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Evaluation of Diverse Cumin (*Cuminum cyminum* L.) Ecotypes for Seed Yield under Normal and Water Stress Condition

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ABSTRACT

Cumin (*Cuminum cyminum* L.) is the second most popular spice in the world and one of the important medicinal plants in Iran. Cumin seed yield is highly affected by water stress, which is one of the most important abiotic stresses affecting seed yield. So far, drought tolerance studies in cumin have been done on limited cumin ecotypes. In the present investigation, forty-nine diverse cumin ecotypes were tested under normal and water stress conditions during 2013 and 2014. The experiment was conducted under two different irrigation regimes of normal irrigation and mid/late season water stress i.e., during flowering. Each of experiments was conducted in a simple lattice design with two replications. The combined analysis of variance showed significant differences among all sources of variation. Twelve drought tolerance indices were calculated based on seed yield under drought and irrigated conditions. Yield under stress and non-stress conditions was significantly and positively correlated with Geometric Mean Productivity Geometric Mean Productivity (GMP), Stress Tolerance Index (STI), Harmonic Mean (HM), Drought Resistance Index (DI), modified Stress Tolerance Index in normal irrigation (K1STI), modified Stress Tolerance Index in stress irrigation (K2STI), Stress Non-stress Production Index (SNPI) and Stress Tolerance Score (STS). PCA and cluster analysis were followed to reveal the relationship among different indices. To visualize the GE interaction effects on cumin seed yield, the data were subjected to GGE-Biplot analysis. Finding superior ecotypes in each environment was done using GGE-Biplot. Regarding mean yield and drought tolerance indices, ecotypes from Maneh (Northern Khorasan), Shahmirzad (Semnan), and Rafsanjan (Kerman) were identified as the most favorable candidates for further research in cumin breeding programs. GC/MS analyses of elite ecotype Kerman (Rafsanjan) was also done for both conditions, the main components of essential oil were found to be *γ*-terpinene, *β*-pinene, m-cymene, and cuminic aldehyde.

Keywords: Drought tolerance indices, Ecotypes, GC/MS, GGE-Biplot, Terminal water stress.

INTRODUCTION

Cumin (*Cuminum cyminum* L.), the king of seed spices (Lal *et al.*, 2014), belongs to Apiaceae (Umbelliferae) family and is valued for its aroma and medicinal and therapeutic properties (Sowbhagya, 2013). It is the second most popular spice in the world after black pepper (Lodha and Mawar, 2014). Today, India, Iran and Turkey are the main exporters of cumin seeds in the world. Iran, is one of the leading producers of cumin in the world (Sowbhagya, 2013). In Iran, cumin is cultivated on approximately 22,000 hectares, which is much different in terms of the area under cultivation compared to other medicinal plants from Apiaceae family (Anonymous, 2009). In Iran, it is grown mainly in arid and semi-arid regions in Eastern, South-Eastern and Central provinces (Hashemian *et al.,* 2013). Iran and Turkey have the same level of production and both stand third in the leading producers' list in the world (Sowbhagya, 2013). The value of exports of this product

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for Iran was estimated at 4,728,842 dollars in 2013 (Tehran Chamber of Commerce, Industries, Mines & Agriculture, 2013). Cumin is a very good source of iron and manganese (Parthasarathy *et al.*, 2008) and possess antioxidant, anticancer, stimulant, and carminative pharmacological properties (Ravi *et al.*, 2013). Cumin flavor is due to volatile oil present in the seeds which varies depending on the variety and its origin (Sowbhagya, 2013). The chemical composition of oil of cumin (*C. cyminum*) shows variations which is attributed to the difference in the geographical localities or varieties (Hanafi *et al.*, 2014). Drought is probably the most important abiotic stress limiting plant growth and crop productivity globally (Saint Pierre *et al.*, 2012). Climate change induced temperature increase is estimated to reduce plant yields all over the world (Shiferaw *et al.*, 2013). Meanwhile, there are some reports indicating the positive effect of moisture deficiency in biosynthesis of secondary metabolites, antioxidant accumulation, and enzyme activities in medicinal plants (Sangwan *et al.*, 2001).

Drought tolerance is a complex quantitative trait, involving interactions of many metabolic pathways related to stress tolerance genes (Abdolshahi *et al.*, 2013). If the strategy of a breeding program is to improve yield in both stress and non-stress environments, it is noted that selection should be based directly on yield parameter under both conditions, when a breeder is looking for genotypes adapted to a wide range of environments (Sio-Se Mardeh *et al.*, 2006). Crop breeding for complex traits require accurate selection criteria based on appropriate biometric models (Cruz, 2013).

Different selection indices on the basis of mathematical relationships between stress and non-stress conditions have been suggested for screening stress-tolerant genotypes. Stress Susceptibility Index (SSI), Yield Stability Index (YSI), Tolerance Index (TOL), Stress Tolerance Index (STI), (GMP), Harmonic Mean (HM), Drought Resistance Index (DI), modified Stress Tolerance Index in normal irrigation (K1STI), modified Stress Tolerance Index in stress irrigation (K2STI), Stress Non-stress Production Index (SNPI), Abiotic Tolerance Index (ATI) and Stress Tolerance Score (STS) have all been employed under various conditions. Fischer and Maurer (1978) explained that cultivars with an SSI of less than one are stress tolerant, since their yield reduction under stress conditions is smaller than the mean yield reduction of all cultivars (Bruckner and Frohberg, 1987). The genotypes with a high *YSI* are expected to have high yield under both stress and non-stress conditions (Bouslama and Schapaugh, 1984). *STI* and *GMP* were reported as preferred criteria in selection of drought-tolerant cumin genotypes (Motamedi-Mirhosseini *et al.*, 2011). The landraces with high value of *HM* are considered desirable (Gholinezhad *et al.*, 2014). Lan (1998) defined *DI*, which was commonly accepted to identify genotypes producing high yield under both stress and non-stress conditions. *K1STI* and *K2STI* are modified stress tolerance index indicating the ideal genotypes in normal and stress conditions, respectively. Mousavi *et al.* (2008) introduced *SNPI* and *ATI* as powerful indices for screening drought tolerant genotypes in stress and non-stress conditions. The genotypes with high value of this index will be suitable for drought stress condition. Since multivariate techniques were too complicated, Abdolshahi *et al.* (2013) proposed linear equation, *STS*, based on several indices. In this equation, indices in which large values represent more tolerance to stress have positive sign, while the sign is negative for other indices in which smaller value represent more tolerance (Abdolshahi *et al*., 2013). The Genotype and Genotype-by-Environment (GGE) Biplot method is a multi-faceted tool in quantitative genetic analyses and plant breeding has strongly captured the imagination of plant breeders and production agronomists (Yan and Kang, 2003). It helps to visualize the interrelationships among genotypes or environments. Moreover, it is a versatile tool

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to find superior genotypes in each environment visually (Mortazavian *et al*., 2014).

The objectives of the present study were: (1) To investigate the performance of 49 major cultivated cumin ecotypes under normal irrigation and water stress after flowering during two years, (2) Identification of the best promising cumin ecotypes for drought prone areas using GGE-Biplot analysis, (3) To compare selection indices for their relative effectiveness, and (4) To investigate the effect of different water regimes on volatile composition of essential oil and characterization of compounds in elite ecotype of *Cuminum cyminum* L.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

The study was conducted during crop years 2013 and 2014 at research farm of College of Aburaihan, University of Tehran, Iran, in Pakdasht (33º 28′ N, 51º 46′ E and 1,180 m altitude). Forty nine cumin ecotypes from different provinces of Iran (Table 1) were evaluated under two irrigation regimes i.e., normal irrigation and moisture stress in both years of the study. The experimental design for both conditions was lattice design with two replications and efficiency of lattice design over Randomized Complete Block Design (RCBD) was calculated. After data adjustment, combined analysis of variance under two water conditions (low water and normal) according to RCBD was followed for both years. The seeds were sown manually during the third week of February in both years at a depth of 1.5 to 2 cm of soil in plots of 2 m long with four rows for each ecotype. There was 60 cm distance between each experimental plot and the distance between plants was 5 cm in each row. Soil texture was silty clay and was sampled from zero to 50 cm depth and analyzed for various properties (pH 7.4 and

Table 1. Province, city and codes of evaluated cumin ecotypes.

Code	Province	City
E01	Esfahan	Ardestan
E02	Esfahan	Feridan
E03	Esfahan	Khansar
E04	Esfahan	Naien
E05	Esfahan	Natanz
E06	Esfahan	Semirom
E07	Golestan	Aq-Qala
E08	Golestan	Gonbad
E09	Golestan	Jat
E10	Golestan	M-Tapeh
E11	Kerman	Baft
E12	Kerman	Bardsir
E13	Kerman	Chatrood
E14	Kerman	Joopar
E15	Kerman	K-banan
E16	Kerman	Mahan
E17	Kerman	Rafsanjan
E18	Kerman	Ravar
E19	Kerman	Sirjan
E20	Kerman	Zarand
E21	Khorasan-R	Bardsekan
E22	Khorasan-R	Ferdows
E23	Khorasan-R	Gonabad
E24	Khorasan-R	Kashmar
E25	Khorasan-R	Taybad
E26	Khorasan-R	Torbat-H
E27	Khorasan-R	Torbat-J
E28	N-Khorasan	Maneh
E29	N-Khorasan	Bojnord
E30	N-Khorasan	Esfarayen
E31	N-Khorasan	Shirvan
E32	Fars	Estahban
E33	Fars	Sarvestan
E34	Fars	Sepidan
E35	Fars	Sivand
E36	Semnan	Ivanaki
E37	Semnan	Kalateh
E38	Semnan	Shahmirzad
E39	Semnan	Sorkheh
E40	S-Khorasan	Birjand
E41	S-Khorasan	Darmian
E42	S-Khorasan	Nahbandan
E43	S-Khorasan	Qaen
E44	S-Khorasan	Sarayan
E45	Yazd	Ardekan
E46	Yazd	Bafq
E47	Yazd	Khatam
E48	Yazd	Sadoq
E49	Yazd	Sadroea

EC 3.55 mS cm^{-1}, no manure applied).

Field Capacity (FC) of soil was determined before the experiment. Water stress was applied from flowering stage and there was no rainfall from this stage until plant harvest. To determine the volume of water to be applied per irrigation, soil was sampled from 0 to 30 cm depth, the day before the anticipated irrigation time and soil moisture content was determined. The amount of water needed to reach the field capacity (normal condition) and 30% of field capacity (drought stress) was applied. Cumin is semi drought-tolerant plant and normally planted in arid area, therefore, 30% of *FC* (severe stress) was selected. Amount of water was calculated by Michael and Ojha (1966) formula. Weeds were controlled by hand from the beginning of spring and up to the growth cycle, as per need. Seed yield (g) was calculated on the basis of plot area (m^2).

Drought Resistance and Susceptibility Indices

Twelve drought resistance indices were calculated using the following formulas:

$$SSI = \frac{1-\left(\frac{Ys}{Yp}\right)}{1-\left(\frac{\bar{Ys}}{\bar{Yp}}\right)}$$ (Fischer and Maurer, 1978) (1)

$$YSI = \frac{Ys}{Yp}$$ (Bouslama and Schapaugh, 1984) (2)

$$TOL = Yp - Ys$$ (Hossain *et al.*, 1990) (3)

$$STI = \frac{Ys \times Yp}{\bar{Yp}^2}$$ (Fernandez, 1992) (4)

$$GMP = \sqrt{(Yp)(Ys)}$$ (Fernandez, 1992) (5)

$$HM = \frac{2\,(Yp)(Ys)}{Yp + Ys}$$ (Schneider *et al.*, 1997) (6)

$$DI = \frac{Ys \times \left(\frac{Ys}{Yp}\right)}{\bar{Ys}}$$ (Lan, 1998) (7)

$$K1STI = \frac{(Y_p)^2}{(\bar{Y}_p)^2} \times STI$$ (Farshadfar and Sutka, 2002) (8)

$$K2STI = \frac{(Y_s)^2}{(\bar{Y}_s)^2} \times STI$$ (Farshadfar and Sutka, 2002) (9)

$$SNPI = \left[\sqrt[3]{(Yp + Ys)/(Yp - Ys)}\right]\left[\sqrt[3]{Yp \times Ys \times Ys}\right]$$ (Mousavi *et al.*, 2008) (10)

$$ATI = \left[(Yp - Ys)/\left(\frac{\bar{Yp}}{\bar{Ys}}\right)\right] \times \left[\sqrt{Yp \times Ys}\right]$$ (Mousavi *et al.*, 2008) (11)

STS= YSI+STI+GMP+HM+DI+K1STI+K2STI+SNPI+ATI–TOL–SSI (12)

Where, Y_S is the yield of ecotypes under stress, Y_P the yield of ecotypes under normal conditions, $\bar{Ys}$ and $\bar{Yp}$ are the mean yields of all ecotypes under stress and non-stressed conditions, respectively. Equation (12) is not accurate for raw data (Abdolshahi *et al.*, 2013); hence, all indices in this equation were standardized and then *STS* was calculated.

Relationship among Indices and Multivariate Analysis

To understand relationships among indices for drought tolerance and seed yield in stress and normal conditions, correlation and multivariate Principal Component Analysis (PCA) were used. Biplot of two main components and quantitative indices of drought stress *Yp* and *Ys* were drawn to determine the best ecotype for each environment. All calculations were performed using IBM SPSS Statistics 21, SAS-based program and Excel software.

Graphic Presentation Using GGE-Biplot

GGE-Biplot based on the Sites REGression (SREG) linear-bilinear (multiplicative) model (Cornelius *et al.*, 1996) was used as below:

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$$Y_{ij} = \mu + \sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij} \quad (13)$$

Where, Y_{ij} is the mean response of genotype i in the environment j; μ is the overall mean; g_i is the fixed effect of genotype i (i= 1, 2, ... g); e_j is the random effect of environment j (j= 1, 2, ... e); ε_{ij} is the average experimental error; the $G\times E$ interaction is represented by the factors; λ_k is a unique value or singular value of k^{th} Interaction Principal Component Analysis (IPCA), (k= 1, 2, ... t, where t is the maximum number of estimable main components), α_{ik} is a singular value for the i^{th} genotype in the k^{th} IPCA, y_{jk} is a unique value of the j^{th} environment in the k^{th} IPCA; r_{ij} is the error for the $G\times E$ interaction (noise present in the data); and k is the characteristic non-zero roots, $k= [1, 2, ... min (G - 1, E - 1)]$. The model used for the *GGE* interaction Biplot analysis was the environment-centered model and no-scaling.

Essential Oil Isolation and GC/MS Analysis

Cumin seeds of elite ecotype, Kerman (Rafsanjan), from both conditions were finely grounded in an electric grinder. Fifty grams of each ground sample was subjected to hydro-distillation with 400 mL distilled water for 120 minutes using a Clevenger-type apparatus. The extraction was protected against light and kept in refrigerator at 5ºC. The yield of essential oil was expressed in % (w w^{-1}) dry basis. The essential oil of candidate ecotype, Kerman (Rafsanjan), in both water treatments, was analyzed by GC/MS using an Agilent 7890N GC coupled with a 5975C MS equipped with a Capillary, HP-5MS column (30 m×0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed in 60°C for 4 minutes raising 3°C min^{-1} to 100°C for 2 minutes and then 4°C min^{-1} to 225°C; injector temperature, 260ºC; detector temperature 270ºC; injection volume, 1 µL; carrier gas, Helium (1 mL min^{-1}); split ratio, 50:1. The identity of oil components was assigned by comparison of their retention indices relative to (C8–C22) *n*-alkanes with those in literatures or with those of authentic compounds already available. Compounds were identified by use of NIST, Wiley, NBS mass spectral library of the GC–MS data system and other published mass spectra.

RESULTS AND DISCUSSION

Analysis of Variance and Nature of GE Interaction

Conducting the experiment based on lattice design showed more efficiency (in average 127%) rather than RCB design. For further analysis, each data was adjusted using blocks within replications and intra

Table 2. Combined analysis of variance for the seed yield of 49 cumin ecotypes across four environments (normal and stress conditions during two years).

Source	*DF*	*MS*	Explained (%) of G+E+GE
Genotype	48	1713.03 **	16.50
Environment	3	118493.22 **	71.34
Condition	1	61629.33 **	
Year	1	283422.93 **	
Condition×Year	1	10427.39 **	
Genotype×Environment	144	420.62 **	12.15
Genotype×Condition	48	181.57 *	
Genotype×Year	48	917.40 **	
Genotype×Condition×Year	48	162.89 *	
Error	192	113.04	
Total	465		

**, * and ns: Respectively significant at the 0.01 and 0.05 probability levels and non-significant.

block error source of variations in lattice output. Then, combined analysis of variance was carried out for the drought stress treatments (moisture stress and control) from 2013 to 2014 based on RCBD. Condition×year combinations (i.e., four) were considered to be environments. Highly significant differences were detected among the tested ecotypes for seed yield, suggesting the presence of genetic variability among the ecotypes (Table 2). The significant interactions of *GEI* indicated some ecotypes possessing better performance than the tolerant ecotypes under moisture stress condition. There was significant genotype × year interaction indicating that genotype behavior in a condition between years had been different. The large seed yield variation due to environment is the main source of variation in most of the multi-environment trials (Gauch and Zobel, 1997). In the present study, the cumin seed yield was affected by environment, which accounted for 71.34% of sum of squares (E+G+GEI), whereas G and GE captured 16.5 and 12.15% of total sum of squares, respectively. It indicated that conditions and years contributed more to yield variance than genotypes and GE in this experiment. The focus on GE interactions as a component of plant adaptation is largely a consequence of the uncertainty they introduce into the process of selection among genotypes, particularly where this is based on their phenotypic performance in a relatively small sample of environments taken from the target population of environments (Cooper and Delacy, 1994). Taking the mean general yield as the first parameter for the assessment of the ecotypes, the mean yield of 49 cumin genotypes in four environments ranged from 11.24 g m^{-2} (E15) (the lowest value in stress 2014 environment) to 144.30 g m^{-2} (E28) (the highest value obtained in non-stress 2013 environment). Over all environments (stress and non-stress), ecotype 28 recorded the highest seed yield with an average of 105.07 g m^{-2} (Supplementary Table 1). On the other hand, ecotype 30 gave the lowest yield (20.53 g m^{-2}). Ecotypes 32 and 30 had the lowest and the highest yield variation across environments, respectively. For better evaluation of 49 cumin ecotypes for drought tolerance, twelve selection indices were used.

Resistant and Susceptibility Indices

As shown in supplementary Table 1, the greater the *TOL* and *SSI* values, the larger yield reduction under stress conditions and the higher drought sensitivity. A selection based on minimum yield reduction under stress condition in comparison with on-stress condition (TOL) failed to identify the most tolerant genotypes (Rizza *et al*., 2004). Rosielle and Hamblin (1981) reported that selection based on the tolerant index often leads to selecting cultivars which have low yields under non stress conditions. The greater *TOL* and *SSI* values, the greater sensitivity to stress, thus, a smaller value of these indices is favored. Five ecotypes with the lowest *SSI* and *TOL* values (ecotypes E17, E25, E41, E27 and E32) were identified as the most tolerant ecotypes. Ecotype 30 with the highest value of SSI and TOL identified as the most sensitive ecotype. The tolerant indices (STI, GMP, HM, DI, K1STI, K2STI, SNPI and STS) measure the higher stress tolerance and yield potential. Ecotypes E28, E38, E17 and E08 were the most tolerant ecotypes, whereas some ecotypes such as E30, E01, E12, E02, and E37 were the least relatively drought-tolerant ecotypes. Among these ecotypes, ecotype 28, with the highest mean yield in both conditions, was found as the most tolerant ecotype based on almost all quantitative indices.

Correlation Analysis among Indices

An important factor for the success of a plant breeding program in stressed environments is good performance of genotypes under severe stress conditions and

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maximum yield under optimum conditions. Seed yield under non stress condition was positively correlated with stress conditions (r= 0.8**) (Supplementary Table 2). Negative and positive correlations were observed between *SSI* and *YSI* with Yield under stress (Ys), respectively; while there were no significant correlations between *SSI* and *YSI* with irrigated Yield (Yp). In addition, a positive correlation was observed between *TOL* and irrigated Yield (Yp) and negative correlation between TOL and Yield under stress (Ys) (Supplementary Table 2), which suggested that SSI and YSI discriminate drought sensitive genotypes under stress condition and TOL discriminate drought sensitive genotypes under both conditions. Bouslama and Schapaugh (1984) stated that *YSI* evaluates the yield under stress of a cultivar relative to its non-stress yield, and can be an indicator of drought resistant genetic materials. So, the genotypes with a high *YSI* are expected to have high yield under both stress and non-stress conditions. In the present study, ecotypes E38 and E17 with the high *YSI* exhibited high yield under stress and non-stress conditions (Supplementary Table 1). Stress Tolerance Score (STS) showed high positive correlation with seed yield under both conditions. There was high coincidence between *STS* values and seed yield under both conditions. Abdolshahi *et al.* (2013) and Sardouie-Nasab *et al.* (2014) showed identical results of STS index to those of factor analysis. They noted that using *STS* was much easier to use than factor analysis. Totally, GMP, STI, HM, DI, ATI, K1STI, K2STI, SNPI, and STS were significantly correlated with both stress and non-stress yields in both years (Supplementary Table 2). STI, GMP, HM, K1STI, K2STI, and STS were better predictors of *Yp* and *Ys* than other indices under both drought stressed conditions and can be introduced as the most suitable indices to identify high yielding genotypes for both normal and stress conditions.

Principal Components Analysis

Principal component analysis was performed to assess the relationships between all attributes to identify superior ecotypes in both years. The first and the

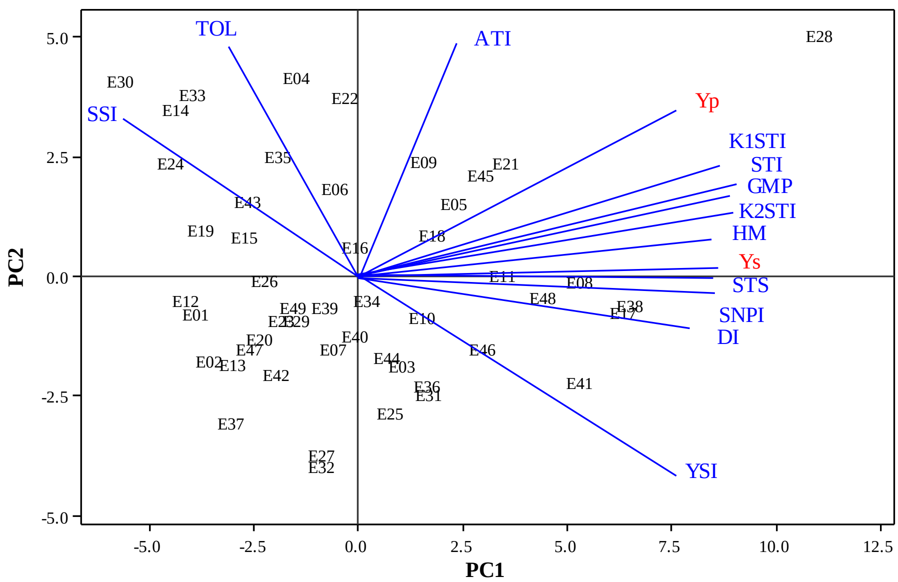

Figure 1. Biplot of principal component analysis of cumin genotypes and various drought tolerance indices in both years.

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second components justified, respectively, 72.7 and 24.6% of total variation (97.3% of total variation). Pattern analysis indicated that there were three distinct groups based on indices and mean yield under both conditions. The relationships among indices were graphically displayed in a Biplot of PC1 and PC2 (Figure 1). The *ATI*, TOL and SSI indices clustered in group I. YSI, SNPI, DI, Ys and STS were associated with group II and K1STI, STI, GMP, K2STI, HM and Yp were grouped in the third cluster. Indices in each group can select the same ecotypes because they consider similar aspects of ecotypes behavior, so, from each group an individual index could be used.

GGE Biplot Analysis of Cumin Ecotypes

Yield data from multi-environment trials are usually large, and their graphical presentation helps in understanding the pattern involved in particular data set. The GGE Biplot allows visual examination of *GE* interaction pattern of multi environment trials data (Sawargaonkar *et al.*, 2011). The GGE Biplot is shown in Figure 2. The first two principle components derived by subjecting the double-centered yield to Singular Value Decomposition (SVD), which make up a Genotype plus *GE* interaction (GGE) Biplot, explained 75% and 13% of the total *G+GE*, respectively (Figure 2). The acute angles between two stress vectors and two normal vectors suggested that *GE* was affected by conditions more than years, and each condition, regardless of each year, tend to discriminate among genotypes in a similar manner. Stress condition in the second year had the longest environment vector which demonstrated more discriminating ability

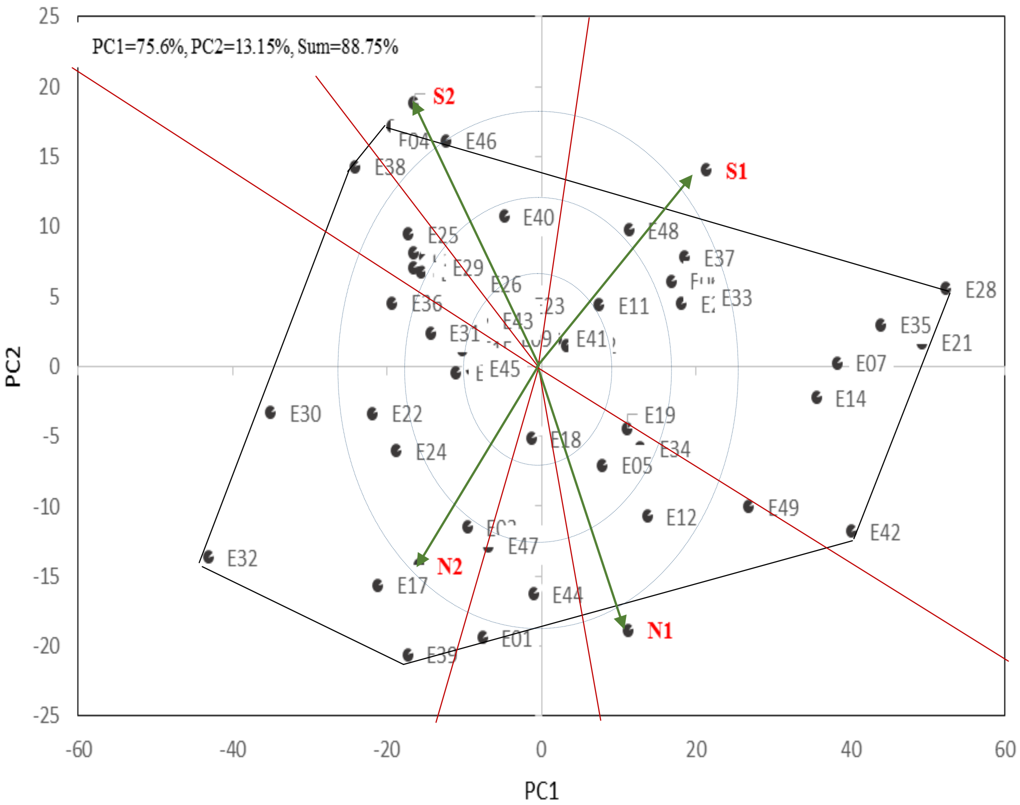

Figure 2. GGE Biplot identification of winning ecotypes and their related environments.

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than the other environments (Figure 2). The Biplot for seed yield was divided into 6 sectors with ecotypes E28, E42, E39, E32, E38, and E4 as the corner ecotypes. The corner ecotypes that are the most responsive ones can be visually determined from the Biplot shown in Figure 2. In each sector, the corner ecotypes that are farther along the positive direction of vector tend to give higher yields, and are better adapted to those environments. Those ecotypes within the polygon (for example, ecotype 41) were less responsive to environments than the corner ecotypes. The performance of ecotypes in relation to candidate ecotypes (E17 and E28) is reflected in Figure 3. Two candidates were connected by a line and a perpendicular line which passed through the biplot origin was made. Based on the figure, N2 is located on the same side of the perpendicular line as E17, suggesting that E17 should have a greater value than E28 relative to N2. On the contrary, S1 is located on the other side of the perpendicular line, i.e., on the same side as E28, suggesting that E28 has greater value than E17 with regard to S1 (Figure 3).

Oil Yield and Chemical Composition in Both Conditions

The characterization of essential oil from candidate ecotype (e.g. E17, Kerman-Rafsanjan) by GC/MS analyses, allowed the identification of 19 volatile constituents, accounting for higher than 95% of the total oil composition. The essential oil in low irrigation condition (1.59%) was higher than normal condition (1.2%) indicating that water stress caused more accumulation of essential oils in seed organ. An increase of essential oil under a limited water relative to non-water-stressed controls and heating of soil (Bettaieb *et al.*, 2011) has been reported earlier in cumin (Bettaieb Rebey *et al.*, 2012) and other plants, e.g. lima bean (Viuda-Martos *et al.*, 2007), *Pimpinella anisum* L. (Križman *et al.*, 2006) and *C. carvi* L. (El-Sawi and Mohamed, 2002). In normal condition, there were 17 components while in stress condition it decreased to 12 components (Table 3). Bettaieb Rebey *et al.* (2012) also showed change in composition of cumin essential oils under water deficit.

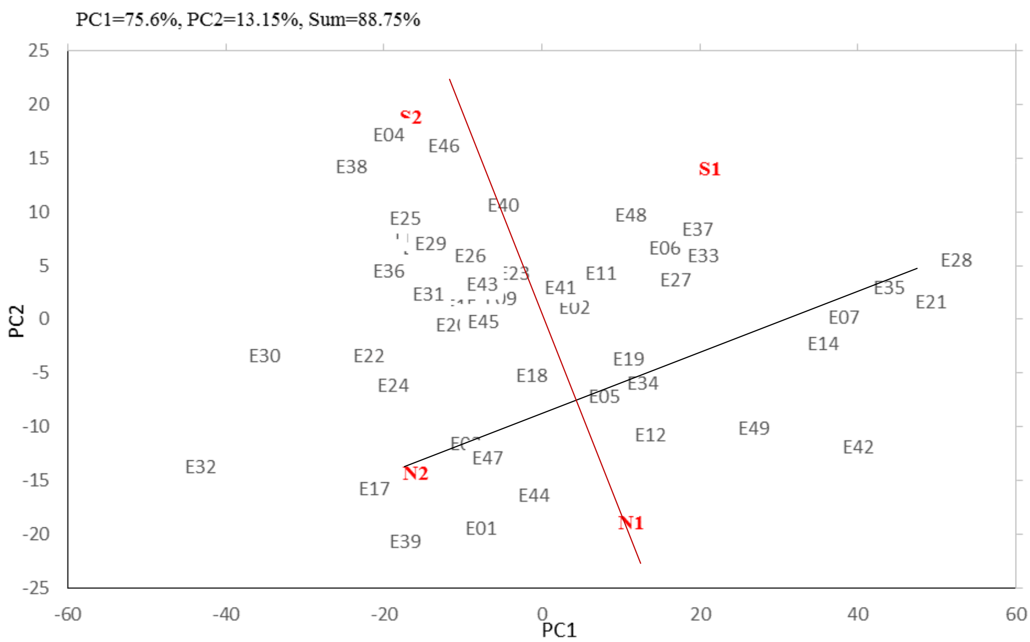

Figure 3. Visual comparison of two candidate ecotypes (E17 and E28) in different environments. GGE Biplot obtained from Site REGression (SREG) analysis.

Table 3. Essential oil composition of selected ecotype (Kerman-Rafsanjan) of *C. cyminum* L. in both conditions [Normal irrigation (N) and low water Stress conditions (S)].

Component			Water Condition		Component			Water Condition	
monoterpenic hydrocarbons	Formula	RI[a]	N	S	Monoterpenic Oxygenate	Formula	RI[a]	N	S
α-Pinene	$C_{10}H_{16}$	935	0.3639	0.3787	1,8-Cineole	$C_{10}H_{18}O$	1028	0.2224	-
Sabinene	$C_{10}H_{16}$	970	0.647	0.5005	cis-Sabinene hydrate	$C_{10}H_{18}O$	1066	0.2628	-
β-Pinene	$C_{10}H_{16}$	976	6.5913	7.4258	Pulegone	$C_{10}H_{16}O$	1230	-	2.2183
β-Myrcene	$C_{10}H_{16}$	991	0.7077	0.4869	Terpinene-4-ol	$C_{10}H_{18}O$	1175	0.4246	-
α-Phellandrene	$C_{10}H_{16}$	1001	3.4372	0.4193	Cuminic aldehyde	$C_{10}H_{12}O$	1223	57.482	53.32
α-Terpinene	$C_{10}H_{16}$	1017	0.2224	-	Phellandral	$C_{10}H_{16}O$	1274	0.2426	-
m-Cymene	$C_{10}H_{14}$	1021	6.935	16.935	Sesquiterpenic Oxygenate				
dl-Limonene	$C_{10}H_{16}$	1023	0.465	-	Carotol	$C_{15}H_{26}O$	1588	-	0.6763
β-Phellandrene	$C_{10}H_{16}$	1027	0.5257	-	Aldehyde				
γ-Terpinene	$C_{10}H_{16}$	1052	17.004	13.134	Nonanal	$C_9H_{18}O$	1101	0.5863	0.5951
Sesquiterpenic hydrocarbons					Oil yield [% (w w^{-1})]			1.2	1.59
β-Farnesene	$C_{15}H_{24}$	1450	-	0.5005					

[a] **RI:** Retention Index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

Change in secondary metabolism of *C. cyminum* under drought stress could be a defense mechanism and a biochemical adaptation to environmental constraints (Beis *et al.*, 2000). The main chemical compositions extracted from cumin seeds in both conditions were monoterpenic oxygenate (58.6% in normal and 55.5% in stress conditions) represented by cuminic aldehyde and monoterpenic hydrocarbons (36.9% in normal and 39.3% in stress conditions) mainly by *γ*-terpinene, *β*-pinene and m-cymene. *β*-pinene and *γ*-terpinene, which have antifungal activity against various fungi when treated as a sole component (Patra *et al.*, 2002) and cuminic aldehyde is the main factor of cumin odor and shows fungitoxic, fungicidal, antibacterial and larvicidal activity (Jirovetz *et al.*, 2005; Lawrence, 1992). Pulegone, β-Farnesene and Carotol were found only in stress condition, while α-Terpinene, dl-Limonene, β-Phellandrene, 1,8-cineole, cis-Sabinene hydrate, Terpinene-4-ol, and Phellandral were identified only in normal irrigated condition (Table 3).

In conclusion, farming systems in south Asia suffer mostly from heat stress and water scarcity due to climate change (Shiferaw *et al.*, 2013). The production of stable yield and economic profitability is an important and complicated issue for plant breeders and also agronomists. Using tolerant indices is the rational way to precise selection of the best genotypes. Because of multivariate nature of yield, many scientists suggest using multivariate approach on this trait. On the other hand, application of all tolerant indices simultaneously is a good approach for screening tolerant genotypes. *STS* index includes all tolerant indices, concomitantly. This index shows the same result as complicated multivariate analysis like factor analysis and linear discriminant function, beside, it is much easier. Then, it can be introduced as an efficient screening tool for identification of cumin drought tolerant ecotypes. We identified three ecotypes (E28, E38, and E17 belonging to

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Maneh, Shahmirzad and Rafsanjan, respectively) as the most drought tolerant ecotypes with the highest seed yield in both years and both conditions. These ecotypes can be recommended as promising ecotypes for drought areas or under limited available water conditions of Iran and can also be utilized in cumin breeding programs for further improvement of cumin germplasm for drought tolerance.

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ارزیابی اکوتیپ‌های مختلف زیره سبز (*Cuminum cyminum* L.) از نظر عملکرد دانه تحت شرایط نرمال وتنش آبی

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چکیده

زیره سبز (*Cuminum cyminum* L.) دومین گیاه ادویه‌ای مهم در دنیا و یکی از گیاهان دارویی مهم در ایران است. عملکرد دانه زیره به شدت متاثر از تنش آبی است که یکی از مهمترین تنش‌های غیرزنده موثر بر عملکرد دانه است. تاکنون، مطالعات مرتبط با تحمل به تنش خشکی در زیره بر روی تعداد محدودی از اکوتیپ‌های زیره انجام شده است. در مطالعه حاضر، ۴۹ اکوتیپ متنوع زیره طی دو سال متوالی تحت شرایط آبیاری نرمال و تنش آبی طی دو سال ۲۰۱۳ و ۲۰۱۴ مورد ارزیابی قرار گرفت. آزمایش تحت دو رژیم آبیاری نرمال و تنش آبی اواسط/انتهای فصل یعنی طی دوره گلدهی اجراشد. هر آزمایش در قالب یک طرح لاتیس ساده با دو تکرار پیاده شد. تجزیه واریانس مرکب اختلافات معنی‌دار بین کلیه منابع تغییر نشان داد. دوازده شاخص تحمل به خشکی براساس عملکرد دانه تحت شرایط خشکی و آبیاری نرمال محاسبه شد. عملکرد تحت شرایط تنش و بدون تنش همبستگی مثبت و معنی داری باGMP ، STI، HM، DI، K1STI، K2STI، SNPI و STS داشت. تجزیه خوشه‌ای و تجزیه به مولفه‌های اصلی برای نشان دادن ارتباط بین شاخص‌های مختلف محاسبه شد. برای نمایش اثرات برهمکنش متقابل GE بر عملکرد دانه زیره داده ها مورد تجزیه-GGE Biplotقرار گرفتند. شناسایی اکوتیپ‌های برتر در هر محیط با استفاده از GGE-Biplot انجام شد. با در نظر گرفتن عملکرد دانه و شاخص های تحمل به تنش خشکی، اکوتیپ‌های خراسان شمالی-مانه،

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