Physiological responses of *Nigella Sativa* ecotypes to drought stress condition

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Abstract

In order to study the effect of drought stress at budding stage on five black cumin (*Nigella sativa* L.) ecotypes (Arak, Yasuj, Semirom, Azarbaijan, and Torbat Heydarieh), an experiment was conducted in based on a factorial randomized complete block design (RCBD) with four replications in Islamic Azad University, Arak branch, Arak, Iran. Treatments included control (normal irrigation) and drought stress (stop irrigation at budding stage until the end of the growth period). Oil percentage, oleic acid, linoleic acid, and linolenic acid were affected by drought and the highest values of oil percentage (2.6%), oleic acid (0.27%), linoleic acid (13.02%) and linolenic acid (1.57%) were observed in Arak, Torbat Heydarieh, Yasuj, and Torbat Heydarieh ecotypes, respectively. Results of tolerance indices showed that Torbat Heydarieh had the highest value of GMP (233.8), STI (2.26), and SSI (84.64). The correlation analysis showed Yp with GMP (r=+0.89**), STI (r=+0.95**), and MP (r=+0.89**). Based on the present study, Torbat Heydarieh ecotype is recommended for planting in arid and semi-arid regions.

Keywords: oleic acid; linoleic acid; linolenic acid; oil percent; black cumin

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Introduction

The black cumin seed, *Nigella sativa* L., is an annual aromatic plant native to Southwest Asia and the Mediterranean region. Nowadays, it is cultivated in various parts of the world, including Asia, the Middle East, and North Africa. *Nigella sativa* belongs to the family *Ranunculaceae* with a height of 60 to 70 cm, and capsule fruit (follicles) that contains large number of fragrant black seeds within (Soltan and Mansourifar, 2017). Seeds of black cumin contain about 21% protein and 35% carbohydrates (Ahmad and Ghafoor, 2007). They also contain all essential amino acids and are a rich source of vitamins and minerals (Heshmati and Namazi, 2015). As fatty components, linoleic acid (50-60%), oleic acid (20%), di homo linoleic acid (10%), and eicosadienoic acid (3%) are the main unsaturated fatty acids. The palmitic acid and stearic acid belong to two main saturated fatty

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acids. Some other fatty acids such as myristic acid, palmitoleic acid, linoleic acid, linolenic acid, arachidonic acid, cholesterol, campesterol, β-sitosterol, Δ5-avenasterol, Δ7-stigmasterol, and Δ7- avenasterol are also reported by Gharby et al. (2015) in *N. sativa*.

Black cumin is also an important plant in medicine (Mahdavi et al., 2015). Studies have shown that extract of black cumin seeds have many therapeutic effects such as anti-inflammatory (Amin and Hosseinzadeh, 2016), antioxidant, anticancer and immune system stimulation (Gholamnezhad et al., 2015), and antitumor (Gupta et al., 2012), antibacterial (Aljabre et al., 2015), antifungal (Randhawa, 2005; Aljabre et al., 2015), and anti-parasitic properties (Nadaf et al., 2015). Black cumin is originally grown in arid and semi-arid regions especially in Iran (Ghamarnia et al. 2010).

Drought stress is one of the most important environmental factors which limits plant growth, development, and production as well as the active ingredient of medicinal plants (Omidbeygi, 2005). Iran, with an average rainfall of 240 mm, is classified as one of the dry regions in the world. High rates of evapotranspiration, water resources constraints, and other factors have made researchers to pay more attention to the studies on the effects of drought stress on this plant(Mozzafari et al., 2000).

Some studies have shown that, black cumin is able to tolerate moderate levels of water stress (Mozzafari et al., 2000; Bannayan et al., 2008; Ghamarnia et al., 2010). A group of researchers have focused on the response of black cumin to different irrigation intervals (Mozzafari et al., 2000) and irrigation scheduling based on developmental stage of the plant (Bannayan et al., 2008). Also, drought stress was shown to increase grain oil percentage while it reduced total oil yield due to lower yield following the stress (Mozzafari et al., 2000). Several reports also showed an increase in extracted oil per unit area by reducing irrigation water (Mozzafari et al., 2000).

The grain yield of ecotypes in drought and normal conditions seems to be a selection tool in breeding program in dry environments (Clarke et al., 1992). According to Fernandez (1992), varieties can be divided into four groups based on their yield response to stress conditions: (1) ecotypes producing high yield under both water stress and non-stress conditions (group A), (2) ecotypes with high yield under non-stress (group B) or (3) stress (group C) conditions, and (4) ecotypes with poor performance under both stress and non-stress conditions (group D).

To distinguish drought tolerance ecotypes, several selection indices have been suggested. Stress susceptibility index (SSI) (Fischer and Maurer, 1978), tolerance (TOL) (McCaig and Clarke, 1982; Clarke et al., 1992), geometric mean productivity (GMP), stress tolerance index (STI) (Fernandez, 1992), mean productivity (MP) (McCaig and Clarke, 1982), and yield stability index (YSI) (Bouslama and Schapaugh, 1984) have been used in different conditions. Sio-Se Mardeh et al. (2006) reported under moderate stress, MP, GMP, and STI were more effective in identifying high yielding bread wheat cultivars in both drought-stressed and irrigated conditions (group A cultivars). Hajvand Ghassemabadi et al. (2018) evaluated salinity tolerance of different cultivars of clover based on mean germination time (MGT) and uniformity of germination.

Therefore, the aim of the present study is to investigate the physiological responses of black cumin ecotypes to water limitation including oil percentage and some major fatty acids; also the study seeks to evaluate the drought tolerance of different *Nigella sativa* varieties according to the drought tolerance indices.

Material and Methods

A field experiment was performed in the Agricultural Research Station of Islamic Azad University, Arak Branch situated at 49° 51′ N' and 33° 52' E, at the elevation of 1880 m in Iran in April 2016. The factorial experiment was conducted in based on a randomized complete block design (RCBD) with four replications. The seeds were procured from five different regions of Iran: Arak, Yasuj, Semirom, Azarbaijan, and Torbat Heydarieh. Treatments included control (normal irrigation according to plant requirements) and

Table 1
Analysis of variance for oil percent and fatty acids of five black cumin ecotypes under normal and drought stress conditions

Source of variance	df	Oil Percent	Oleic Acid	Linoleic Acid	Linolenic Acid
Rep	3	34.03ns	0.0005*	0.0001ns	0.0001ns
condition	1	2.63ns	217.1**	4.4**	0.11**
ecotype	4	9.48ns	193.3**	7.3**	0.07**
condition* ecotype	4	34.9ns	210.43**	0.02**	0.04**
Error	27	17.23	0.0001	0.0003	0.00008
cv		16.5	0.05	0.03	3.25

* and **: significant at 5% and 1% probability levels, respectively; ns: non-significant

drought stress (stop irrigation at budding stage until the end of the growth period).

Each plot consisted of five rows in 4 m length. The distance between the rows was 20 cm, the distance of two plants in each row was 7 cm, the distance between the plots was 60 cm, and the distance between replication was 50 cm. The irrigation was conducted as surface drip tape irrigation. All agronomical activities were done in the same way in both the normal and water limited plot. In budding stage, the irrigation stopped. After harvest, the seeds of the trial plants were collected and submitted to laboratory for evaluation of fatty acids. Also, seed yields were measured from 1 m^{-2} of each plot. Then, drought tolerance indices were calculated as follows.

Stress susceptibility index (SSI) was calculated based on the equation given by Fisher and Maurer (1978):

$SSI= [1-(Ysi/Ypi)]/SI$
$SI=1-(Ys/Yp)$

where Ypi is the yield of individual ecotypes without stress, Ysi is the yield of individual ecotypes with stress, Ys is the average yield of all ecotypes with stress, and Yp is the average yield of all ecotypes without stress.

Stress tolerance index (STI) and tolerance against stress (TOL) indices were calculated according to Fernandez (1992):

$STI = (Ypi) (Ysi)/(Yp)^2$
$TOL= (Ypi- Ysi)$

Geometric mean productivity (GMP) and mean productivity (MP) were calculated according to Fernandez (1992):

$GMP = \sqrt{(Ysi) (Ypi)}$,

$MP = (Ysi+Ypi) / 2$

Yield stability index (YSI) was calculated according to Bouslama and Schapaugh (1984):

$YSI = Ysi/Ypi$

Data analysis was carried out using SAS (Statistical Analysis System ver 9.1) and means were compared using Duncan's multiple range tests ($P<0.05$).

Results

Results of the analysis of data indicated that the effects of condition, ecotype, and the condition × ecotype interaction were not statistically significant on oil percentage, but they were significant for oleic acid, linoleic acid, and linolenic acid ($P<0.01$) (Table 1).

Among trial ecotypes, Arak had the highest oil content (2.6%). Torbat heydarieh, Yasuj, and Torbat heydarieh had the highest values of oleic acid, linoleic acid, and linolenic acid, respectively (0.27, 13.2, and 1.57%, respectively). Also, Azarbaijan, Semirom, and Azarbaijan had the lowest values of oleic acid, linoleic acid, and linolenic acid (0.21, 12.36, and 0.14%, respectively) (Table 2). Results showed that the the highest value of oleic acid was in normal × Yasuj treatment (Fig. I). The highest value of linoleic acid was in normal × Azarbaijan (Fig. II) and the highest value of linolenic acid was observed in normal × Semirom treatment (Fig. III).

Table 2
Mean comparison of oil percent and fatty acids of five black cumin ecotypes under normal and drought stress conditions

	Oil Percent	Oleic Acid	Linoleic Acid	Linolenic Acid
Condition				
Normal condition	2.33±0.04 a	0.24± 0.008 a	12.9±0.087 a	1.2±0.2 a
Drought stress condition	2.26± 0.03 b	0.23± 0.002 b	12.6±0.05 b	0.35± 0.11 b
Ecotype				
Arak	2.6±0.013 a	0.22±0.003 c	12.6±0.05 d	0.21±0.08 d
Yasuj	2.17±0.02 d	0.24±0.003 b	13.02±0.01 a	1.5±0.06 b
Semirom	2.3±0.03 b	0.22±0.002 c	12.36±0.04 e	0.6±0.22 c
Azarbaijan	2.25±0.04 c	0.21±0.002 d	12.09±0.13 b	0.14±0.05 e
Torbat Heydarieh	2.13±0.004 e	0.27±0.015 a	12.83±0.15 c	1.57±0.42 a

*Means in each column followed by similar letter are not significantly different at 5% probability level according to Duncan's Multiple Range Test.

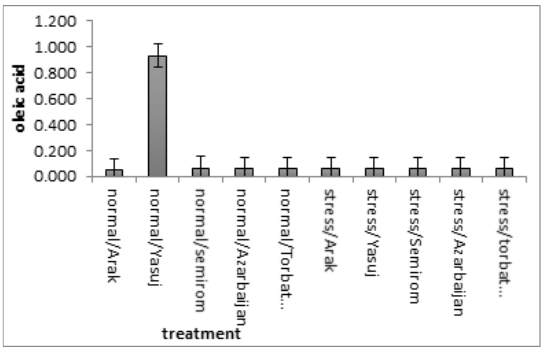

Fig. I. Effect of condition × ecotype on oleic acid content of black cumin plant

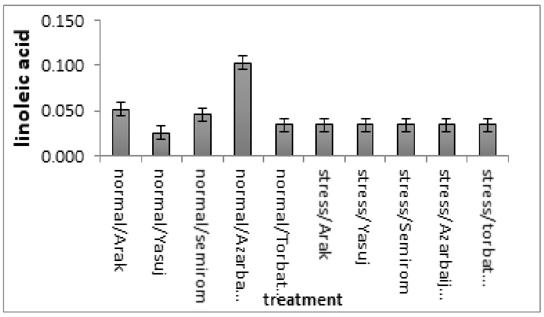

Fig. II. Effect of condition × ecotype on linoleic acid content of black cumin plant.

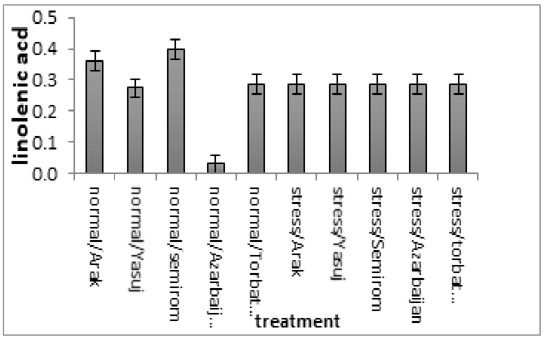

Fig. III. Effect of condition × ecotype on linolenic acid content of black cumin plant

Drought Tolerance Indices Stress susceptibility index (SSI)

The least SSI (-184.93) was resulted by Semirom ecotype with the yield of 133.45 and 213.73 kg/ha under non-stress and stress conditions, respectively. The next minimum values were related to Yasuj (-95.64) with the non-stress yield of 144.38 kg/ha and stress yield of 189.30 kg/ha (Table 1). The highest SSI values were related to Arak (183.53) with the non-stress and stress yield of 274.65 and 199.03 kg/ha (Table 4).

Tolerance index (TOL)

TOL indicates the tolerance of black cumin ecotypes under stress, however in a reverse correlation. The greater TOL values indicate the ecotypes with a less tolerance and the less TOL values indicate the ecotypes with higher tolerance under stress. The least SSI (-80.28) was resulted by Semirom ecotype with the yield of 133.45 and 213.73 kg/ha under non-stress and stress conditions, respectively. The next minimum values were related to Yasuj (-44.92) with the non-stress yield of 144.38 kg/ha and stress yield of 189.30 kg/ha (Table 1). The highest SSI values were related to Arak (75.62) with the non-stress and stress yields of 274.65 and 199.03 kg/ha (Table 4).

Geometric mean productivity (GMP)

GMP is a similar index to STI and its higher values indicates higher crop tolerance under

Table 3
Mean grain yield and drought tolerance indices of the studied genotypes under irrigated and drought stress conditions.

cultivar	Yp	Ys	tol	MP	GMP	YSI	STI	SSI
Arak	81.59	32.88	48.71	57.24	51.79	0.40	0.11	183.53
Yasuj	143.68	140.28	3.40	141.98	141.97	0.98	0.83	7.27
Semirom	133.45	213.73	-80.28	173.59	168.89	1.60	1.18	-184.93
Azarbaijan	144.38	189.30	-44.92	166.84	165.32	1.31	1.13	-95.64
Torbat Heydarieh	274.65	199.03	75.62	236.84	233.80	0.72	2.26	84.64

Table 4
Correlation coefficient between yields under irrigated (Yp) and drought stress (Ys) conditions and drought tolerance indices

Variables	Yp	Ys	tol	MP	GMP	YSI	STI	SSI
Yp	1							
Ys	0.607	1						
tol	0.415	-0.471	1					
MP	0.893*	0.900*	-0.038	1				
GMP	0.892*	0.900*	-0.040	0.999**	1			
YSI	-0.038	0.770	-0.924*	0.415	0.417	1		
STI	0.959**	0.795	0.155	0.977**	0.973**	0.228	1	
SSI	0.038	-0.770	0.924*	-0.415	-0.417	-1.000**	-0.228	1

* and **: Significant at 5% and 1% probability levels, respectively

stress. Accordingly, Torbat Heydarieh with the GMP index of (233.80) was the ecotypes with the highest GMP values. According to this index the least tolerant ecotype was Arak 51.79 (Table 3).

Stress tolerance index (STI)

The STI index is the stress tolerance index and its higher values indicate the ecotypes that are more tolerant under stress. Accordingly, Torbatheidarieh (2.26) was the ecotype with the highest stress tolerance index and Arak (0.11) was the ecotype with the least rate of tolerance (Table 3).

Mean productivity (MP)

MP is the average of ecotype yield under non-stress and stress conditions and its higher values indicate higher yield potential under such conditions. Accordingly, Torbat Heydarieh with the MP index of 236.84 was the ecotype with the highest rate of MP values. The least rate of MP values was related to Arak (57.24) (Table 3).

Correlation coefficients between the stress indices of black cumin ecotypes grown under drought stress conditions are shown in Table 4. The performance of ecotypes under normal conditions (Yp) highly correlated with the MP (0.89), GMP (0.89), and STI (0.95). The GMP index also highly correlated with the STI (0.97). Alternatively, the Ys correlated with the MP (0.9) and GMP (0.9). There was a high positive correlation between the TOL and SSI (0.92) as well as YSI (-0.92). The correlations between the yield under normal and stress conditions were found not significant. Such insignificant correlations indicate that the ecotypes with high performances under normal conditions did not yield similar responses under stress conditions, and thus the tolerance levels of the ecotypes were different from each other.

Principal Component Analysis (PCA) and Biplot Display

Table 5 shows the data of PCA analysis. The first second components with Eigen value more than 1, could explain 99.8% of total variance. The PC1 contributed to 63.5% of the explained

Table 6
Eigen values, percent of variation, cumulative percentage and Eigen vectors for drought tolerance indices

	F1	F2	F3	F4
Yp	0.727	0.686	-0.020	0.008
Ys	0.987	-0.161	0.001	-0.010
tol	-0.322	0.946	-0.023	0.020
MP	0.958	0.286	-0.010	-0.002
GMP	0.958	0.283	-0.040	-0.013
YSI	0.658	-0.753	-0.010	0.016
STI	0.882	0.466	0.076	0.005
SSI	-0.658	0.753	0.010	-0.016
Eigen value	5.086	2.905	0.009	0.001
Variability (%)	63.570	36.307	0.108	0.016
Cumulative %	63.570	99.876	99.984	100.000

variance which mainly correlated with Ys (0.98), MP (0.95), STI (0.88), GMP (0.95), and Yp (0.85) indices. The PC2 loading plot contributed to 36.3% of the explained variance and mainly contributed to TOL (0.94) and a negative correlation with the YSI (-0.75).

The score plot PC1 and PC2 distinguished three groups of populations of black cumin. The first group was Torbat Heydarieh which was distinguished by the vectors of the Yp, GMP, STI, and MP (Fig. I). The second group was Semirom, Yasuj and Azarbaiejan which was separated by the vectors of Ys and YSI. The third group consisted of Arak which was further separated by the vectors of Tol and SSI (Fig. IV).

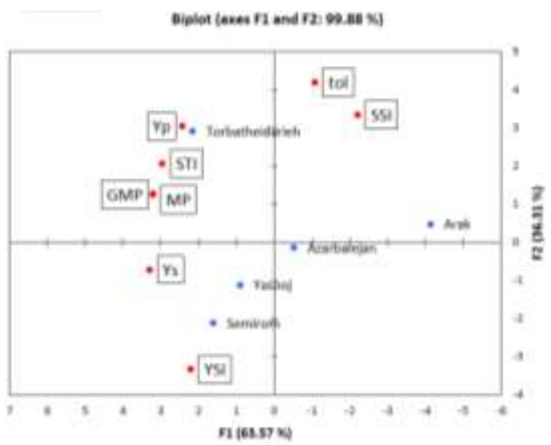

Fig. IV. Principal component analysis of trial cultivars based on the first two components.

Discussion

This paper was performed to investigate physiological responses of 5 ecotypes of black cumin plant to drought stress condition. The value of 3 major unsaturated fatty acids namely, oleic acid, linoleic acid, and linolenic acid were analyzed. As shown in Table 2, there were differences among trial ecotypes for the oil percentage and fatty acids. This variation in ecotypes in response to drought stress is found in the studies reported by other researchers (e.g., Bagheri and Sam– Daliri, 2011). Based on our research, in water stress condition, Torbat Heydarieh had more potential to produce oleic acid (Table 2). Therefore, the oil produced by N. sativa in this condition had more resistance to high temperature. Oleic and linoleic acid production reduced during stress condition while linolenic content increased during drought stress condition. Similarly, Torbat Heydarieh had the highest value of linolenic acid among the plants under study. This evidence shows the prime position of Torbat Heydarieh regarding the quality of fatty acid content. However, the interaction between condition × ecotype indicated that stress had no significant effect on Torbat Heydarieh regarding these fatty acids.

According to drought tolerance indices, Torbat Heydarieh obtained the highest MP, GMP, and STI; also, significant correlations between yields under normal and drought stress conditions and MP and GMP indices were observed. Sio-Se

Mardeh et al. (2006) showed that under moderate drought stress condition, STI, GMP, and MP were able to identify bread wheat cultivars producing high yield in both normal and drought stress conditions. While in severe drought stress condition, SSI was a more useful index to distinguish tolerant cultivars. So considering the climatic condition in Iran which is affected by drought stress, Torbat Heydarieh variety is recommended for planting in arid and semi-arid regions.

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