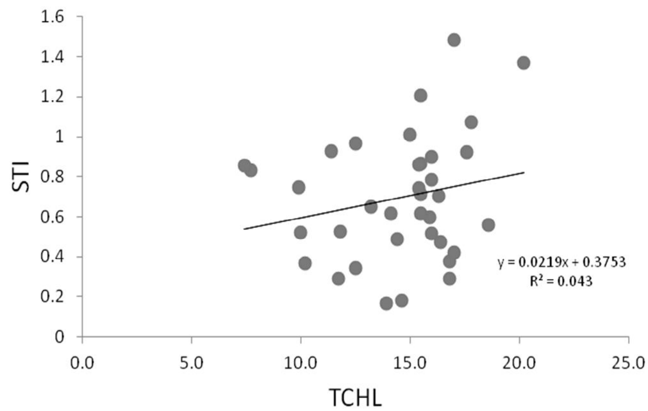
	PCV%			GCV%			h^2 (%)			h^2 (%)
	Non stress	Moderate stress	Severe stress	Non-stress	Moderate stress	Severe stress	Non stress	Moderate stress	Severe stress	Total
SY	55.6	43.9	41.13	51.5	39.2	37	69	35	35	55
TSW	32.8	33.2	32.9	31.6	30	26.2	44	56	52	84
PP	62.4	57.1	48.1	54.3	50.7	42.7	48	48	40	72
BP	76.7	69.2	53.1	75.6	68.6	52.8	24	24	07	45
Proline	47.6	42.3	38.8	25.9	25.1	18.5	22	22	22	44
Chla	51.2	40.1	33.2	21.4	10.2	10	40	40	38	39
Chlb	58.6	46.3	37.2	27.8	22.4	11.6	15	15	10	38
Car	5.8	4.1	3	2.2	2.1	1.82	42	34	28	24
RWC	11.5	10.8	9.9	2.4	2.2	2.2	08	07	07	07
TChl	42.8	32.8	29.3	42.6	32.5	29.1	32	25	16	39
Chla/Chb	20	15.1	12.1	12	10.2	8.1	39	32	16	41
TChl/Car	25	14.2	14.6	23.6	14.2	14.6	25	22	18	32
Oil content	33.4	21.2	18.2	18.3	17.6	16.5	67	20	16	23

PCV: Phenotypic coefficients of variation GCV: Genetic coefficient of variation. h^2: Broad sense heritability (%). SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car.

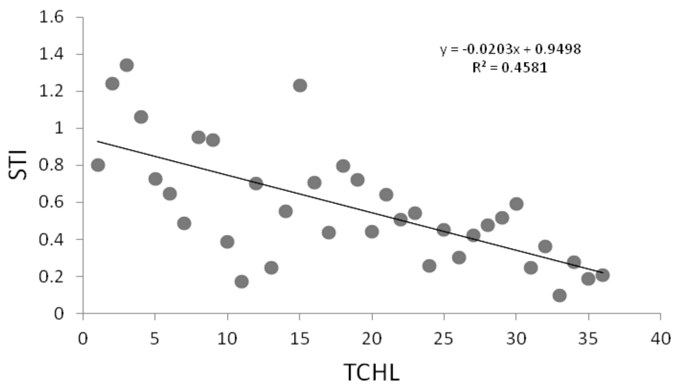
Table 4. Correlation coefficients between different traits with drought susceptibility index and stress tolerance index in *Brassica* species.

Trait	Drought susceptibility index		Stress tolerance index	
	Sever stress	Moderate	Sever stress	Moderate stress
SY	-0.17ns	-0.05ns	0.60**	0.50**
TSW	0.03ns	-0.03ns	-0.18ns	0.14ns
PP	-0.02ns	-0.03ns	-0.31ns	0.23ns
BP	0.29ns	0.05ns	0.31ns	0.23ns
Proline	0.36*	0.38*	-0.32ns	-0.29ns
Chla	-0.12ns	0.13ns	0.29ns	0.23ns
Chlb	0.22ns	0. 29ns	0.42*	-0.31ns
Car	-0.19ns	-0.19ns	0.34*	0.32ns
RWC	-0.12ns	-0.09ns	0.55**	0.61**
TChl	0.03ns	-0.15ns	0.54*	0.38*
Chla/Chb	-0.14ns	-0.17ns	-0.24ns	-0.27ns
TChl/Car	-0.13ns	-0.08ns	-0.31ns	-0.28ns
Oil content	-0.09ns	-0.12ns	-0.13ns	-0.09ns
STI	-0.15ns	-0.12ns	1	1

*: Significant at 5% level of probability, **: Significant at 1% level of probability, ns: Non-Significant. SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car, STI: stress tolerance index.



(a)



(b)

Figure 1. Relationship between total chlorophyll content (TCHL) and stress tolerance index (STI) under moderate (a) and severe (b) drought stress.

Result of stepwise multiple liner regression (Table 7) showed that BP, RWC and proline content explained more than 63 percent of the total variation of SY (SY=1.58 BP + 0.41 RWC +18.51 proline -34.9) under moderate stress condition. The most important component of SY was BP (Partial R^2=36%) while RWC and proline content justified only 27% of SY variation. Under severe stress condition TChl content and TSW could be considered as independent variables that explained most of the SY variation (71%) (SY= -0.73 TChl content + 2.7 TSW + 15.24). Of the observed variation for SY, 37 percent was justified by TChl content and 34 percent was justified by TSW.

Principal component analysis (PCA) revealed that the first component explained 56% and 76% of the variation for moderate and severe stress, respectively (Data not shown). High correlation was observed between PC1 with PP and TSW under moderate and severe stress conditions. Thus, the first dimension (PC1) could be named as the "yield components". The second PC (PC2) explained 41% and 22% of the total variation in moderate and severe stress, respectively. High and positive correlation was found between PC2 and photosynthetic pigments (Chla, Chlb, TChl and Car content) (Data not shown). The second dimension (PC2) could be named as the "photosynthetic index" under both stress conditions. Thus selections of species with high PC1 and PC2 are suitable to use moderate and severe stress conditions.

The biplot of first and second principal components to classify species was constructed for moderate and severe stress condition (Figures 2 and 3). *B. carinata* and *B. juncea* were identified as the ones with moderately high yield components (moderately PC1) and potential to photosynthesis (moderately high PC2) under moderate stress condition. These species had high SY and RWC than other studied species. Results indicated that *B. napus* had low PC1 (low yield components) and very high PC2 (high potential to photosynthesis) while *B. nigra* was identified as the ones with very high yield components (high PC1) and very low potential to photosynthesis (low PC2). Also species of *B. napus* had the high value for TSW, oil and proline content but *B. nigra* had the high value for PP and BP traits. *B. fruticulosa* was introduced as the most susceptible species. According to results of this biplot under severe stress condition, species of *B. oleracea* had the high value for PC1 and PC2 (Figure 3). *B. napus* had the lowest value for PC1 and moderate PC2. *B. juncea* was identified as the one with the high value for proline content. In this study *B. fruticulosa* was destroyed in severe stress condition.

Table 5. Correlation coefficients of different traits under non-stress (above diagonal) and moderate stress (below diagonal) conditions.

Trait	SY	TSW	PP	BP	Proline	Chl a	Chl b	Car	RWC	TChl	Chla/Chb	TChl/Car	Oil
Seed yield	1	0.67^{**}	0.56^{**}	0.78^{**}	0.18^{ns}	0.24^{ns}	0.17ns	0.32^{ns}	0.23^{ns}	0.46^{**}	0.30^{ns}	-0.26^{ns}	-0.59^{*}
1000-seed weight	0.84^{**}	1	-0.64^{**}	-0.67^{**}	0.57^{*}	-0.45^{*}	0.60^{*}	-0.65^{**}	-0.66^{**}	-0.59^{*}	-0.58^{*}	0.64^{**}	0.19^{ns}
Number of pods per plant	0.55^{**}	-0.58^{**}	1	0.93^{**}	-0.73^{**}	0.46^{*}	-0.75^{**}	0.95^{**}	0.92^{**}	0.91^{**}	0.87^{**}	-0.92^{**}	-0.58^{*}
Number of branches per plant	0.50^{*}	-0.46^{**}	-0.59^{**}	1	-0.74^{**}	0.46^{*}	-0.77^{**}	0.94^{**}	0.95^{**}	0.94^{**}	0.82^{**}	-0.93^{**}	-0.58^{*}
Proline content	0.37^{*}	0.78^{**}	-0.13^{ns}	-0.44^{*}	1	-0.19^{ns}	0.97^{**}	-0.73^{**}	-0.64^{*}	-0.69^{**}	-0.64^{*}	0.78^{**}	0.31^{*}
Chlorophyll a content	0.24^{ns}	-0.18^{ns}	-0.19^{ns}	-0.15^{ns}	-0.10^{ns}	1	-0.23^{ns}	0.50^{**}	0.51^{*}	0.24^{*}	0.48^{*}	-0.55^{*}	-0.17^{ns}
Chlorophyll b content	-0.14^{ns}	0.29^{ns}	-0.18^{ns}	-0.19^{ns}	-0.11^{ns}	-0.11^{ns}	1	-0.76^{**}	-0.68^{**}	-0.71^{**}	-0.69^{**}	0.84^{**}	0.31^{*}
Carotenoids contents	0.89^{**}	-0.89^{**}	0.58^{**}	-0.45^{*}	-0.90^{**}	-0.15^{ns}	-0.09^{ns}	1	0.92^{**}	0.92^{**}	0.92^{**}	-0.91^{**}	-0.50^{*}
Relative water content	0.88^{**}	0.88^{**}	-0.57^{**}	-0.43^{*}	-0.87^{**}	-0.19^{ns}	-0.10^{ns}	-0.93^{**}	1	0.90^{**}	0.82^{**}	-0.95^{**}	-0.60^{*}
Total chlorophyll	0.63^{**}	0.79^{**}	-0.31^{ns}	-0.37^{*}	0.62^{**}	-0.15^{ns}	-0.14^{ns}	-0.61^{**}	-0.78^{**}	1	0.78^{**}	-0.90^{**}	-0.55^{*}
Ratio of Chla/Chb	0.17^{ns}	0.14^{ns}	-0.11^{ns}	0.07^{ns}	-0.14^{ns}	0.17^{ns}	-0.17^{ns}	-0.17^{ns}	-0.11^{ns}	-0.04^{ns}	1	-0.81^{**}	-0.23^{*}
Ratio of TChl/Car	0.38^{**}	-0.18^{ns}	0.45^{**}	-0.14^{ns}	0.34^{*}	-0.14^{ns}	0.02^{ns}	-0.19^{ns}	-0.18^{ns}	-0.06^{ns}	-0.08^{ns}	1	0.48^{*}
Oil Content	-0.56^{**}	-0.86^{**}	-0.72^{**}	-0.67^{**}	0.89^{**}	0.02^{ns}	-0.15^{ns}	-0.96^{**}	0.95^{**}	0.88^{**}	-0.08^{ns}	-0.12^{ns}	1

*: Significant at 5% level of probability, **: Significant at 1% level of probability, ns: non-Significant. SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car.

Table 6. Correlation coefficients of different traits under non-stress (above diagonal) and severe stress (below diagonal) conditions.

Trait	SY	TSW	PP	BP	Proline	Chl a	Chl b	Car	RWC	TChl	Chla/Chb	TChl/Car	Oil
Seed yield	1	0.67^{**}	0.56^{**}	0.78^{**}	0.18^{ns}	0.24^{ns}	0.17^{ns}	0.32^{ns}	0.23^{ns}	0.46^{**}	0.30^{ns}	-0.26^{ns}	-0.59^{*}
1000-seed weight	0.43^{*}	1	-0.64^{**}	-0.67^{**}	0.57^{**}	-0.45^{*}	0.60^{**}	-0.65^{**}	-0.66^{**}	-0.59^{*}	-0.58^{*}	0.64^{**}	0.19^{ns}
Number of pods per plant	-0.13^{ns}	-0.49^{**}	1	0.93^{**}	-0.73^{**}	0.46^{*}	-0.75^{**}	0.95^{**}	0.92^{**}	0.91^{**}	0.87^{**}	-0.92^{**}	-0.58^{*}
Number of branches per plant	-0.16^{ns}	-0.35^{*}	0.21^{ns}	1	-0.74^{**}	0.46^{*}	-0.77^{**}	0.94^{**}	0.95^{**}	0.94^{**}	0.82^{**}	-0.93^{**}	-0.58^{*}
Proline content	0.44^{*}	-0.26^{ns}	-0.15^{ns}	-0.09^{ns}	1	-0.19^{ns}	0.97^{**}	-0.73^{**}	-0.64^{*}	-0.69^{**}	-0.64^{*}	0.78^{**}	0.31^{*}
Chlorophyll a content	0.26^{ns}	-0.16^{ns}	-0.17^{ns}	-0.16^{ns}	-0.08^{ns}	1	-0.23^{ns}	0.50^{**}	0.51^{*}	0.24^{*}	0.48^{*}	-0.55^{*}	-0.17^{ns}
Chlorophyll b content	-0.12^{ns}	0.30^{*}	-0.15^{ns}	-0.17^{ns}	-0.14^{ns}	-0.09^{ns}	1	-0.76^{**}	-0.68^{**}	-0.71^{**}	-0.69^{**}	0.84^{**}	0.31^{*}
Carotenoids contents	-0.13^{ns}	-0.13^{ns}	0.12^{ns}	0.18^{ns}	-0.17^{ns}	-0.15^{ns}	-0.07^{ns}	1	0.92^{**}	0.92^{**}	0.92^{**}	-0.91^{**}	-0.50^{*}
Relative water content	0.36^{*}	-0.12^{ns}	0.44^{*}	0.32^{*}	-0.16^{ns}	-0.17^{ns}	-0.14^{ns}	-0.17^{ns}	1	0.90^{**}	0.82^{**}	-0.95^{**}	-0.60^{*}
Total chlorophyll	0.35^{*}	-0.10^{ns}	-0.10^{ns}	0.29^{ns}	0.12^{ns}	-0.13^{ns}	-0.13^{ns}	-0.93^{**}	-0.16^{ns}	1	0.78^{**}	-0.90^{**}	-0.55^{*}
Ratio of Chla/Chb	-0.13^{ns}	0.17^{ns}	-0.12^{ns}	-0.12^{ns}	-0.12^{ns}	0.19^{ns}	-0.14^{ns}	-0.14^{ns}	-0.22^{ns}	-0.01^{ns}	1	-0.81^{**}	-0.23^{*}
Ratio of TChl/Car	-0.14^{ns}	-0.15^{ns}	0.48^{**}	-0.12^{ns}	-0.13^{ns}	-0.13^{ns}	0.04^{ns}	-0.16^{ns}	-0.15^{ns}	-0.10^{ns}	-0.05^{ns}	1	0.48^{*}
Oil Content	-0.12^{ns}	0.15^{ns}	-0.16^{ns}	-0.17^{ns}	-0.48^{*}	-0.14^{ns}	-0.13^{ns}	0.15^{ns}	-0.15^{ns}	0.12^{ns}	-0.11^{ns}	-0.08^{ns}	1

*: Significant at 5% level of probability, **: Significant at 1% level of probability, ns: non-Significant. SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car.

Table 7. Relative contribution of traits to predicting seed yield of *B*rassica species using stepwise regression in severe and moderate stress conditions.

Conditions	Variable entered	Parameter Estimate	Partial R^2	Model R^2	F Value
Moderate stress	Number of branches per plant	1.58	0.36	0.26	12.15**
	Relative water content	0.41	0.17	0.53	9.6*
	Proline content	18.51	0.10	0.63	4.07*
	Intercept	-34.9			10.72**
Severe stress	Total chlorophyll content	-0.73	0.37	0.37	8.35*
	1000-seed weight	2.79	0.34	0.71	8.82*
	Intercept	15.24			13.64**

*: Significant at 5% level of probability, **: Significant at 1% level of probability, ns: non- Significant.

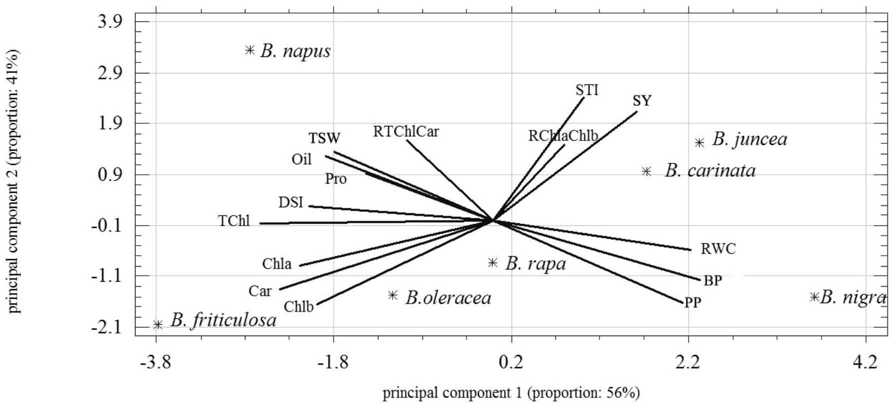


Figure 2. The biplot display of physiological attributes, stress tolerant indices and *Brassica* species under moderate drought stress (SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car, STI: Stress tolerance index, DSI: drought susceptibility index). Definition of the codes and origin of the genotypes can be seen in Table 1.

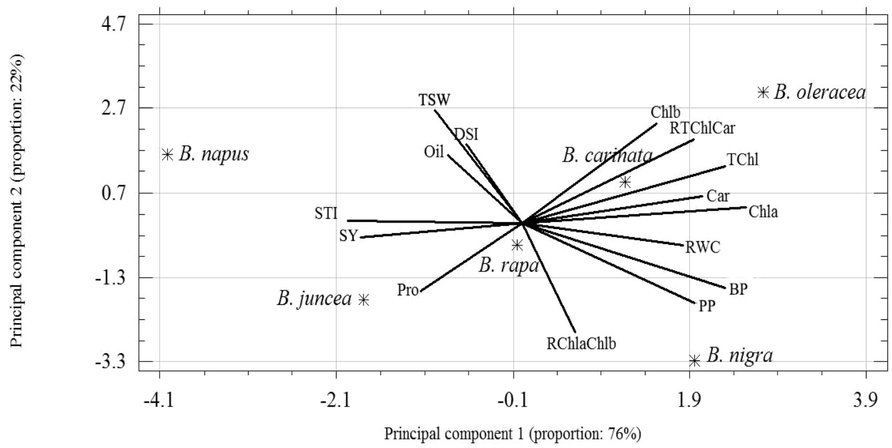


Figure 3. The biplot display of physiological attributes, stress tolerant indices and *Brassica* species under severe drought stress (SY: seed yield, TSW: 1000-seed weight, PP: Number of pods per plant, BP: Number of branches per plant, RWC: Relative water content, Chll-a: Chlorophyll a content, Chll-b: Chlorophyll b content, Car: Carotenoids contents, TChl: Total chlorophyll, Chl-a/b: Ratio of Chla/Chb, TChl/Car: Ratio of TChl/Car, STI: Stress tolerance index, DSI: drought susceptibility index). Definition of the codes and origin of the genotypes can be seen in Table 1.

Discussion

Water deficit stress accelerates phenological growth stages and reduces the normal growth and development periods (Ghobadi et al., 2006). The physiological changes have been considered as an important adaptation mechanisms for plant to resist drought (Ebrahimiyan et al., 2012). In the present study considerable reduction in almost all the studied traits was observed as a result of severe stress condition while this reduction was lower under moderate stress condition. Also considerable genetic variation was found among the species. This variation can be used for selecting drought tolerant species or genotypes. *B. fruticulosa* entirely was destroyed under severe stress condition while this species had the lowest SY under moderate stress condition. Decrease of SY has been reported in *Brassica* under stress condition by other researchers (e.g. Tohidi-Moghadam et al., 2009; Din et al., 2011).

Reduction of chlorophyll content has been considered as a commonly observed phenomenon in response to drought stress (Bayat et al., 2009; Ebrahimiyan et al., 2012), but some studies have documented increased chlorophyll content under moderate and intensive drought stress which was similar to our findings (Jiang and Huang, 2001; Garcıa-Valenzuela et al., 2005). Present study indicated that the effect of drought stress on chlorophyll depends on plant species and stress conditions. Drought stress not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (Ladjal et al., 2000). Reduction in chlorophyll content under drought stress might be causes reduction of synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (Allakhverdiev et al., 2002) or destruction of chiral macro-aggregates of light harvesting chlorophyll "a" or "b" pigment protein complexes (CHCIIs) which protect the photosynthetic apparatus or due to oxidative damage of chloroplast lipids, pigments and proteins (Tambussi et al., 2000; Guo et al., 2015). Also, increased chlorophyll content following moderate and intensive drought stress could be the result of slowing cellular growth relative to chlorophyll synthesis (Garcıa-Valenzuela et al., 2005).

The Chla/Chlb ratio in most species non- significantly increased under moderate stress while under severe stress it decreased. This is presumably due to faster damage of Chla compared to Chlb under moderate stress condition In this study the highest value of ratio Chla/Chlb was observed in *B. carinata*, *B. oleracea* (under non-stress condition) and *B. juncea* (under moderate and severe stress condition). Also, *B. fruticulosa* and *B. oleracea* species had the lowest value for ratio of Chla/Chlb under non-stress, moderate and severe stress conditions. El-Tayeb (2006) showed that decrease in the Chla/Chlb ratio is faster in drought sensitive than in drought tolerant genotypes.

In this study drought stress significantly increased proline under stress conditions in all species. The increase in proline was more pronounced under severe water deficit stress compared to moderate water deficit stress. In this study seed yield was fairly correlated with proline content under moderate and severe stress conditions indicating selection for this character under stress environment might result in increase of grain yield of *Brassica* species. Reports on the effects of stresses on proline accumulation and its relationship with drought tolerance are very different (Ashraf and Foolad, 2007). Despite the presence of a strong correlation between stress intensity and accumulation of proline in higher plants, relationship between proline accumulation and genetic drought tolerance may not be universal (Ashraf and Foolad, 2007). Some reports suggest that proline accumulation is a reaction to stress and not a plant indicator associated with tolerance (De-Lacerda et al., 2003). Increase in proline during stress in *Brassica* is in agreement with Hsu et al. (2003), Gunes et al. (2008), Bayoumi et al. (2008), Din et al. (2011), Sepehri and Golparvar (2012) and Ebrahimiyan et al. (2012).

In this study GCV and PCV for all studied traits were higher under non-stress condition. Reduction in GCV and PCV under drought conditions may indicate that genetic variation and selection efficiency would depend on stress intensity. Breeding programs depend on the knowledge of key traits, genetic systems controlling their inheritance and genetic and environmental factors that influence their expression (Chaghakaboodi et al., 2012). Heritability estimates provide an indication of the expected genetic gain available in a population that will provide the basis for designing an effective breeding program to maximize genetic improvement (Ebrahimiyan et al., 2012). High heritability for TSW indicating that selection for these traits may be effective for indirect improvement of SY under moderate and severe stress conditions. Also, low heritability estimates were obtained for RWC indicating that these traits were more affected by environmental conditions. Therefore it may not be useful as an index to finding drought tolerant genotypes in *Brassica*. Blum (2011) reported that indirect selection via yield components and other traits could be more efficient than direct selection if these traits are related to yield and have a higher heritability than yield.

Results indicated that non-significant correlation was observed between STI and DSI (r= -0.12 in moderate stress and r= -0.15 in severe stress) suggesting that these indices could be indicators of different biological responses to drought. Significant correlations was observed between RWC, TChl content and SY with STI under moderate stress condition while STI was positively correlated with RWC, TChl, Car contents, Chlb content and SY under severe stress condition. As RWC and Car has low heritability, therefore selection based on higher TChl content under moderate stress and TChl content and Chlb content under severe stress condition may lead to higher yielding genotypes. Thus changes in chlorophyll content can be recognized as a key component affecting drought tolerance which is consistent with the results of Xiao et al. (2008). In this study the first principle component (PC1) had higher correlation with PP and TSW while the second principle component (PC2) had a positive correlation with carotenoids and TChl content and higher negative correlation with TChl/Car under moderate stress condition. The higher value of Car content and lower value of TChl/Car shows more efficient photo protective system (Ebrahimiyan et al., 2012). Car are essential components of the photosynthetic machinery and play multifarious role in drought tolerance including preventing photo oxidative damage light harvesting and protection from oxidative damage caused by drought (Howitt and Pogson, 2006). In this study yield component had a positive correlation with PC1 and photosynthesis pigments had a positive correlation with PC2 indicating that selection of species with high PC1 and PC2 may result in drought tolerant varieties in both stress conditions. Based on these two components and according to the distribution of species on biplot (Figures 2 and 3), *B. carinata* and *B. juncea* with high PC1 and PC2 may be suggested as the superior species in moderate stress condition while *B. oleracea* can be introduced as the superior species in severe stress.

In conclusion, the results of this study suggest that drought stress greatly influences yield components and physiological functions that affect plant growth and biomass production of *Brassica*. This effect is highly dependent on drought stress intensity. However large genotypic variation was observed among species for most of the studied traits indicating that selection in this germplasm would be useful. The moderate heritability for seed yield suggests that indirect selection based on related traits which had moderate to high heritability would be more effective. Although chlorophyll content might act as part of a survival mechanism under stress conditions however stronger relationship was found between drought tolerance (as estimated by STI) and chlorophyll content under severe stress condition. Results of principle component analysis indicated that *B. juncea* species are more tolerant to drought stress condition and can be used in development of breeding varieties.

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