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Breeding cumin landraces (*Cuminum cyminum* L.) for drought tolerance based on physiological and genetical traits

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ABSTRACT

The effect of water stress on the different physiological and biochemical traits, including the essential oil (EO) content, the relative water content (RWC) of leaf, soluble sugars, chlorophyll *a* and *b*, and carotenoid besides seed yield, was studied in 49 landraces of cumin (*Cuminum cyminum* L.). The genetic bases of distinct traits were estimated to get an overview of the genetic variability for cumin breeding programs. Heritability, genetic advance, and genotypic and phenotypic correlation coefficients were determined for all the traits. The combined analysis of variance showed significant differences among all the sources of variation. The seed yield and EO content, as well as other traits, were affected by water stress. The GC–MS analysis of the elite landrace, Golestan (Jat), revealed that the main chemical compositions in both conditions were γ-terpinene, β-pinene, m-cymene and cuminic aldehyde. The landrace Golestan (Gonbad) was introduced as a good candidate for further breeding research on RWC. However, this landrace was clustered in medium-oil-yield group, while Semnan (Ivanakey), Yazd (Bafq) and Southern Khorasan (Ghaen) were grouped in the top-ranking landraces for EO. They were also suggested as suitable candidates for studying the physiological mechanisms and breeding involved in pigment and sugar accumulation. According to the results, we suggest carotenoid content, soluble sugars and RWC as drought-tolerance indices in cumin improvement programs.

1. Introduction

Cumin belongs to the Apiaceae (Umbelliferae) family and its seeds are valued for their aroma and pharmaceutical applications (Banerjee and Sarkar, 2003; Kedia et al., 2014; Rahman et al., 2015; Sowbhagya, 2013). It is the second most popular spice in the world after black pepper and has been used for thousands of years (Hashemian et al., 2013). Iran is one of the leading producers of cumin in the world (Sowbhagya, 2013). It is mainly grown in arid and semi-arid regions of the eastern, southeastern and central states of Iran. Khorasan (North, Razavi, and South) is the largest cumin-producing region in Iran (Hashemian et al., 2013). However, there is no improved cumin variety in Iran. One of the reasons for that is insufficient genetics and breeding data on this crop.

Among the different environmental stresses worldwide, drought is one of the significant limiting factors, inhibiting plant growth and productivity, and altering biochemical properties of the spice (Azhar et al., 2011; Lipiec et al., 2013). Reports show seed-yield reduction of cumin when it is faced with drought stress, especially in the spring cultivation of this crop (Nezami et al., 2009).

Understanding the physiological and biochemical responses to a drought condition, as well as having information on genetic parameters, including the extent of variations, heritability and expected genetic advance are important not only for a comprehensive knowledge about the plant's resistance mechanisms to limited water conditions, but also for developing selection criteria for screening drought tolerance in plant breeding programs (Cha-um et al., 2012; Huseynova, 2012). One of the biochemical mechanisms to cope with water-defi

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cit stress is osmotic adjustment by the accumulation of sugars (Mahajan et al., 2005; Mohammadpour et al., 2015; Rivero et al., 2014). Hence, one of the methods to assess plant tolerance to drought stress is the comparison of sugar contents between plants under normal and drought-stress conditions. In addition, the relative water content (RWC) is an indicator of the level of water deficit in the plant (Abdi et al., 2013). Among the photosynthetic traits, chlorophyll concentration is an index for estimating the effects of environmental stress on growth and yield (Abdi et al., 2013). The assessment of pigment accumulation in the response to drought stress is also important to evaluate plants that were subjected to drought. Pigment loss during drought

Abbreviations: GA, genetic advance; PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; ECV, environmental coefficient of variation; RWC, relative water content; SCH, sugar content; EO, essential oil; YLD, seed yield; CAR, carotenoids; CHA, chlorophyll *a*; CHB, chlorophyll *b*; TCH, total chlorophyll

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stress can be attributed to the sensitivity of the plant to the loss of light-harvesting chlorophyll *a/b*-protein. Moreover, the accumulation of reactive oxygen species (ROS) in response to drought stress causes not only membrane lipid peroxidation and degradation of chlorophyll, but also the accumulation of non-enzymatic antioxidants like carotenoids (Askari and Ehsanzadeh, 2015), as well as certain osmoprotectant molecules (Rivero et al., 2014).

Knowledge of the genetic nature of traits helps breeders select the correct breeding strategy for the genetic improvement of plants. Broad-sense heritability (H_b) determines the type of breeding technique and the usefulness of the selection method. Genetic advance is also very important in breeding programs. It helps breeders get a clear idea about the extent of improvement between two generations (Tahir and Razi, 2016). Traits with high heritability and genetic advance are much more efficient in screening programs. Without genetic advance, heritability is not a very effective approach for selection (Ahmad et al., 2014). It is important to consider heritability along with the phenotypic and genotypic coefficients of variation to avoid bias in heritability estimates (Liu et al., 2015).

The majority of experiments done on cumin have used only one or a few cumin cultivars. Few studies have covered several cultivars from the major cumin cultivation areas of Iran. Moreover, few reports have been filed on the physiological and biochemical traits, such as RWC and pigment change, of this plant under drought stress for breeding purposes. There is no report on the genetic parameters of the biochemical and physiological traits of cumin. Hence, this study was designed to assess, for the first time, the broad heritability and genetic advance of the physiological and biochemical traits of the landraces of *Cuminum cyminum* to be used in breeding programs. The evaluation of the drought-tolerance ability of the 49 landraces and the selection of suitable genotypes were followed for the introduction and cultivation process, which can also improve the quality of plants and significantly ensure effective ingredient stability. In addition, the genetic parameters of the evaluated traits were obtained to select the future breeding strategy.

2. Materials and methods

2.1. Plant materials and growth conditions

This study was conducted in the research field of the College of Aburaihan, the University of Tehran, Iran (33°28′N, 51°46′E and 1180 m altitude) for two years. Forty-nine cumin landraces from different provinces of Iran (Table 1) were evaluated under two irrigation regimes: normal irrigation (field capacity) and water stress (30% of field capacity).

Furrow irrigation was used in the field. The seeds were sown manually in two-meter-long plots, with four rows 30 cm apart, for each landrace on February 22 in the first year (2013) and on February 19 in the second year (2014). The area of each plot was 2.4 m^2. The distance between two adjacent plots was 60 cm. The experimental design of each site was the simple lattice (7 × 7, 49 plots). Two replications in each site were arranged in the same direction with irrigation gradients. The two sites were three meters apart to ensure prevent irrigation interference. The field capacity (FC) of the soil was determined before the experiment. Drought stress was applied from the flowering stage. There was no rainfall from this stage until plant harvest in early June (Table 2). The soil texture was sandy loam (Table 3).

The wilting point (WP) of the soil, the moisture content of 0.14 (w/w), and FC were determined by the pressure plate method (Sandhya et al., 2009). The soil moisture content was determined with soil moisture tensiometer (2725ARL JET FILL, Soilmoisture Co.) in 15- and 30-cm depth of the soil. The soil was irrigated when the water content at a depth of 30 cm was 30% of FC (water stress). Whenever the soil moisture level reached 30% of FC, the soil was re-watered. The amounts of water needed for FC (normal irrigation) and 30% of FC (water stress) were applied. The amount of water was calculated with the formula of Michael and Ojha (1966). Weeds were controlled by hand, beginning in spring and continued throughout the growth cycle, whenever needed. Sampling for physiological traits was done randomly from two middle rows in each plot (1.2 m^2). Whole plants were harvested manually in early June in both years. The seeds were separated from the rest of the

Table 1
Geographic profile of origins of 49 Iranian cumin landraces.

Sample	Populations	Sub-Populations/ genotypes	Altitude (m)	Longitude	Latitude
1	Fars	Sarvestan	1547	53 13 E	29 16 N
2	Fars	Sepidan	2118	52 16 E	30 3 N
3	Fars	Sivand	1706	52 55 E	30 4 N
4	Fars	Estahban	1740	54 2 E	29 7 N
5	Yazd	Ardakan	1011	53 57 E	32 21N
6	Yazd	Bafq	992	55 24 E	31 36 N
7	Yazd	Sadoq	2091	53 28 E	32 1 N
8	Yazd	Khatam	1613	54 22 E	30 2 N
9	Yazd	Sadroea	1215	54 20 E	31 54 N
10	Golestan	Maraveh-Tapeh	253	55 57 E	37 53 N
11	Golestan	Aq-Qala	−17	54 26 E	37 0 N
12	Golestan	Jat	490	54 30 E	36 48 N
13	Golestan	Gonbad	38	55 9 E	37 14 N
14	Kerman	Baft	2219	56 36 E	29 12 N
15	Kerman	Bardsir	2036	56 34 E	29 56 N
16	Kerman	Chatrood	1863	56 55 E	30 36 N
17	Kerman	Joopar	1885	57 7 E	30 3 N
18	Kerman	Kooh-banan	1990	56 16 E	31 24 N
19	Kerman	Mahan	1890	57 16 E	30 3 N
20	Kerman	Ravar	1185	56 47 E	31 16 N
21	Kerman	Rafsanjan	1541	55 59 E	30 21 N
22	Kerman	Sirjan	1744	55 40 E	29 27 N
23	Kerman	Zarand	1672	56 34 E	30 49 N
24	Southern-Khorasan	Qaen	1440	59 10 E	33 44 N
25	Southern-Khorasan	Nahbandan	1180	60 1 E	31 31 N
26	Southern-Khorasan	Birjand	1466	59 13 E	32 51 N
27	Southern-Khorasan	Sarayan	1433	58 30 E	33 51 N
28	Southern-Khorasan	Darmian	1521	60 7 E	33 2 N
29	Esfahan	Feridan	2589	50 18 E	33 1 N
30	Esfahan	Semirom	2331	51 34 E	31 23 N
31	Esfahan	Ardestan	1154	52 22 E	33 23 N
32	Esfahan	Naien	1560	53 5 E	32 50 N
33	Esfahan	Khansar	2074	50 19 E	33 17 N
34	Esfahan	Natanz	1659	51 54 E	33 30 N
35	Semnan	Shahmirzad	2102	53 19 E	35 46 N
36	Semnan	Sorkheh	1174	53 12 E	35 28 N
37	Semnan	Ivanaki	1080	52 4 E	35 20 N
38	Semnan	Kalateh	1161	55 33 E	36 23 N
39	Northern-Khorasan	Esfarayen	1252	57 30 E	37 4 N
40	Northern-Khorasan	Shirvan	1094	57 55 E	37 24 N
41	Northern-Khorasan	Bojnord	1069	57 19 E	37 28 N
42	Northern-Khorasan	Maneh	675	56 44 E	37 39 N
43	Khorasan-Razavi	Gonabad	1095	58 42 E	34 20 N
44	Khorasan-Razavi	Ferdows	1278	58 10 E	34 1 N
45	Khorasan-Razavi	Torbat-Heidareh	1363	59 12 E	35 16 N
46	Khorasan-Razavi	Torbat-Jam	905	60 38 E	35 13 N
47	Khorasan-Razavi	Kashmar	1057	58 28 E	35 14 N
48	Khorasan-Razavi	Taybad	805	60 47 E	34 44 N
49	Khorasan-Razavi	Bardsekan	969	57 58 E	35 15 N

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