



# Genetic Dissection and Half-Sib Progeny Test for Selecting Elite Parents to Develop Drought-Tolerant Varieties of *Cuminum cyminum* L.

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## ABSTRACT

Cumin (*Cuminum cyminum* L.) is an annual, outcross plant with medicinal properties. It is adapted to arid and semi-arid regions of the world. This research aimed to evaluate genetic parameters such as GCA and genetic distance based on molecular markers and important traits for parent selection among diverse genotypes of cumin. For this purpose, ISSR markers and a polycross assay were conducted. Forty-nine half-sib families were obtained from the polycross test and were evaluated for two years under normal and drought stress conditions. The measurements were aimed at 1000-seed weight, mechanized harvest height, and seed yield. The statistical analysis guided a selection of elite parents due to the high genetic diversity among half-sib families. An estimation of narrow sense heritability and general combining ability of the studied traits revealed the contribution of both additive and non-additive effects in genetically controlling the evaluated traits. According to the general combining ability, drought tolerance indices and genetic distance of 49 genotypes, the top 10 candidates were selected for the production of drought-tolerant seeds. From a molecular perspective, the banding patterns obtained from ISSR markers showed significant genetic diversity among all genotypes and indicated a proper distance among ten selected parents. The findings of this experiment provided a starting point for cumin breeding. It is likely that a breeding program would proceed with random pollinations among the ten selected genotypes under controlled conditions to produce high-yield drought-tolerant varieties.

## Introduction

Drought stress usually causes damage to plants and affects plant health, especially in countries that are more vulnerable to climate change. Developments in cultivar breeding for drought tolerance has been a major strategy to counter the threat posed by climate change, especially in dry and semi-dry lands (Knight et al., 2006). Cumin (*Cuminum cyminum* L.) is a low-water demand medicinal plant with a short growth period and high economic value. It is suitable for cultivation in arid and semi-arid areas around the world. Despite its relatively good drought tolerance,

cumin plants suffer from drought stress, especially in the reproductive stage. Therefore, the development of drought-tolerant cultivars is a crucial step in reducing the impact of drought stress on cumin yield (Darvishzadeh et al., 2011). Despite the importance of cumin in the pharmaceutical, health, and chemical industries (Kafi, 2002), no research has been conducted on breeding and producing drought-tolerant varieties of cumin in Iran. A high level of diversity in cumin has been reported in terms of genetics (Ebrahimiyan et al., 2017), morphology (Safari et al., 2015), and phytochemicals (Kazemi et al., 2018). The existence of sufficient diversity while

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knowing the level and type of diversity in any germplasm are requirements for managing induced changes in the genetic structure of plants (Vojdani, 1993). Therefore, this diversity can be explored to achieve important goals in cumin breeding, including improvements in seed yield, mechanization of harvesting, and drought tolerance. The genetic nature of cumin is heterozygous-heterogeneous and self-fertilization has a negative effect on its development. Therefore, it is necessary to use breeding methods that minimize self-fertilization and purity, thereby facilitating the production of hybrid varieties (Nematzadeh and Kiani, 2005). Since the inflorescence of cumin is umbrella-shaped and its small flowers are without stamens, it is very difficult to control pollination in this plant. The result of each crossing may eventually lead to the production of several seeds. Therefore, crossing parental lines with each other for hybrid production may improve seed yield, plant height for mechanized harvests and drought tolerance. An important step in the production of hybrid varieties is the selection of suitable parents among multiple genotypes. This evaluation can be done by evaluating the parents themselves, the offspring resulting from their self-fertilization, or estimating the general combining ability obtained from testing half-sibs (Aastveit and Aastveit, 1990). The half-sib (HS) test includes polycross, topcross, and open-pollinated tests. The polycross test involves a sufficient replication and suitable isolated crossing blocks due to its simplicity in implementation, sufficient seed production, the possibility of evaluating a large number of parents, and estimation of genetic variance components, which make it more useful than top cross and open-pollinated designs in the production of half-sib families (Comstock and Robinson, 1952). In the polycross test, after performing a random crossing between a set of individuals in isolated conditions, the best parents in terms of general combining ability (GCA) are selected and used to produce a hybrid cultivar (Acquaah, 2012). Also, the average progeny performance of each maternal parent in the polycross test is used for determining the variance components. The calculated heritability provides a decision guide for the usefulness of the polycross test in the breeding program (Falconer and Mackay, 1996).

In medicinal plants, the half-sib test and polycross mating design have been successfully performed to determine the parents of hybrid cultivars. In a breeding program aimed at *Artemisia scoparia*, by performing a polycross test between 25 genotypes and evaluating their progeny, genotypes with appropriate GCA were selected to

create a hybrid variety (Heuberger, 2010). Similarly, hybrid cultivars of two species of *Salvia miltiorrhiza*, *Leonurus japonica* and black cumin (*Carum carvi*) were introduced (Heuberger, 2010). The hybrid variety production method has also been used for improving *Lavandula angustifolia*, resulting in the introduction of a hybrid variety 'Etherio', which had high adaptability, and viability, as well as flower and essential oil yield, with a high percentage of linalool and linalyl acetate compounds (Hassiotisa et al., 2010). In *Satureja rechingeri* Jamzad, with the selection of 20% of half-sib families in a polycross test for essential oil yield and GCA evaluation, suitable parents were selected for the production of hybrid varieties (Eghlima et al., 2015). Also, in various studies, polycrosses have been used in crop and fodder plants to increase yield, improve important traits, and produce hybrid cultivars, including *Festuca aurondinace* Schreb (Amini, 2015), *Onobrychis viciifolia*, (Tourchi et al., 2007), alfalfa (Monirifar, 2010), onion (Mansouri, 2008) and other fodder and pasture plants (Kölliker et al., 2005; Vogel and Mitchell, 2008; ShahNazari et al., 2010; Zarabian and Majidi, 2010; Araghi, 2011).

The height of cumin is usually between 15 and 30 cm. This short height is an issue for the mechanized harvest of this plant. Previous studies have shown that drought stress has a negative effect on plant height, 1000-seed weight, and seed yield of cumin. Due to the short height of cumin, increasing the height of the plant can help the mechanized harvesting of cumin and the development of its cultivation. Thus, the mechanized harvesting height, i.e. the distance of the first branch to the soil surface, was measured and evaluated as an important breeding trait. In addition to plant height, seed yield and 1000-seed weight were also studied as important breeding traits that can be affected by drought stress. Producing a drought-tolerant hybrid variety in cumin not only enabled a variety-exploration approach to desirable characteristics of various genotypes, but also brought a relative uniformity and better performance in terms of yield and height. The present experiment was carried out to evaluate cumin genotypes through a half-sib progeny test and selection of superior parents as a pre-breeding program for the production of drought-tolerant hybrid varieties of cumin.

## Material and Methods

### *Plant materials and evaluation of half-sib families*

In the present study, half-sib families were derived from the polycross test of 49 parental

genotypes of cumin. The parental seeds were collected from different climatic regions of Iran (Table 1) and were sown during two years under two conditions of normal irrigation and water stress (30% of field capacity) in a research field in the College of Aburaihan, University of Tehran. To form a polycross mating scheme and to randomly distribute plant materials in both normal and stress conditions, a randomized complete block design with two replications was applied. Each experimental plot consisted of four two-meter-long rows with a row distance of 30 cm apart and a density of 120 plants per square meter from

each half-sib family.

Irrigation was done based on the soil field capacity (FC). Changes in the irrigation cycle for drought stress began from the flowering stage by irrigating when the soil water content decreased to 30% of FC. After ripening, three important agronomic traits related to the breeding goals of the cumin plant, including 1000-seed weight (g), seed yield ( $\text{g m}^{-2}$ ), and mechanized harvesting height (cm) were measured in half-sib families in both normal and stress conditions during two years.

**Table 1.** Province and genotype number of evaluated cumin genotypes

Genotype Number	Province	Genotype Number	Province	Genotype Number	Province
1	Fars	18	Kerman	35	Semnan
2	Fars	19	Kerman	36	Semnan
3	Fars	20	Kerman	37	Semnan
4	Fars	21	Kerman	38	Semnan
6	Yazd	23	Kerman	40	North Khorasan
7	Yazd	24	South Khorasan	41	North Khorasan
8	Yazd	25	South Khorasan	42	North Khorasan
9	Yazd	26	South Khorasan	43	Khorasan Razavi
10	Golestan	27	South Khorasan	44	Khorasan Razavi
11	Golestan	28	South Khorasan	45	Khorasan Razavi
12	Golestan	29	Isfahan	46	Khorasan Razavi
13	Golestan	30	Isfahan	47	Khorasan Razavi
14	Kerman	31	Isfahan	48	Khorasan Razavi
15	Kerman	32	Isfahan	49	Khorasan Razavi
16	Kerman	33	Isfahan		
17	Kerman	34	Isfahan		

### ***Analysis of variance and estimation of heritability***

The analysis of variance was performed to examine the differences between half-sib families using SPSS (v.25) software. The variance components were estimated based on the expected value of the mean square (Lothrop et al., 1985). Since genotypes are half-sib families, the genetic variance and genotype  $\times$  environment interaction can be interpreted as an additive genetic variance and additive  $\times$  additive interaction variance. Considering that the parents in this study were non-inbred and in random mating, the genetic variance among half-sib

families was equal to the covariance of half-sib families and a quarter of the additive genetic variance, assuming there is no additive  $\times$  additive effect of epistasis variance, i.e.  $\sigma^2_F = COV(HS) = 1/4\sigma^2_A$ . Accordingly, the variances of the interaction effect of family  $\times$  environment ( $\sigma^2_{FE}$ ), family  $\times$  year ( $\sigma^2_{FY}$ ) and family  $\times$  environment  $\times$  year ( $\sigma^2_{FEY}$ ) were equal to a quarter of the additive genetic variance  $\times$  environment ( $\sigma^2_{AE}$ ), the additive genetic  $\times$  year ( $\sigma^2_{AY}$ ), and the variance of the additive genetic  $\times$  environment  $\times$  year ( $\sigma^2_{AEY}$ ), for which the interaction was  $\sigma^2_{FE} = 1/4\sigma^2_{AE}$ ,  $\sigma^2_{FY} = 1/4\sigma^2_{AY}$  and  $\sigma^2_{FEY} = 1/4\sigma^2_{AEY}$ , respectively (Nguyen and Sleeper,

1983). Narrow-sense heritability based on phenotypic average and a prediction of genetic yield from a selection of families were estimated via equations 1 and 2, respectively (Nguyen and Sleper, 1983):

$$\text{Equation 1} \quad H^2_{PFM} = \frac{\sigma^2_F}{\sigma^2_F + \frac{\sigma^2_{FE}}{E} + \frac{\sigma^2_{FY}}{Y} + \frac{\sigma^2_{FEY}}{EY} + \frac{\sigma^2_E}{rYE}}$$

$$\text{Equation 2} \quad \Delta G = cK h^2_{PFM} PFM \sigma = k \frac{2\sigma^2_F}{\sigma_{PFM}} = K \frac{\frac{1}{2}\sigma^2_A}{\sigma_{PFM}}$$

### **General combining ability and drought tolerance indices**

General combining values of different traits were calculated from the difference between the average of each genotype from the total average in both conditions (Lothrop et al., 1985). Finally, using the stress tolerance indices, a comparison was made between the performance of genotypes under control and stress conditions. Reactions of half-sib families to drought stress were evaluated according to the following formulae.

$$\text{Equation 3} \quad SSI = \frac{1 - \left(\frac{Y_s}{Y_p}\right)}{1 - \left(\frac{\bar{Y}_s}{\bar{Y}_p}\right)}$$

$$\text{Equation 4} \quad TOL = Y_p - Y_s$$

$$\text{Equation 5} \quad MP = \frac{Y_s + Y_p}{2}$$

$$\text{Equation 6} \quad STI = \frac{Y_p \times Y_p}{\bar{Y}_p^2}$$

$$\text{Equation 7} \quad HARM = \frac{2(Y_p)(Y_s)}{Y_p + Y_s}$$

$$\text{Equation 8} \quad K2 = Y_s^2 / \bar{Y}_s^2$$

$$\text{Equation 9} \quad GMP = \sqrt{(Y_p)(Y_s)}$$

$$\text{Equation 10} \quad K1 = Y_p^2 / \bar{Y}_p^2$$

$$\text{Equation 11} \quad YSI = \frac{Y_s}{Y_p}$$

$$\text{Equation 12} \quad DI = \frac{Y_s \times \left(\frac{Y_s}{Y_p}\right)}{\bar{Y}_s}$$

$$\text{Equation 13} \quad ATI = \left[ (Y_p - Y_s) / \left(\frac{\bar{Y}_p}{\bar{Y}_s}\right) \right] \times \left[ \sqrt{Y_p \times Y_s} \right]$$

In the formulae, YS and YP are the yield under drought stress and normal conditions, respectively.  $\bar{Y}_s$  and  $\bar{Y}_p$  are the average performance of all tested genotypes under drought stress and non-stress conditions, respectively.

### **Evaluation of genetic diversity of half-sib families by ISSR markers**

From a molecular perspective, ten polymorphic inter-simple sequence repeat (ISSR) primers were used for determining genetic diversity in 49 genotypes. Genomic DNA was extracted from the young leaf tissue of ten plants per genotype according to an established method, with some modifications (Pirttila et al., 2001). The quality and quantity of extracted DNA were checked on 1% agarose gel via spectrophotometry. Reproducible and well-defined bands were obtained after PCR amplification using ten ISSR

primers (Table 4) scored as 1 and 0 for the presence or absence of bands, thereby generating a binary matrix. Molecular indices were calculated using the binary matrix. The amplification of PCR was performed in a reaction volume of 25 microliters which included one microliter of DNA (25 ng  $\mu\text{l}^{-1}$ ), 1.25 microliters of primer, and 12.50  $\mu\text{l}$  of Mastermix 2x (1.50 mM magnesium chloride), 0.4 mM dNTPs and 0.2 units/ $\mu\text{l}$  Taq DNA polymerase. The amplification of PCR in a thermocycler at a denaturing temperature of 94 °C for 5 minutes and then 40 cycles at a temperature of 94 °C for 1 minute, followed by 2 minutes of annealing temperature per primer, and 2 minutes at a temperature of 72 °C as an extension temperature and finally 5 minutes at 72 °C as a final expansion. The PCR product was separated in 2% agarose gel with TAE buffer for 3 hours at a voltage of 100. Amplified bands were observed after staining in ethidium bromide. The molecular indices were calculated, including polymorphism percentage ((number of polymorphic fragments/total number of fragments)  $\times 100$ ), PIC polymorphism content ( $PIC = -1 \sum p^2 - q^2$ , where p is the frequency of presence and q is the frequency of absence of a band) and MI is the indicator index ( $MI = PIC \times n\beta$ , where: n is the number of bands and  $\beta$  is the ratio of polymorphic bands). To estimate statistics and draw a dendrogram, genotype grouping was done based on the Jaccard similarity coefficient, via the Centroid Linkage method, using NTYSY 2.02-PC software. Also, principal coordinate analysis (PCoA) was performed to obtain a graphical representation of the relationship structure between different cumin genotypes via Gen Alex 6.5 software (Peakall et al., 2012).

## **Results**

### **Analysis of variance**

The results of combined analysis of variance indicated highly significant differences among the evaluated traits in different irrigation conditions and among half-sib families (Table 2). In addition, the interaction between water condition and year in half-sib family for all traits was significant, suggesting different responses of half-sib families under different years and irrigation conditions (Table 2). Mean comparisons among genotypes in both average of years and in distinct years showed ten top-yielding, tall genotypes, although the ranking of superiority in different years and conditions was different (data not shown). Genotypes 5, 7, 12, 14, 21, 28, 35, 37, 42, and 49 were the top ten genotypes in terms of the evaluated traits.

**Table 2.** Results of variance analysis for measured traits in 49 half-sib families of cumin

Sources of variation (S.O.V)	Degrees of Freedom (DF)	MS		
		1000-seed weight	Seed yield	Mechanized height of harvesting
Year	1	2.81**	196821.48**	131.22**
Irrigation Conditions	1	1.35**	42798.15**	310.47**
Year × Irrigation Conditions	1	1.35**	7241.25**	0.05**
Replication × Irrigation Conditions × Year	4	0.78**	15276.96**	1.48**
Half-sib family	48	0.70**	1189.60**	19.04**
Half-sib family × Irrigation Conditions	48	0.01**	126.09**	1.461**
Half-sib family × Year	48	0.25**	637.09**	0.98**
Half-sib family × Year × Irrigation Conditions	48	0.01**	113.12**	0.30**
Error	192	0.01	78.50	0.18
Coefficient of variation (%)	-	2.02	8.21	2.95

### *Heritability and genetic advance*

The narrow-sense heritability was 0.66, 0.34, and 0.89 for 1000-seed weight, seed yield, and mechanized harvesting height, respectively (Table 3). These values can be attributed to variations in the additive effects of genes, particularly for estimations about selection, which make it more important than broad-sense heritability. The moderate to high narrow-sense heritability for the studied traits indicates a greater contribution of additive effects than non-additive effects. In half-sib progeny testing, the best parents are selected based on the performance of half-sib progeny cross with each other in an isolated cross-breeding block, so the parental control coefficient equals 2. The most important difference between the selection of half-sib families and the selection based on the

half-sib test is the recombination unit. This method has been described by Falconer (Falconer, 1983) as a family selection method. It is highly effective in each period. This method is especially useful and important for the selection of parents in the production of hybrid varieties (Nguyen and Slepser, 1983). In the current research, the genetic efficiency ( $\Delta G$ ) was measured by applying a selection intensity of 20% for different traits. The expected response for 1000-seed weight, seed yield, and mechanized harvesting height was calculated as 1.89, 1.61, and 2.48, respectively. It is expected to have an increase of 5.72, 4.92, and 17.80%, compared to the average of 1000-seed weight, seed yield, and mechanized harvesting height, respectively (Table 3).

**Table 3.** Estimation of heritability and genetic advance for the studied traits of cumin

Traits	Heritability	Genetic advance ( $\Delta G$ )
1000-seed weight	0.66	1.85
Seed yield	0.34	0.95
Mechanized height of harvesting	0.89	2.50

### *General combining ability (GCA)*

GCA values for the evaluated traits showed a wide range among the families, especially regarding seed yield (Table 4). As shown in Table 3, for at least one of the traits, i.e. 1000-seed weight, seed

yield, and mechanized harvesting height, genotypes 3, 5, 7, 12, 13, 14, 20, 21, 28, 35, 37, 42, 44 and 49 showed a highly positive general combining ability in normal irrigation and drought stress.

**Table 4.** The results related to general combining ability and drought tolerance indices in 49 cumin genotypes

Drought Tolerance Indicators											General combining						Genotype Number
K <sub>2</sub> STI	K <sub>1</sub> STI	ATI	DI	YSI	MP	Harm	STI	Toll	GMP	SSI	Mechanized height of harvesting		Seed yield		1,000-seed weight		
											Normal	stress	Normal	stress	Normal	stress	
0.20	0.54	3336.46	0.26	0.40	80.62	66.12	0.47	68.38	73.01	1.80	1.61	1.47	2.54	-14.16	0.07	0.13	<b>1</b>
0.75	0.74	2146.85	0.69	0.67	91.41	87.90	0.71	35.84	89.64	0.99	0.71	0.83	-0.25	1.44	0.09	0.12	<b>2</b>
0.31	0.65	3274.86	0.35	0.46	84.85	73.51	0.55	62.04	78.98	1.61	2.5	2.26	4.12	-6.25	0.28	0.16	<b>3</b>
0.17	0.20	1282.06	0.44	0.62	65.23	61.72	0.35	30.23	63.45	1.13	0.71	0.02	-17.34	-5.32	0.03	0.01	<b>4</b>
1.77	1.52	2621.09	0.94	0.72	110.62	107.70	1.05	35.93	109.15	0.84	1.49	1.85	17.16	12.18	0.34	0.41	<b>5</b>
1.79	1.18	1464.92	1.11	0.82	106.39	105.38	0.98	20.70	105.88	0.53	0.83	0.63	4.8	11.73	0.30	0.19	<b>6</b>
3.46	2.33	2168.45	1.29	0.81	125.90	124.57	1.38	25.91	125.23	0.56	1.79	1.93	12.66	17.39	0.24	0.20	<b>7</b>
0.19	0.19	1134.05	0.48	0.66	65.12	62.40	0.36	26.62	63.75	1.02	0.45	-1.12	-14.11	-10.38	-0.05	-0.04	<b>8</b>
0.39	0.42	1778.10	0.55	0.64	78.86	75.07	0.52	34.58	76.94	1.08	0.12	-1.62	-6.72	-5.65	-0.20	-0.31	<b>9</b>
0.61	0.59	1888.82	0.66	0.68	86.51	83.30	0.63	33.29	84.89	0.97	-2.41	-2.89	2.8	6.54	0.57	0.49	<b>10</b>
0.64	0.61	1862.11	0.68	0.69	87.31	84.29	0.65	32.48	85.79	0.95	-1.89	-1.34	-6.71	-2.15	-0.06	0.01	<b>11</b>
1.55	1.51	3026.18	0.84	0.68	109.32	105.24	1.01	42.21	107.26	0.98	1.27	1.00	14.29	7.09	0.05	0.12	<b>12</b>
1.42	1.00	1553.53	1.00	0.80	101.45	100.14	0.89	23.06	100.79	0.62	2.11	2.36	15.97	20.55	0.05	0.12	<b>13</b>
2.90	2.13	2449.04	1.17	0.78	122.13	120.26	1.29	30.24	121.19	0.66	2.43	2.03	11.55	13.96	0.31	0.32	<b>14</b>
0.14	0.21	1596.82	0.35	0.54	64.89	59.16	0.34	38.56	61.96	1.38	-0.47	-1.60	-15.86	-16.01	-0.12	-0.19	<b>15</b>
0.15	0.19	1327.86	0.39	0.59	63.54	59.43	0.33	32.33	61.45	1.22	-1.74	-1.18	-16.88	-12.22	0.12	-0.06	<b>16</b>
0.17	0.28	1938.11	0.34	0.52	69.31	62.28	0.38	44.14	65.70	1.46	-0.85	-0.45	-0.01	-15.63	-0.32	-0.26	<b>17</b>
0.43	0.40	1480.06	0.63	0.69	78.75	76.15	0.53	28.60	77.44	0.93	0.14	-2.57	-5.15	-9.78	0.07	0.08	<b>18</b>
0.98	0.92	2241.60	0.77	0.69	96.81	93.61	0.80	35.23	95.20	0.93	0.44	-0.36	2.98	0.62	-0.16	-0.15	<b>19</b>
0.86	0.80	2113.00	0.74	0.69	93.68	90.53	0.74	34.33	92.09	0.93	1.75	1.70	9.57	7.83	0.05	0.10	<b>20</b>
5.25	3.19	1924.23	1.53	0.86	137.61	136.81	1.65	20.98	137.21	0.43	2.66	1.53	16.76	24.00	0.41	0.46	<b>21</b>
0.14	0.20	1554.85	0.35	0.55	64.58	59.09	0.34	37.66	61.78	1.36	-1.04	-1.29	-8.39	-13.85	0.03	-0.04	<b>22</b>
0.26	0.36	1950.83	0.44	0.57	74.64	69.10	0.45	40.65	71.82	1.29	-1.83	-1.60	-12.57	-9.22	-0.22	-0.16	<b>23</b>
0.22	0.34	2027.68	0.39	0.54	73.26	66.84	0.43	43.36	69.97	1.38	0.64	-0.09	-1.97	-9.22	-0.15	-0.12	<b>24</b>
0.21	0.20	1079.00	0.52	0.68	66.23	63.91	0.37	24.82	65.06	0.95	-1.35	-1.04	-13.78	-8.13	-0.16	-0.15	<b>25</b>
0.49	0.47	1697.19	0.63	0.68	81.87	78.82	0.57	31.61	80.33	0.98	-1.75	-1.03	-3.78	0.07	0.31	0.35	<b>26</b>
1.14	0.89	1748.62	0.89	0.76	97.54	95.66	0.82	27.09	96.59	0.74	2.26	2.46	-3.01	2.83	-0.36	-0.25	<b>27</b>
4.31	2.84	2263.30	1.38	0.82	132.55	131.31	1.53	25.67	131.93	0.53	2.34	2.49	9.02	19.95	0.55	0.67	<b>28</b>
0.10	0.18	1628.92	0.29	0.50	62.25	55.33	0.30	41.53	58.69	1.51	-1.28	-0.44	-18.97	-14.57	-0.31	-0.29	<b>29</b>
0.28	0.34	1761.85	0.47	0.60	74.09	69.53	0.45	36.73	71.77	1.20	-1.64	-1.59	6.02	-1.02	0.28	0.26	<b>30</b>

0.10	0.21	1883.77	0.27	0.46	64.33	55.71	0.31	47.08	59.87	1.62	-2.07	-1.91	-15.46	-14.99	-0.04	-0.16	<b>31</b>
0.12	0.38	2963.47	0.21	0.37	73.67	58.07	0.38	67.80	65.41	1.90	-1.28	-1.44	11.68	-4.53	0.15	0.18	<b>32</b>
1.24	1.01	1982.25	0.89	0.74	100.31	98.09	0.86	29.90	99.19	0.78	0.76	1.90	-2.5	4.19	-0.46	-0.54	<b>33</b>
1.86	1.31	1770.78	1.08	0.80	108.59	107.20	1.02	24.56	107.89	0.61	-0.87	0.00	13.41	9.85	-0.17	-0.20	<b>34</b>
5.57	2.46	-65.30	1.89	1.01	133.85	133.85	1.57	-0.73	133.85	-0.02	1.3	0.91	17.39	24.65	0.44	0.50	<b>35</b>
0.27	0.38	2045.82	0.43	0.57	75.72	69.87	0.46	42.09	72.73	1.31	-1.44	0.11	-4.08	-2.51	0.23	0.13	<b>36</b>
1.51	0.80	554.55	1.22	0.92	98.84	98.66	0.86	8.40	98.75	0.25	2	2.84	-2.13	6.25	-0.40	-0.43	<b>37</b>
0.10	0.08	540.73	0.48	0.75	53.65	52.57	0.25	15.24	53.10	0.75	-1.62	-1.20	-22.22	-13.50	-0.43	-0.50	<b>38</b>
0.07	0.30	2777.23	0.16	0.33	69.10	51.56	0.31	69.62	59.69	2.02	-2.38	-1.95	-1.91	-20.79	-0.62	-0.62	<b>39</b>
0.65	0.69	2243.14	0.64	0.65	89.39	85.25	0.67	38.45	87.29	1.07	0.12	0.54	-2.17	6.34	-0.08	-0.01	<b>40</b>
0.41	0.54	2317.22	0.50	0.59	82.83	77.15	0.56	43.37	79.94	1.25	0.32	-0.68	-7.58	-5.49	-0.15	-0.20	<b>41</b>
8.53	5.68	3282.09	1.63	0.82	157.49	155.93	2.16	31.34	156.71	0.55	2.03	2.67	42.85	37.75	0.42	0.45	<b>42</b>
0.31	0.37	1768.71	0.50	0.62	75.82	71.56	0.48	35.93	73.66	1.16	-0.77	-1.80	-8.76	-6.85	-0.08	-0.08	<b>43</b>
0.89	0.97	2714.53	0.68	0.64	97.09	92.35	0.79	42.90	94.69	1.09	-0.12	1.72	13.65	-0.03	0.33	0.36	<b>44</b>
0.27	0.39	2091.41	0.42	0.56	75.78	69.65	0.46	43.07	72.65	1.33	-2.09	-1.09	-7.07	-8.17	-0.36	-0.44	<b>45</b>
0.25	0.23	1134.48	0.54	0.69	68.56	66.24	0.40	25.19	67.39	0.94	-2.47	-1.15	-17.15	-5.23	-0.31	-0.31	<b>46</b>
0.16	0.39	2731.41	0.27	0.43	74.52	62.48	0.41	59.90	68.23	1.73	-1.83	-1.94	-5.11	-16.30	-0.30	-0.18	<b>47</b>
0.82	0.72	1853.16	0.76	0.71	91.71	89.15	0.72	30.67	90.42	0.86	-0.74	-1.36	-7.1	2.44	-0.43	-0.38	<b>48</b>
2.85	2.25	2816.99	1.11	0.75	122.90	120.45	1.30	34.64	121.67	0.74	1.16	1.48	19.49	14.32	0.25	0.30	<b>49</b>

### ***Drought tolerance indices***

In this study, 49 half-sib families of cumin for drought tolerance were evaluated based on 11 drought tolerance indices, including Stress Susceptibility Index (SSI), Geometric Mean Productivity (GMP), Tolerance (ToL), Stress Tolerance Index (STI), Harmonic mean (Harm), Mean Productivity (MP), Yield Stability Index (YSI), Zinc Deficient resistance Index (DI), Abiotic Tolerance Index (ATI), Modified Stress Tolerance Index for drought stress conditions (K2STI), and Modified Stress Tolerance Index for optimum conditions (K1STI) (Table 4). These indices were calculated based on the performance of genotypes under drought stress (Ys) and normal (Yp) conditions. High values of YSI, Harm, STI, GMP, DI, MP, ATI, K1STI, and K2STI indices and low values of SSI and TOL indices indicated stress tolerance. In this study, based on SSI, TOL, and YSI indices, genotypes 35 and 42 were identified as the most tolerant based on Harm, STI, GMP, DI, MP, ATI, K1STI, and K2STI indices. In general, according to all drought tolerance indices, genotypes 5, 7, 12, 14, 21, 28, 35, 37, 42, and 49 may potentially create a genetically modified population. Also, the highest seed yield ( $\text{g m}^{-2}$ ) in the normal irrigation condition was related to genotypes 21, 28, 35, and 49. Under drought stress, the highest seed yield ( $\text{g m}^{-2}$ ) was related to genotypes 7, 21, 35, and 42. The cluster analysis of 49 half-sib families was evaluated based on drought tolerance indices and also based on the studied traits (1000-seed weight, mechanized harvest height, and seed

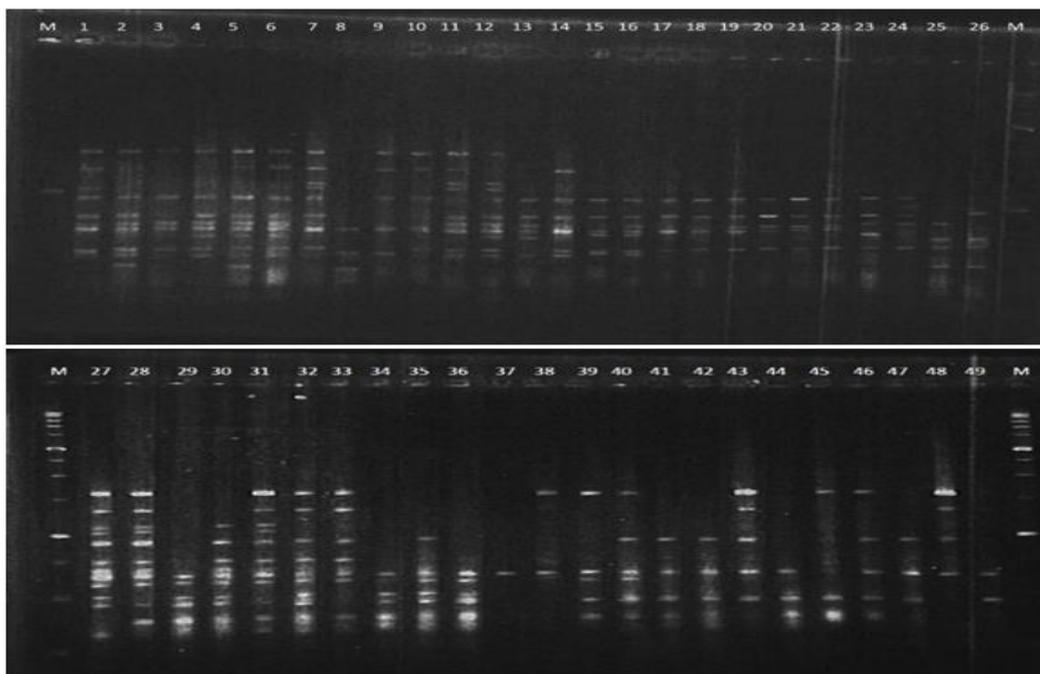
yield) under normal and stress conditions. The distances between hybrids were determined by the square of the Euclidean distance and dendrogram, drawn by the Ward method, and the half-sib families were grouped into four clusters. The results of cluster analysis showed that half-sib families 5, 7, 12, 14, 21, 28, 35, 37, 42 and, 49 were placed in one cluster. Therefore, it can be concluded that these genotypes, in addition to having a relatively high tolerance to drought stress, are superior in terms of the studied traits in response to normal and drought stress conditions, compared to other genotypes (data not shown).

### ***Genetic diversity of half-sib families using ISSR markers***

Ten ISSR primers were screened to study genetic relationships among half-sib families of cumin. All primers produced reproducible polymorphic bands in 49 half-sib families. A total of 90 amplified polymorphic bands were generated. In this marker system, 94.30% polymorphism was observed. The size of the amplified fragments ranged from 200 to 3000 bp. The highest and lowest number of polymorphic bands were 12 for UBC840 and 6 for USB825 and UBC827, with an average of 9 polymorphic bands per primer (Table 5). The PIC and MI statistics of each primer showed that the highest content of polymorphic information was related to the UBC 857 primer and the lowest value was related to the UBC 834 primer (Fig. 1).

**Table 5.** Information obtained from the ISSR primers used in the study of the genetic diversity of 49 half-sib families of cumin

	Marker Name	Marker sequence	Temperature Annealing	Total number of bands	Number of polymorphic bands	PIC	MI	Polymorphism percentage
1	UBC 818	(CA)8G	48	8	8	0.33	2.64	100
2	UBC 824	(GA)8YG	48	12	12	0.35	4.22	100
3	USB 825	(AC)8CT	52	7	6	0.38	2.27	68.72
4	UBC 827	(AC)8G	52	7	6	0.28	1.66	74.31
5	UBC 834	(AG)8YT	50	7	7	0.20	1.41	100
6	UBC 840	(GA)8YT	48	12	12	0.27	3.19	100
7	UBC 844	(CT)8RC	48	9	9	0.30	2.69	100
8	UBC 845	CT)8RG	48	8	8	0.41	3.28	100
9	UBC 848	(CA)8RG	48	11	11	0.40	4.41	100
10	UBC 857	(AC)8YG	48	11	11	0.42	4.59	100
	mean	-	-	-	-	0.33	3.04	94.30



**Fig. 1.** The banding pattern of Iranian cumin genotypes by primer UBC857

For classifying genotypes, Mantel's test was performed for Dice, Jaccard, and simple matching similarity coefficients based on the centroid linkage clustering method. The Jaccard similarity coefficient had the highest cophenetic correlation coefficient (0.96), indicating a good fit and high correlation between the similarity matrix and the final dendrogram. The genetic similarity of the populations based on the Jaccard similarity coefficient ranged from 0.29 to 0.84. According to the dendrogram, the genotypes were classified into four major groups (Fig. 2). The first group mainly included North Khorasan and Razavi Khorasan genotypes and a small number of Isfahan and Semnan genotypes. The second group

mainly included genotypes from South Khorasan along with a number of genotypes from other regions such as Kerman, Yazd, and Semnan. The third group mainly included Yazd, Fars, and Golestan genotypes. The fourth group was related to Kerman genotypes. According to the dendrogram, the clustering of genotypes can be adapted and justified by geographical location, because genotypes with the same genetic origin were placed in one group. Also, the principal coordinate analysis showed that 23 principal components explained 86.22% of the total variation, indicating that the primers were not continuous and had a suitable distribution at the genome level.

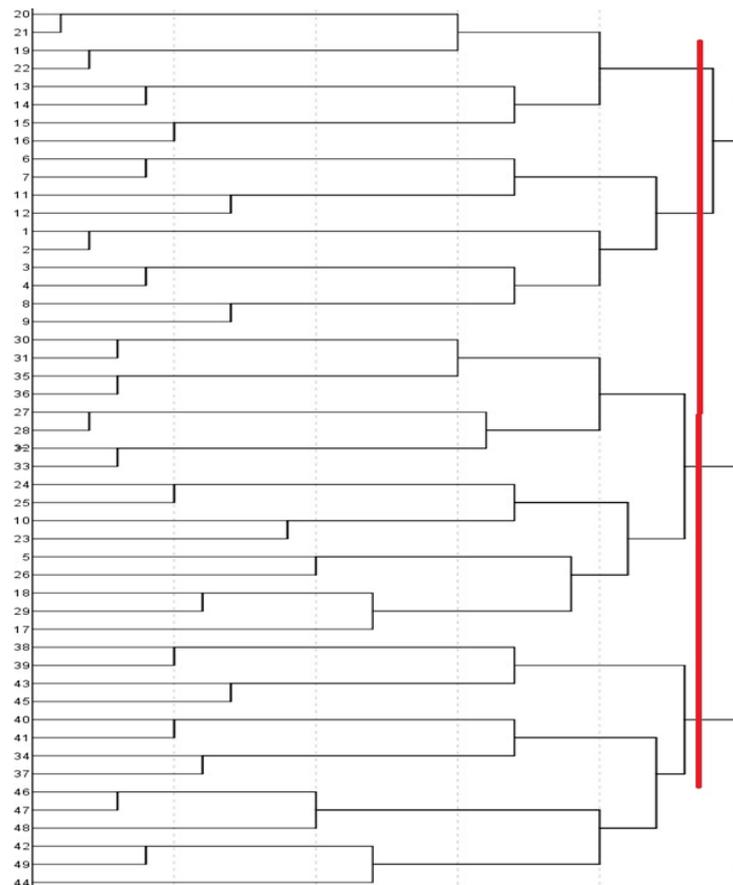


Fig. 2. Clustering of 49 cumin genotypes

## Discussion

Drought stress in plants may lead to physiological disorders such as lowered levels of transpiration and photosynthesis. Environmental factors such as drought stress usually affect the one-thousand seed weight, seed yield, and mechanized harvesting height in medicinal and aromatic plants (Burbott and Loomis, 1969; Nadjafi et al., 2009). The effects of drought stress on various plant species are commonly recognized (Khalid, 2006; Khazaie et al., 2008).

The cultivation of cumin is usually in dry areas, but it is sensitive to drought stress at the end of the season. Therefore, evaluating and improving cumin genotypes for drought tolerance and the mechanization of harvesting is necessary. Generally, plant breeding is a cheaper and more stable approach to overcome the harmful effects of drought stress through developing cultivars with high-yield production under water-deficit conditions. While crossing blocks in the cumin is not easy because of the small flowers, producing commercial F1 hybrids is not presently applicable in the case of cumin. In aiming to breed new cumin cultivars, half-sib mating is effective with poly-cross designs. Due to the relatively simple crossing plan, producing adequate numbers of

individuals and using a much larger set of parents is possible in half-sib poly-cross designs (Comstock and Robinson, 1952). Half-sib mating, including poly-cross, top-cross, and open pollination, is frequently used in forage grass breeding to evaluate the general combining abilities of parental clones for developing hybrid cultivars (Nguyen and Sleper, 1983). This study is unique for using half-sib families derived from the poly-cross of cumin genotypes to identify drought-tolerant half-sib families as a source of elite parents for hybrid cultivars.

In this study, differences between different conditions, sufficient irrigation, and drought stress were due to the effects of drought stress. So, a high genetic variation among half-sib families of cumin could be a valuable resource for the selection of drought-tolerant families as a source of elite parents for hybrid cultivars (Table 2).

Narrow sense heritability plays an important role in the calculation of genetic improvement. In some respects, it is more important than broad sense heritability. Without genetic improvement, the heritability values would not be of practical importance in selections based on phenotypic appearance. So, genetic advances should be considered along with narrow sense heritability

in coherent selection breeding programs. In the present research, high heritability values, coupled with high genetic advances (Table 3), were recorded for the mechanized harvesting height. This indicated that the additive nature of genetic variation was transmitted from parents to progeny. Also, this trait can easily be fixed in the genotypes by selection in early generations. However, the mechanized harvesting height is an important feature because it increases the development of mechanization, reduces seed loss, and ultimately produces a higher yield. High heritability, accompanied by small genetic improvement, regarding the 1000-seed weight, is indicative of non-additive gene relations and dominance which could be explored through heterosis breeding. Moderate heritability with low genetic advances was observed in seed yield, indicating slow progress through the selection for this trait. The reason for the moderate heritability of seed yield is a result of variances constituting the environmental variance. Brown et al. (1996) reported similar results in estimating the narrow sense heritability of F2 and F1 populations in rapeseed.

Broad variability of the GCA for the study traits indicated that the poly-cross test is suitable for screening primary populations and identifying superior parents for producing hybrid cumin varieties. A high positive GCA indicates the additive gene effect, the ability to effectively transfer traits to the next generation, and determine suitable parents as good combiners in breeding programs. According to the results of the GCA obtained from the half-sib progeny test and considering both normal and stress conditions, genotypes 5, 7, 12, 14, 21, 28, 35, 37, 42, and 49 can be introduced as valuable assets to future hybrid development programs. Cross-breeding among these ecotypes is expected to result in an enhanced yield. In a relevant study, to create a hybrid variety in the medicinal plant *Coriandrum* (*Coriandrum sativum*), the GCA was estimated in the poly-cross among 12 different genotypes. Then, according to the GCA of the genotypes and especially the seed yield, the superior genotypes were selected to create a hybrid variety (Kermanshahani et al., 2018). Also, Toorchi et al. (2007) determined the GCA of native *Onobrychis* genotypes, based on which six genotypes had the highest GCA for fodder-related traits and were introduced to produce hybrid cultivars.

One of the strategic goals of breeding programs in stressed areas is to introduce genotypes that have high yield and yield stability in response to changes in environmental conditions. The average seed yield under normal irrigation

conditions and drought stress was 70.71 and 71.31 grams per square meter. Accordingly, in the stress condition, the seed yield decreased by about 50%, compared to the normal irrigation conditions. The decrease in seed yield under drought stress in cumin was consistent with previous research (Mohamed and Abdu, 2004; Osman, 2009; Pouryousef et al., 2012).

Water is a critical limiting factor in grain yield. With the decrease in soil water content, the seed yield decreased significantly. The occurrence of drought stress during different stages of development, especially the reproductive stage, caused a decrease in the length of the photosynthesis period, the transfer of materials resulting from current photosynthesis to the seed, contribution to the retransfer of stored materials from the stem to the seed, and finally, a decrease in seed yield. Therefore, considering the 11 selection indices of drought tolerance, half-sib genotypes 5, 7, 12, 14, 21, 28, 35, 37, 42, and 49 were selected as drought-tolerant. Therefore, these half-sib genotypes can be used as a source of elite parents for hybrid cumin cultivars.

The results indicated the diversity of cumin genotypes and the strong ability of ISSR primers to detect differentiation among plant samples. The highest and lowest similarity was observed between half-sib genotypes 23 and 20, and between half-sib genotypes 40 and 5, respectively. The low similarity showed that the two genotypes had large genetic differences, compared to each other. Thus, it was suggested that they can serve as parents in cross-breeding programs. It is assumed that by performing cross-breeding among distantly related genotypes, different progenies can be produced in breeding programs. One of the most reliable ways of achieving high levels of heterozygosity in cumin genotypes is to select parents that are less genetically similar to each other. The identification of crossbreeds with high levels of heterozygosity is an important step in producing hybrid crops. Parents with higher cross-breeding abilities and greater genetic distances can produce hybrids capable of producing higher yields (Brünjes and Link, 2021). Grouping half-sib families into 4 groups indicated the existence of a considerable genetic diversity that allows the production of hybrid cumin varieties. Rostami Ahmadvandi et al. (2013) used the centroid linkage method to draw a dendrogram based on the ISSR marker system when studying cumin ecotypes and divided the genotypes into six main groups, thereby confirming the results of the current research.

In general, the results of examining half-sib families by ISSR markers from a genetic viewpoint indicated the optimal sampling of

markers from the genome level. The ISSR markers are reportedly suitable and effective in evaluating the genetic diversity of cumin genotypes. Through this experiment, it was concluded that exploring the genetic diversity of cumin genotypes using the ISSR marker can be useful. ISSR markers can be used effectively in detecting genetic diversity and relationships among plants in future breeding programs.

## Conclusion

In cumin, the estimation of genetic parameters such as heritability and general combining ability can be obtained through the evaluation of half-sib families, resulting from the poly-cross test. This is usually aimed to create a hybrid variety or a new genetic resource. In this research, all statistical and molecular analyses showed a large genetic diversity among half-sib cumin genotypes. It is possible to select parents for producing new hybrids. The study of narrow-sense heritability and genetic progress showed the additive nature of genetic diversity. Therefore, by selecting superior parents, it is possible to transfer traits from parents to progeny. According to the GCA of half-sib families, and especially by considering drought tolerance indices, the 10 elite genotypes were 5, 7, 12, 14, 21, 28, 35, 37, 42, and 49. These were selected for producing drought-tolerant hybrid seeds. Ten elite genotypes had significant genetic diversity in terms of ISSR markers and were placed in four different clusters. Thus, they can be used as parents in cumin breeding programs. This experiment may continue by providing random crossing for ten elite genotypes in controlled conditions to produce drought-tolerant hybrid seeds.

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## Conflict of interest

The authors indicate no conflict of interest for this work.

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