

Research Paper

Evaluation Drought Stress Indices and Yield Stability in Some Chamomile Ecotypes

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Abstract

Breeding for drought tolerance is an important objective of crop breeding programs in arid and semi-arid regions. A factorial experiment based on a randomized complete blocks design with three replicates was carried out to determine suitable drought tolerance indices in chamomile ecotypes under stress and non-stress conditions in 2018. According to the results of the analysis of variance, there was a significant difference among the ecotypes regarding plant yield under both environmental conditions (stress and non-stress). The highest average yield (0.568 and 0.665 g plant⁻¹) under stress and non-stress conditions was associated with Arak and Kerman ecotypes, respectively. Based on the plant yield of ecotypes under non-stress environments (Y_p) and moisture stress (Y_s), ecotypes were evaluated in terms of drought tolerance by six different indices of geometric mean productivity (GMP), stress tolerance index (STI), stress sensitivity index (SSI), tolerance index (TOL), mean productivity (MP), and harmonic mean (HM). According to the results obtained under both stress and non-stress conditions, SSI and STI were selected as the best indices for isolation of tolerant ecotypes. Based on these indices, biplot diagram, and mean comparison table, ecotypes of Mashhad, Khuzestan, and Kerman were identified as tolerant. Isfahan, Arak and Shiraz ecotypes were the most sensitive ecotypes to drought stress. Also, cluster analysis by Ward's method was used to group ecotypes based on plant yield; based on which the ecotypes were divided into 3 separate groups in both environments. Considering the results of this study, it is recommended to exploit the drought stress tolerance of indigenous chamomile populations of the country and conserve this valuable plant as valuable genetic resources.

1. Introduction

Nowadays, many studies have been carried out on medicinal plants. Medicines with natural active ingredients have opened new horizons before the pharmacists and medical practitioners. More than a third of the medicines used by people have plant origins, and their use is increasing (Hajizadeh-Sharafabad *et al.*, 2020). Chamomile (*Matricaria chamomile* L.) is among these medicinal plants.

Iran is among the regions in which major abiotic stress such as drought, salinity, heat, cold, lead to yield loss in a significant part of the country (Kafi, 2005). In most parts of the country, only a few crops may be cultivated due to limited water exploitation and inappropriate rainfall distribution. Without a doubt, the introduction of new plants for such regions may help to achieve

sustainable agricultural systems as well as diversifying the cultivated crops (Pezeshkpour and Mosavi, 2005).

Abiotic stresses are one of the most important factors leading to yield loss in crops in arid and semi-arid regions, and ameliorating their adverse effects have been considered a feasible method to improve crop yield. Among the abiotic stresses, water deficit, i.e. drought stress is the most important yield-limiting factor in arable crops and horticultural products all over the world (Blum, 2011; Longenberger *et al.*, 2006). Therefore, the development of drought-tolerant cultivars is a major goal in plant breeding programs. Selection of drought-tolerant cultivars also seems necessary for the medicinal plants due to the importance of their domestication and cultivation (Wei *et al.*, 2020). Irregular rainfall patterns in arid regions of the world make these regions prone to various intensities of drought stress. High temperature and poor nutritional

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status often exacerbate the adverse effects of drought. Development of drought-tolerant cultivars is highly regarded in Iran and is among the most important breeding programs, as approx. Two-third of the arable lands in Iran are arid and semi-arid (Anwaar *et al.*, 2020b). Thus, studying the tolerance of crops to drought is an important goal in plant breeding programs (El-Mouhamady *et al.*, 2019).

One of the methods to increase the quality and quantity of arable crops and horticultural products is plant breeding. Plant breeding is the art of selecting the best, which requires the presence of diversity regarding the studied attributes in the community. Awareness of diversity, in turn, requires germplasm evaluation (Bagheri *et al.*, 2020). (Alwala *et al.*, 2010) have suggested various methods for the evaluation of ecotypes under arid conditions, which include selection based on the potential yield, stability indices, a combination of yield and attributes correlating with yield, and yield under stress and non-stress conditions. (Sadat Sayyah *et al.*, 2012) defined drought tolerance as the ability of a genotype to produce higher yields compared with the other genotypes under similar moisture conditions, and this definition is highly considered by the breeders. (Aslam Khyber *et al.*, 2019) stated that the evaluation of yield under stress and optimum conditions is a starting point in the determination of drought-tolerant cultivars.

Various quantitative indices have been suggested to evaluate the response of ecotypes under different environmental stresses. In general, indices which have high correlations with yield under both stress and non-stress conditions are introduced as the best indices, as these indices are capable of determining the high-yielding plants under both conditions and may be used to estimate yield stability. One of the important issues in the evaluation of cultivars regarding drought tolerance is to measure these indices (Rab Faisal Sultan *et al.*, 2012). Accordingly, (Angelini *et al.*, 2019) suggested tolerance index (TOL) and Mean productivity (MP). High values of TOL indicate the sensitivity of the genotype to stress. Thus, the ecotypes with low TOL values are selected. (Mohammadi and Abdulahi, 2017) carried out an experiment to investigate the drought tolerance of some wheat cultivars in various stress intensities and introduced stress sensitivity index (SSI) as an index to screen the genotypes for drought tolerance. These researchers reported that all drought stress treatments significantly decreased the grain yield. Lower SSI values indicate lower variation in the yield of a genotype under stress and non-stress conditions. (Salehi *et al.*, 2016) suggested stress tolerance index (STI) and geometrical mean productivity (GMP) indices distinguish the drought-tolerant genotypes. Genotypes with higher STI values have higher stability. These

researchers reported that the correlation coefficient between GMP and STI is 1. (Ashkani, 2002) investigated spring safflower cultivars under optimum and limited irrigation conditions and suggested that MP, GMP and STI are the best quantitative drought tolerance indices. In another study, GMP and STI were determined as the best indices to evaluate drought tolerance of sunflower genotypes (Rezaee-Zadeh, 2007). According to a study on the wheat genotypes under stress and non-stress conditions, STI, MP and GMP were the most efficient indices to detect drought-tolerant genotypes (Mohammadi *et al.*, 2010). (Sio-se Mardeh *et al.*, 2006) evaluated 11 bread wheat genotypes and reported that STI, MP and GMP were suitable to detect high-yielding genotypes under mild stress. (Daneshvar Hosseini *et al.*, 2019) mentioned STI as a suitable index for breeding programs. (Moghadas-Zadeh Ahrabi *et al.*, 2012) evaluated inbred and hybrid lines of spring wheat and reported STI, MP and GMP as the most efficient indices to detect the best and worst lines. (Mahdavi *et al.*, 2012) suggested four indices of MP, GMP, HM and STI as the most suitable indices to screen barley genotypes due to their high correlation with grain yield under stress and non-stress conditions. Determination of yield adaptation and stability is a crucial and costly step of crop breeding programs which is rather difficult due to the interaction of genotype and the environment. This interaction will be important in the case of significance, and some genotypes show their superiority in different environments (Ghaedi Jeshni *et al.*, 2017). In recent decades, the utilization of graphical methods such as GGE biplot has become common in the investigation of genotype \times environment interaction. In this method, the effect of genotype and genotype \times environment interaction are not separated from each other, and the selection of stable cultivars is made based on both of the mentioned effects (Purdad and Jamshid Moghaddam, 2013).

Since the most of lands in Iran are arid and semi-arid and water shortages make the production of crops and medicinal plants difficult, the present study was carried out to determine the suitable indices to select the best chamomile ecotypes in terms of drought tolerance.

2. Material and Methods

This experiment was conducted in 2018 at the research greenhouse of Zabol University, Iran (31°13' N, 61°29' E) with 498.2 m elevation using eight chamomile ecotypes. The studied soil was sandy loam with a pH and organic matter of 7.3 and 0.46 percent, respectively. The experiment was carried out as factorial based on a randomized complete blocks with three replicates. The treatments included two drought stress levels (90% field capacity (FC) as control and 70% FC as stress) and

eight chamomile ecotypes (Esfahan, Mashhad, Shiraz, Kerman, Arak, Khuzestan, Germany and Hungary). All chamomile seeds were provided by Pakan Bazr company, Esfahan, Iran. The seeds were sown in 35 * 30 cm plastic pots, each pot serving as a replicate. The soil was first screened by sieve. Then, the pots were filled with the soil, and the seeds were sown in at 1 cm depth. The minimum and maximum temperatures of the greenhouse was regulated at 9 and 35°C and the pots were irrigated regularly when required. The stress was applied when the plants reached the 4-leaf stage using a time domain reflectometry device (TRIME type, IMKO, Germany). The plants were thinned at 4 and 8 leaf stages and four leaves remained in the pots in the last stage. To measure the plant yield, the plants were cut from the soil surface after flowering and were dried in the open air. Then, the total dry matter was calculated as yield per plant. Drought tolerance indices were calculated using the equations presented below:

$$SSI = \frac{1 - \frac{Y_{si}}{Y_{pi}}}{SI} \text{ and } SI = 1 - \frac{Y_{si}}{Y_{pi}} \quad (\text{Fischer and Maurer, 1987})$$

In which Y_{si} and Y_{pi} are the average yields of all genotypes under stress and non-stress conditions.

$$TOL = Y_P - Y_S \quad (\text{Rosielle and Hamblin, 1981})$$

In which Y_p and Y_s are the yield of a genotype under stress and non-stress conditions.

$$MP = \frac{Y_P + Y_S}{2} \quad (\text{Rosielle and Hamblin, 1981})$$

$$GMP = \sqrt{Y_p \cdot Y_s} \quad (\text{Fernandez, 1992})$$

$$STI = \frac{Y_p \cdot Y_s}{(Y)^2} \quad (\text{Fernandez, 1992})$$

$$\text{Harmonic mean} = HM = \frac{2 \cdot Y_p \cdot Y_s}{Y_p + Y_s}$$

Comparison of means was made using the LSD methods at $p < 0.05$. Cluster analysis based on Ward's method was used to classify the genotypes and determine the diversity among them as well as their distance. SAS V. 9.2, SPSS V. 19 and GGEbiplot V.7.0 software was used for the statistical analysis.

3. Results

The results of ANOVA for plant yield under stress and non-stress conditions showed that there was a significant difference among the ecotypes in terms of drought tolerance indices, except for HM. This indicates a high genetic variation among the ecotypes and the possibility of selection for stress tolerance (Table 1).

Comparison of means showed that the highest plant yields under stress and non-stress conditions were associated with Kerman (0.65 g plant⁻¹) and Arak (0.567 g plant⁻¹), respectively, whereas Mashhad ecotype had an acceptable yield under both stress and non-stress conditions (Table 2). The highest and lowest yield loss due to drought stress were observed in Kazeron (58.59 %) and Esfahan (27.16 %) ecotypes, respectively.

Among the studied ecotypes, Ardestan, Kazeron, and Kerman had the lowest plant yield under drought stress (Table 2). In terms of the quantitative drought tolerance indices, the highest value for TOL, GP and GMP were associated with Mashhad ecotype, and the lowest value for SSI and STI was related to Arak ecotype. The highest TOL and SSI were observed in Kerman and Kazeron biotypes, respectively, whereas the lowest value for these indices was associated with Arak biotype (Table 2).

Ardestan ecotype had the lowest MP value (Table 3). Selection based on MP results in the selection of ecotypes with high yields under non-stress and low yields under stress conditions. Mashhad ecotype had the highest MP, SSI, GMP and STI values.

The highest and lowest HM was obtained in Shiraz and Ardestan ecotypes, respectively. Ardestan ecotype had the lowest MP, STI and HM values.

Cluster analysis based on Ward's method was used to investigate the genetic diversity and ecotype classification, and the number of the clusters at 11 distance was determined three. The 1st cluster included Shiraz, Hungary and Arak, whereas the 2nd cluster included Kerman and Germany biotypes. Also, Mashhad, Khuzestan and Esfahan were placed in the 3rd cluster (Fig. 1).

In this study, the highest genetic distance was obtained between Shiraz and Esfahan ecotypes (Fig. 1). Placement of the population from different regions within the 1st, 2nd and 3rd cluster indicates that the phenotypic diversity does not follow the geographic diversity.

In this study, average and standard error were calculated for each cluster (A, B and C) under both conditions to investigate the share of the six studied indices in the generation of clusters (Table 4). The average phenotypic value of the 2nd cluster (B) for all attributes was higher than the total average. The 2nd cluster (B) with two ecotypes, had the highest average SSI value (2.24). The dendrogram showed that these two ecotypes were already distinguished from the others. The first cluster with three ecotypes had the lowest average.

The results of the biplot analysis are discussed below. In each section, it is tried to discuss the diagrams which present more comprehensive information. This type of biplot diagram divides the total study space into smaller parts and determines which attributes should be placed in each part so that the genotype placed in each part could be the best regarding the traits in that specific part.

As seen in Figure 2, the ecotypes are ranked in the cluster according to their yield. Kerman in SSI and Mashhad and Khuzestan in STI, GMP, MP, TOL, YP, YS and HM were the best and Esfahan, Arak and Shiraz were not placed in any indices.

Figure 3 illustrates the difference and similarity of the ecotypes. Kerman, Mashhad, Khuzestan and Hungary were more similar to each other, whereas Esfahan, Shiraz and Arak had a high similarity.

The relationship among the attributes is shown in Figure 4. It may be observed that the indices have a positive relationship with each other and have high correlation coefficients.

Average tester coordinate is used to investigate the stability and yield of the ecotype simultaneously. This type of biplot is also called average vs stability. Figure 5 illustrates the average yield of ecotypes defined by PC1 and PC2 according to all attributes. The blue line which is perpendicular to the red line and has two arrowheads is the average and shows the ecotypes lower and higher than the average. The red line shows the direction of the average increase. According to Figure 5, SSI and TOL indices have higher average yields, whereas STI demonstrated a lower yield. The yield of Mashhad, Kerman, Khuzestan and Hungary genotypes was higher than the average, whereas the yield of Esfahan, Shiraz and Arak genotypes was lower than the average. On the other hand, Mashhad and Khuzestan genotypes were stable whereas Arak genotype was unstable.

An ideal ecotype is an assumptive ecotype with the highest yield and stability and is placed in the centre of the concentric circles of the biplot. The level of ecotype utility depends on their distance from the ideal ecotype. The cultivars are ranked based on the means defined by PC1 and PC2, which according to Figure 6 is as follows: Mashhad ecotype is placed in the centre of the circles, which indicates being the best ecotype, followed by Khuzestan. Also, Arak is placed in the farthest place relative to the concentric circles.

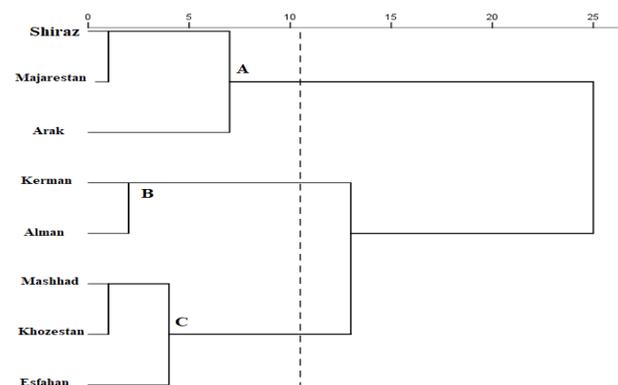


Fig. 1 Dendrogram of the cluster analysis of the chamomile ecotypes of based on the tolerance indices using wards method

Table 1 Analysis of variance for plant yield of chamomile ecotypes under stress and non-stress conditions

S.O.V	df	Mean of Squares							
		Yp	Ys	TOL	MP	SSI	GMP	STI	HM
Block	2	0.001 ^{ns}	0.002 ^{ns}	0.04 ^{ns}	0.49 [*]	0.12 ^{ns}	0.37 [*]	0.37 [*]	0.43 [*]
Ecotype	12	0.026 ^{**}	0.036 ^{**}	2.61 ^{**}	2.67 ^{**}	2.57 ^{**}	2.76 ^{**}	2.76 ^{**}	2.76 ^{ns}
Error	24	0.002	0.001	0.20	0.13	0.21	0.10	0.10	0.09
C.V.%	-	9.96	11.02	14.96	12.17	15.45	10.55	10.55	10.27

ns, * and **: No significant, significant at %5 and %1 level of probability, respectively

Yp= plant yield under non-stress condition, Ys= plant yield under stress condition, TOL=Tolerance Index, MP=Mean Productivity, SSI=Stress Susceptibility Index, GMP=Geometrical Mean Productivity, STI=Stress Tolerance Index and HM=Harmonic mean.

Table 2 Comparison of means for plant yield of chamomile ecotypes under stress and non-stress conditions and different drought tolerance indices

Ecotype	YP	YS	TOL	STI	SSI
Esfahan	0.439±0.022	0.557±0.020	-0.118±0.002	1.332±0.113	-1.420±0.094
Mashhad	0.612±0.058	0.409±0.018	0.204±0.076	1.348±0.070	1.641±0.516
Shiraz	0.378±0.028	0.408±0.029	-0.030±0.056	0.830±0.025	-0.521±0.768
Arak	0.329±0.004	0.568±0.034	-0.238±0.037	1.015±0.050	-3.787±0.623
Kerman	0.665±0.028	0.296±0.001	0.369±0.029	1.071±0.043	2.887±0.101
Khozestsn	0.515±0.012	0.430±0.010	0.021±0.085	1.202±0.024	0.854±0.194
Majarestan	0.524±0.010	0.365±0.004	0.159±0.011	1.038±0.023	1.583±0.078
Alman	0.438±0.022	0.394±0.010	0.028±0.044	0.938±0.039	0.502±0.296
LSD (P≤0.01)	0.100	0.096	1.02	0.72	1.05
LSD (P≤0.05)	0.079	0.071	0.75	0.53	0.78

Yp= plant yield under non-stress condition, Ys= plant yield under stress condition, TOL=Tolerance Index, MP=Mean Productivity, SSI=Stress Susceptibility Index, GMP=Geometrical Mean Productivity, STI=Stress Tolerance Index and HM=Harmonic mean

Table 3 Comparison of means for plant yield of chamomile ecotypes under stress and non-stress conditions and different drought tolerance indices

Ecotype	HM	GMP	MP
Esfahan	0.491±0.021	0.494±0.021	0.498±0.021
Mashhad	0.486±0.007	0.498±0.013	0.510±0.020
Shiraz	0.830±0.005	0.391±0.006	0.393±0.007
Arak	0.416±0.006	0.432±0.010	0.448±0.015
Kerman	0.409±0.005	0.444±0.009	0.481±0.013
Khozestsan	0.468±0.005	0.470±0.005	0.473±0.005
Majarestan	0.430±0.005	0.437±0.005	0.444±0.006
Alman	0.414±0.009	0.415±0.009	0.416±0.010
LSD (P≤0.01)	0.70	0.72	0.83
LSD (P≤0.05)	0.51	0.53	0.61

Yp= plant yield under non-stress condition, Ys= plant yield under stress condition, TOL=Tolerance Index, MP=Mean Productivity, SSI=Stress Susceptibility Index, GMP=Geometrical Mean Productivity, STI=Stress Tolerance Index and HM=Harmonic mean

Table 4 Mean and standard error of drought tolerance indices in different clusters of chamomile ecotypes under both conditions

Indices studied								Mean and standard error	Cluster
HM	STI	GMP	SSI	MP	TOL	Ys	Yp		
0.41	0.93	0.41	-1.27	0.42	-0.07	0.38	0.46	Mean	A
0.01	0.05	0.01	1.29	0.02	0.08	0.03	0.06	Mean standard error	
0.42	1.05	0.44	2.24	0.46	0.26	0.59	0.33	Mean	B
0.01	0.02	0.00	0.65	0.02	0.10	0.03	0.07	Mean standard error	
0.48	1.29	0.49	0.36	0.49	0.06	0.52	0.47	Mean	C
0.01	0.05	0.01	0.92	0.01	0.09	0.05	0.05	Mean standard error	

4. Discussion

The lower the TOL index, the lower the sensitivity of the ecotype to drought. However, selection based on TOL will lead to the selection of ecotypes with low yields under non-stress and high yields under stress conditions (Baghalian et al., 2011). Therefore, this index may not be suitable by itself. Selection based on SSI also leads to the selection of stress-tolerant ecotypes with low yield potential (Baghalian et al., 2011). Thus, this index Results of a study on the bread wheat showed that GMP, MP and STI indices efficiently detected the high-yielding genotypes under both stress and non-stress conditions (Mohammed and Kadhem, 2017). In another study, GMP, MP and STI were efficient in the detection of high-yielding fenugreek genotypes under stress and non-stress conditions, whereas YI, SSI, TOL and SSPI were only efficient under stress conditions (Choudhary et al., 2017). Investigation of the correlation between yield under stress and non-stress conditions and drought tolerance indices showed that STI, MP and GP were suitable for the selection of high-yielding genotypes under stress and non-stress conditions, and STI was the best index (Zabrjadi et al., 2013). is not able to detect the high-yielding ecotypes under both conditions.

Many studies have been done on the diversity and distance of ecotypes, and cluster analysis is among the methods which are very useful in the genetic and geographic diversity, selection of suitable individuals and investigation ecotype interactions studies. Investigation of diversity is possible via studying the similarity of the samples and to classify the individuals, the individuals in each group must have minimum differences and maximum similarities (Habibi et al., 2006). One of the applications of cluster analysis is to determine the genetic distance among the groups (Farshadfar, 2002).

In this study, the highest genetic distance was obtained between Shiraz and Esfahan ecotypes (Fig. 1), and due to this distance, it is expected to achieve the highest genetic diversity by crossing these ecotypes. The offspring of these two ecotypes could be used as the raw material for plant breeding (Farshadfar, 2002). Placement of the population from different regions within the 1st, 2nd and 3rd cluster indicates that the phenotypic diversity does not follow the geographic diversity. This may be due to the probability that the ecotypes collected from a province or country had been imported from another province or country. For instance, in the 2nd cluster, it may be stated that the origin of the Kerman ecotypes may be Germany.

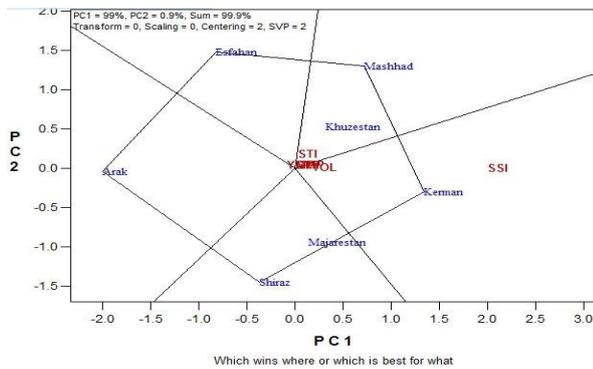


Fig. 2 Evaluation of plant yield among the chamomile ecotypes based on clusters

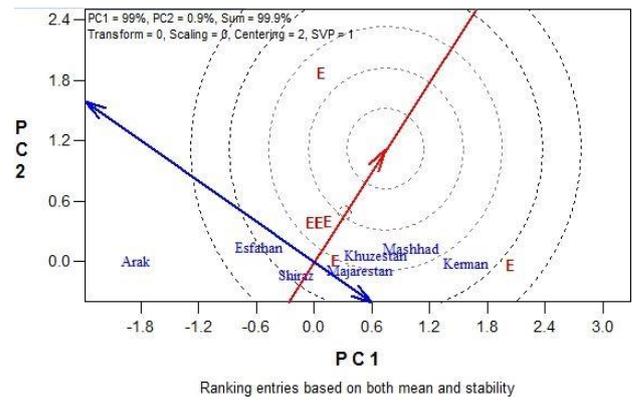


Fig. 6 Identification of the ideal chamomile using biplot analysis

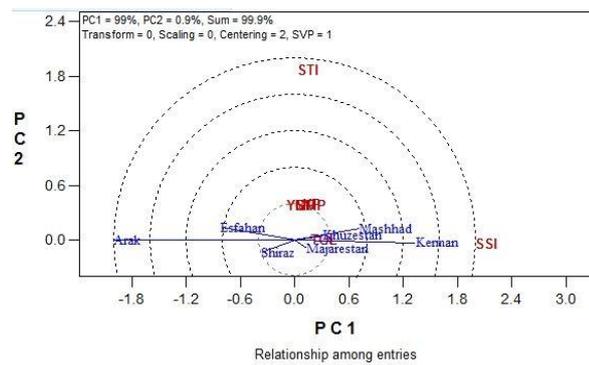


Fig. 3 Relationship between the chamomile ecotypes according to the studied trait

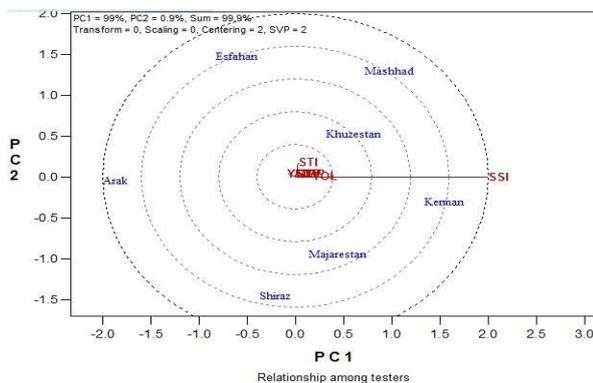


Fig. 4 Relationship between the studied traits using the biplot results

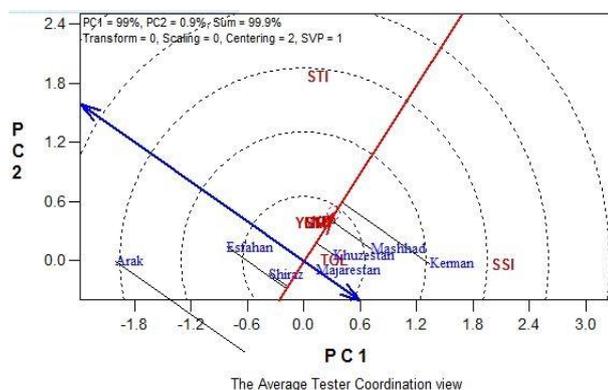


Fig. 5 Average yield of chamomile ecotypes using biplot analysis relative to tolerance indices

The researchers reported that the phenotypic diversity was not in accordance with the geographic diversity in chamomile, sesame and mint), which may be due to the transfer of raw materials between the different regions of the country (Zeynali, 2003; Mehdi Khani *et al.*, 2006).

The yield of Mashhad, Kerman, Khuzestan and Hungary genotypes was higher than the average, whereas the yield of Esfahan, Shiraz and Arak genotypes was lower than the average, On the other hand, Mashhad and Khuzestan genotypes were stable whereas Arak genotype was unstable. The results of an experiment conducted on 15 wheat genotypes showed that there was a significant difference in terms of grain yield among the genotypes. Indices of STI, MP and GMP had the highest correlation with grain yield under stress and non-stress conditions. Results of the principal component analysis showed that the 1st and 2nd components described 97.87% of variations (Hooshmandi *et al.*, 2019).

(Daneshvar Hosseini *et al.*, 2019) reported that the grain yield of wheat under stress and non-stress conditions are highly correlated with MP, GMP and TOL.

In an experiment regarding the grain yield stability of wheat under drought stress, it was reported that the grain yield had a high negative correlation with TOL, drought index (DI) and SSI indices, whereas the correlation of this attribute with MP and GMP was positive. These results indicate that the tolerant genotypes may be selected based on high MP and GMP and low SSI and TOL values (Anwaar *et al.*, 2020a).

5. Conclusion

The results of the present study indicate that the yield of the accessions had significant differences under stress and non-stress conditions, and this variation may be exploited to select the ecotypes suitable for both stress and non-stress conditions. Furthermore, it seems that SSI and STI are suitable indices to select the best ecotype, as the ecotypes selected based on these indices

are expected to have high yields and tolerance to drought stress. In this experiment, Mashhad and Khuzestan were the best ecotypes based on these two indices. They had high drought tolerances and were high-yielding under both conditions. The results of the biplot method to select the high-yielding and drought-tolerant ecotypes based on the indices were in accordance with each other. The dendrogram resulted from the cluster analysis illustrated a significant variation among the ecotypes. Overall, the chamomile ecotypes vary in their response to drought stress based on the studied indices, which indicate the high variation among the ecotypes.

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