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Evaluation of Drought Tolerance in Rapeseed (*Brassica napus* L.) Cultivars Using Drought Tolerance Indices

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Abstract

Drought is the most significant constraint for crop production in arid and semi-arid regions. In order to assess drought tolerance in fifteen rapeseed (*Brassica napus*) cultivars using yield-based drought tolerance indices, two experiments were conducted at the research station of College of Agriculture, Shiraz University, Shiraz, Iran during 2009-2010 growing season. The cultivars were arranged in a randomized complete block design with four replications in each experiment. The experiments differed in respect to irrigation regimes. The well-watered and water-limited experiments were irrigated after 40% and 70% depletion of available soil moisture, respectively. Analysis of variance showed highly significant differences among the rapeseed cultivars for yield in normal and stress conditions as well as all the drought tolerance indices. Karun cultivar had the maximum seed yield in both conditions. Yield in non-stress (Yp) and stress (Ys) conditions showed positive and significant correlations with stress tolerance index (STI), mean productivity (MP), geometric mean productivity (GMP) and Modified stress tolerance indices (K_1STI and K_2STI). Biplot analysis also indicated that STI, MP, GMP, K_1STI and K_2STI were more reliable indices to identify drought tolerant rapeseed cultivars. The results of biplot and cluster analysis revealed that Karun, NK Aviator and NK Octans were the drought tolerant rapeseed cultivars. Therefore, they may be recommended to cultivate in drought prone regions and also can be used in rapeseed breeding programs aimed at improving drought tolerance.

Keywords: *Brassica napus*, Drought stress, Tolerance indices, Seed yield

Introduction

Drought is the most challenging problem for crop production in arid and semi-arid regions such as Iran. It is estimated that 38% of the world area, or 70% of the agricultural production, is influenced by drought (Dilley et al., 2005). Development of drought tolerant cultivars is of great priority to reduce the adverse effects of drought for more consistent levels of crop production. Breeding for yield improvement under water limited conditions is a more complicated scenario than that of under stress-free conditions. Drought tolerance is a complex trait controlled by numerous genes (Blum, 2005; Pinto et al., 2010). Also, plant

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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). From plant breeding point of view, yield loss is the major indicator to evaluate drought tolerance. Thus, various indices have been developed to measure drought tolerance based on yield performance in stress and non-stress environments (Mitra, 2001). These indices are either based on drought tolerance or sensitivity of genotypes (Fernandez, 1992). Fischer and Maurer (1978) proposed stress susceptibility index (SSI) in which genotypes with lower values of SSI have higher levels of drought tolerance. Rosielle and Hamblin (1981) represented stress tolerance (TOL), the yield difference between non-stress (Yp) and stress (Ys) environments, and mean productivity (MP) as the average of Ys and Yp. Based on these indices, genotypes with high MP and low TOL are favored. Yield stability index (YSI) introduced by Bouslama and Schapaugh (1984) evaluates drought tolerance of a given genotype by calculating the ratio of Ys to Yp. Likewise TOL, this indicator may select genotypes with low yield under optimal conditions. Fernandez (1992) defined the two correlated indices, Stress Tolerance Index (STI) and Geometric Mean Productivity (GMP), which are able to identify drought tolerant genotypes with high yield potential. Farshadfar and Sutka (2002) suggested modified stress tolerance index (MSTI) in which STI is multiplied by a correction coefficient (K_i) specific for each stress and non-stress conditions. As a result, K_1STI and K_2STI are the selection indicators for stress and optimal conditions, respectively. The drought tolerance indices are analyzed by different multivariate statistical approaches to obtain more precise selection indicators for drought tolerance. Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops worldwide due to high oil quantity and quality. According to FAO statistics, rapeseed global production has substantially expanded from 34 million tons in 1995 to about 65 million tons in 2012 (http://faostat.fao.org). Despite rapeseed shows a high adaptability to different environmental conditions, many studies have reported significant reduction of rapeseed yield under water deficit stress (Richards and Thurling, 1978; Cheema & Sadaqat 2004; Qifuma et al, 2006). Therefore, improvement of rapeseed productivity under drought stress is a major target in breeding programs (Cheema & Sadaqat 2004). Significant variation has been found between rapeseed cultivars in terms of response to drought stress (Malekshahi et al, 2009; Shirani-rad and Abbasian, 2011). Previous studies reported that STI, MP, GMP and MSTI are useful indices for screening drought tolerant rapeseed genotypes (Malekshahi et al, 2009; Shirani-rad and Abbasian, 2011; Yarnia et al, 2011; Khalili et al, 2012). Once the new rapeseed cultivars are released, it is essential to evaluate their response to water deficit stress, particularly when they are introduced to the regions with limited water resources and erratic rainfall. The present study aimed to assess drought tolerance in fifteen rapeseed cultivars, including some newly introduced ones to Iran, using the yield-based indices.

MATERIALS AND METHODS

Two field experiments were conducted on a silty loam soil at the research station of College of Agriculture, Shiraz University, Shiraz, Iran (29°50′ N, 52°46′ E, Altitude 1810 m above sea level) during 2009-2010 growing season. The experiments differed in respect to irrigation regimes. The well-watered and water-limited experiments were irrigated after 40% and 70% depletion of available soil moisture, respectively. The water deficit stress was imposed from stem elongation stage to crop maturity. Fifteen rapeseed cultivars with different origins were grown in both experiments (Table 1). The cultivars were arranged in a randomized complete block design with four replications in each experiment. Each plot consisted of 6 rows, 5 m long with 30 cm apart. Prior to each irrigation, soil moisture status was measured by gravimetric method.

Table 1. Name and origin of the rapeseed cultivars.

No.	Cultivar	Origin	No.	Cultivar	Origin
1	Karun	France	9	Talaye	Germany
2	NK Aviator	France	10	Zarfam	Iran
3	NK Octans	France	11	Okapi	France
4	Opera	Sweden	12	RGS003	Germany
5	NK Alamir	France	13	NK Karibik	France
6	SLM 046	Germany	14	Champlain	France
7	Sarigol	Germany	15	Licord	Germany
8	Modena	Denmark			

Seed yield was measured by harvesting 2 m^2 of the centre of each plot. The following yield-based drought tolerance indices were calculated in which Yp and Ys denote yield of a given cultivar under non-stress and stress environments; and $\bar{Y}_P$ and $\bar{Y}_s$ are mean yield of all cultivars under non-stress and stress environments, respectively.

1) Tolerance =TOL = $Y_P - Y_S$ (Rosielle and Hamblin, 1981)
2) Mean productivity=MP = $(Y_S + Y_P)/2$ (Rosielle and Hamblin, 1981)
3) Stress susceptibility index= SSI = $[1- (Y_S / Y_P)]/ 1–(\bar{Y}_S / \bar{Y}_P)$ (Fischer and Maurer, 1978)
4) Geometric mean productivity=GMP= $\sqrt{Yp \times Ys}$ (Fernandez, 1992)
5) Stress tolerance index=STI = $[(Y_P) \times (Y_S) / (\bar{Y}_P)^2]$ (Fernandez, 1992)
6) Yield stability index= (YSI) = (Y_S / Y_P) (Bouslama and Schapaugh, 1984).
7) Modified stress tolerance index = MSTI = Ki STI, $K_1= Y_S^2 / \bar{Y}_s^2$, $K_2= Y_p^2 / \bar{Y}_P^2$ (Farshadfar and Sutka, 2002). Ki is the correction coefficient.

The data were subjected to analysis of variance followed by LSD test for means comparisons using SPSS software. Correlation coefficients were calculated to find out the relationships among the indices and the seed yield under both conditions. Based on Principal component analysis, the biplot diagram was depicted to graphically identify the high yielding drought tolerant rapeseed cultivars. Cluster analysis was performed using Ward method to classify the cultivars regarding their drought tolerance based on different indices. Screening of drought tolerant cultivars was also done by ranking the cultivars for each index followed by calculating rank mean and standard deviation of rank.

RESULTS AND DISCUSSION

Comparison of the cultivars for yield and yield-based drought tolerance indices

Analysis of variance showed highly significant differences among the rapeseed cultivars for yield in normal and stress conditions as well as all the drought tolerance indices. This indicated the existence of substantial variation which makes possible to select the drought tolerant cultivars. The means of yield and indices of the cultivars are represented in Table 2. Karun cultivar had the maximum seed yield in both conditions, whereas the minimum seed yield was obtained from Licord, Champlain and NK Karibik in normal and stress environments. The yield rank of some cultivars such as Zarfam and SLM046 dramatically changed in water deficit conditions implying that selection for drought tolerance based solely on yield potential may not be effective. According to all the drought tolerance indices, Karun was the most drought tolerant cultivar. Based on STI, MP, GMP, K_1STI and K_2STI, Licord, Champlain and NK Karibik were the most drought sensitive cultivars, whilst Zarfam and Talaye were known to be the most sensitive cultivars based on SSI, TOL and YSI. Zarfam had already been reported as a drought sensitive cultivar (Shirani-rad and Abbasian, 2011). STI, MP and GMP similarly ranked the cultivars in terms of drought tolerance (Table 3) suggesting that they are comparable for selecting cultivars. Similar trends of these three indices in discriminating drought tolerant genotypes were found in previous studies (Mohammadi et al, 2011; Yarnia et al, 2011; Khalili et al, 2012; Naghavi et al, 2013). However, the drought tolerance ranking of the cultivars was inconsistent over the indices, as reported by previous studies (Shirani-rad and Abbasian, 2011; Yarnia et al, 2011; Farshadfar et al, 2012; Yasir et al, 2013). Therefore, the means and standard deviations of the ranks of the cultivars over all the indices were worked out to provide a better estimation of the level of drought tolerance (Table 3). As a result, Karun, NK Aviator and NK Octans which are relatively newly introduced rapeseed cultivars to Iran, showed the highest level of drought tolerance.

Table 2. The means of yield under stress and non-stress conditions and drought tolerance indices of the fifteen rapeseed cultivars.

Cultivar	Y_P (kg/ha)	Y_S (kg/ha)	SSI	STI	TOL	MP	GMP	YSI	K_1STI	K_2STI
Karun	2821.5	2602.2	0.34	1.53	219.3	2711.8	2709.6	0.92	2.38	3.64
NK Aviator	2336.8	2007.7	0.66	0.99	359.1	2172.2	2179.8	0.86	1.42	1.38
NK Octans	2234.2	1996.4	0.46	0.93	237.8	2115.3	2111.9	0.89	1.40	1.30
Opera	2304.7	1960.6	0.65	0.94	344.1	2132.6	2125.7	0.85	1.35	1.27
NK Alamir	2111.2	1869.2	0.44	0.83	242	1990.2	1986.5	0.89	1.23	1.01
SLM046	2488.2	1679.2	1.41	0.87	809	2083.7	2044	0.67	0.99	0.86
Sarigol	2075.7	1700.7	0.79	0.73	375	1888.2	1887.8	0.82	1.02	0.75
Modena	2276.5	1613.5	1.25	0.76	663	1940.5	1912.7	0.71	0.92	0.70
Talaye	2332.7	1502.5	1.55	0.73	830	1917.6	1872.1	0.64	0.79	0.58
Zarfam	2338.5	1446.5	1.66	0.70	892	1829.5	1839.2	0.62	0.74	0.52
Okapi	2164.4	1497.5	1.38	0.67	666.9	1821.9	1789.4	0.69	0.79	0.53
RSG003	1940.2	1499.5	0.99	0.61	440.7	1719.8	1705.6	0.77	0.79	0.48

NK Karibik	1874.2	1306.3	1.32	0.51	576.9	1590.2	1564.6	0.70	0.60	0.31
Champlain	1851.6	1296.4	1.30	0.50	555.2	1574	1549.3	0.70	0.59	0.30
Licord	1707.8	1315.5	1.00	0.47	392.3	1511.6	1498.8	0.77	0.61	0.28
LSD (5%)	191.7	145.3	0.31	0.09	175.2	118.1	165.4	0.45	0.19	0.21

Table 3. The ranks, rank means ($\overline{R}$) and standard deviation of ranks (SDR) of fifteen rapeseed cultivars for Yp, Ys and the drought tolerance indices.

Cultivar	YP	YS	SSI	STI	TOL	MP	GMP	YSI	K_1STI	K_2STI	$\overline{R}$	SDR
Karun	1	1	1	1	1	1	1	1	1	1	1	0
NK Aviator	4	2	4	2	4	2	2	4	2	2	2.8	1.03
NK Octans	8	3	2	4	2	4	4	2	3	3	3.5	1.78
Opera	6	4	5	3	5	3	3	5	4	4	4.2	1.03
NK Alamir	10	5	3	6	3	6	6	2	5	5	5.1	2.23
SLM046	2	7	13	5	13	5	5	13	7	6	7.6	3.98
Sarigol	11	6	6	8	6	9	8	6	6	7	7.3	1.70
Modena	7	8	9	7	11	7	7	9	8	8	8.1	1.66
Talaye	5	9	14	8	14	8	9	14	9	9	9.9	3.07
Zarfam	3	12	15	10	15	10	10	15	12	11	11.3	3.59
Okapi	9	11	12	11	12	11	11	12	9	10	10.8	1.14
RSG003	12	10	7	12	8	12	12	7	9	12	10.1	2.18
NK Karibik	13	14	11	13	10	13	13	10	14	13	12.4	1.51
Champlain	14	15	10	14	9	14	14	10	15	14	12.9	2.28
Licord	15	13	8	15	7	15	15	7	13	15	12.3	3.53

Correlation analysis

The contradictory drought tolerance ranking of the cultivars according to the different indices led to determine more reliable indices for selection of the drought tolerant cultivars. To do so, correlation coefficients between Yp, Ys and the indices of drought tolerance were calculated (Table 4). A reliable index is presumed to have significant correlations with yield under both stress and non-stress environments (Farshadfar et al, 2001). Yield in non-stress (Yp) conditions had positive and significant correlations with STI, MP, GMP, K_1STI and K_2STI, whereas no significant correlations were observed between Yp and the indices SSI, TOL and YSI indicating that these indices were not good indicators to identify the cultivars with high yield potential. Significant and positive correlations were found between Ys and STI, MP, GMP, YSI, K_1STI and K_2STI. Furthermore, Ys was significantly negatively correlated with SSI and TOL. Consequently, the correlation analysis revealed that STI, MP, GMP, K_1STI and K_2STI were more reliable indices to select high yielding rapeseed cultivars under normal and water deficit conditions. This result was consistent with the studies which indicated reliability of STI, MP and GMP for screening of drought tolerant genotypes in rapeseed (Malekshahi et al, 2009; Shirani-rad and Abbasian, 2011; Yarnia et al, 2011; Khalili et al, 2012) and other crop species such as wheat (Golabadi et al, 2006; Mohammadi et al, 2011; Farshadfar et al, 2013; Yasir et al, 2013), maize (Naghavi et al, 2013) and rice (Rahimi et al, 2013). Also, K_1STI and K_2STI were identified as good indicators of drought tolerance in rapeseed (Khalili et al, 2012), wheat (Farshadfar et al, 2012; Farshadfar et al, 2013) and maize (Naghavi et al, 2013).

Table 4. The correlation coefficients between Yp, Ys and the drought tolerance indices.

	Ys	SSI	TOL	STI	MP	GMP	YSI	K_1STI	K_2STI
Yp	0.75**	-0.12	0.11	0.88**	0.92**	0.90**	0.20	0.99**	0.77**
Ys		-0.74**	-0.56*	0.69**	0.94**	0.96**	0.78**	0.78**	0.94**
SSI			0.96**	-0.54*	-0.49	-0.53*	-1.0**	-0.23	-0.66**
TOL				-0.36	-0.27	-0.32	-0.96**	0.11	0.51*
STI					0.99**	0.99**	0.60*	0.90**	0.96**
MP						0.99**	0.55*	0.93**	0.92**
GMP							0.55*	0.91**	0.93**
YSI								0.22	0.66**
K_1STI									0.81*

Biplot analysis

Principal component analysis was performed to provide the combined indicators for selection of the cultivars suitable for both stress and non-stress environments. The first component (PC1) explained 75.3% of total variation (Table 5). This component was positively correlated with Ys, Yp, MP, GMP, STI, YSI, K1STI and K2STI. Thus, PC1 was named as the yield potential and drought tolerance. The high positive values of PC1 on biplot diagram lead to select genotypes with high yield in stress and non-stress environments. The second component (PC2) explained 23.7% of total variation (Table 5). This component showed strong and positive correlations with TOL, SSI and yield in normal conditions as well as negative correlation with yield in drought stress conditions. Therefore, PC2 was called as the stress susceptibility component which is able to distinguish drought tolerant cultivars from drought sensitive ones. Biplot diagram was depicted based on the first and second components which totally accounted for 98.9% of variation (Fig. 1). The cultivars which have high PC1 and low PC2 are suitable for both stress and non-stress environments (Kaya et al, 2002; Golabadi et al, 2006). As a result, the cultivars 1 (Karun), 2 (NK Aviator), 3 (NK Octans) and 4 (Opera) were known as the high yielding and drought tolerant rapeseed cultivars. Biplot analysis revealed strong positive associations between yield in both water conditions and STI, MP, GMP, K_1STI and K_2STI as evidenced by the acute angles between their vectors. This finding was consistent with the results obtained from correlation analysis. Several reports have used biplot analysis on the basis of the first two principal components for screening drought tolerant genotypes of different crop species (Golabadi et al, 2006; Nazari and Pakniyat, 2010; Malekshahi et al, 2009; Mohammadi et al, 2011; Yarnia et al, 2011; Farshadfar et al, 2012; Rahimi et al, 2013).

Table 5. Principal component analysis for Yp, Ys and drought tolerance indices of fifteen rapeseed cultivars.

Index	PC1	PC2
Yp	0.29	0.37
Ys	0.36	-0.04
SSI	-0.26	0.43
STI	0.35	0.10
TOL	-0.20	0.54
MP	0.35	0.14
GMP	0.35	0.11
YSI	0.26	-0.43
K_1STI	0.30	0.36
K_2STI	0.35	0.02
Eigen value	7.52	2.36
Variance (%)	75.3	23.7
Cumulative variance (%)	75.3	98.9

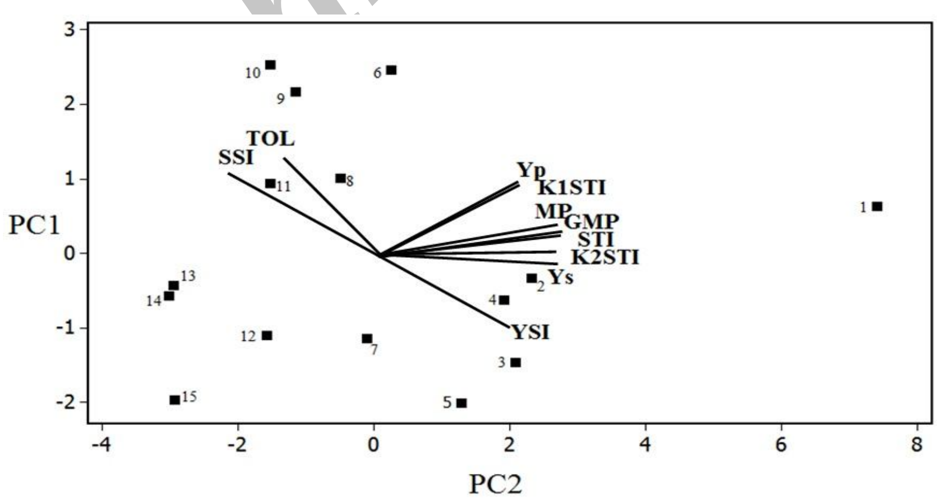

Figure 1. The biplot diagram based on first and second components for fifteen rapeseed cultivars and the indices of drought tolerance. The genotypes are represented by the numbers given in Table 1.

Cluster analysis

Cluster analysis based on drought tolerance indices and seed yield under normal and stress conditions classified the rapeseed cultivars into three groups with 6, 5 and 4 cultivars, respectively (Fig. 2). The first group consisted of the cultivars which had high values of seed yield as well as reliable drought tolerance indices such as STI, MP and K_1STI. Therefore, this group was known as the drought tolerant one. The cultivars of the second group had less stable performance with relatively high yield in non-stress environment, and thus considered as the semi-sensitive cultivars. The third group comprised the cultivars with low values of STI, MP, GMP, K_iSTI as well as seed yield in both conditions and thus they were considered as drought sensitive rapeseed cultivars. The results were generally in accordance with the findings of biplot analysis and ranking method. Cluster analysis was also used by several studies to classify genotypes according to their response to drought stress (Malekshahi et al, 2009; Mohammadi et al, 2011; Khalili et al, 2012; Naghavi et al, 2013; Yasir et al, 2013).

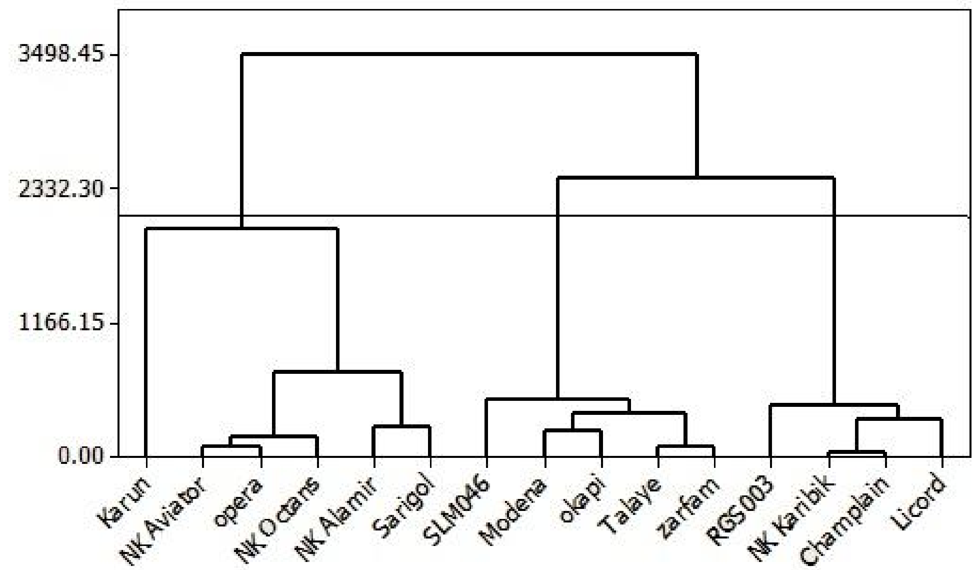

Figure 2. Dendrogram of rapeseed cultivars using Ward method based on drought tolerance indices.

Conclusion

This study evaluated the level of drought tolerance in fifteen rapeseed cultivars. Correlation coefficients and biplot analysis revealed that STI, MP, GMP, K_1STI and K_2STI were the most reliable yield-based indices for screening drought tolerant rapeseed cultivars. Among the cultivars, Karun, NK Aviator, NK Octans and Opera were known as the drought tolerant cultivars. Therefore, if they consistently show such tolerance over multiple trials, they can be introduced to cultivate in drought prone regions and also can be used in breeding programs aimed at improving drought tolerance in rapeseed.

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