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Geographical variation in breaking the seed dormancy of Persian cumin (*Carum carvi* L.) ecotypes and their physiological responses to salinity and drought stresses

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ABSTRACT

Seed germination is a critical stage in the life of crops. The laboratory experiments were conducted to determine whether the severity of seed dormancy in Persian cumin ecotypes and their response to salinity and drought stresses could be influenced the parent plant habitat. Seeds of three Persian cumin ecotypes (Alamut, Khabar, and Tandoureh) were collected from three protected areas in Iran. For each ecotype in seed dormancy-breaking study, the treatments included control, the prechilling of seeds for 20, 40, and 60 days at 5 °C and 10% relative humidity and the inundating of them into 0.72, 1.44, and 2.88 mmol GA_3 L^{-1} for 24 h. In the response to salinity and drought stresses study, two experiments were arranged separately as a completely randomized factorial design to investigate the effect of salinity and drought stress. In the 1st experiment, the prechilled seeds of three Persian cumin ecotypes were subjected to four salinity stress levels (0, 2, 4, and 6 dS m^{-1}). In the 2nd experiment, those were subjected to four drought stress levels (0, −0.2, −0.4 and −0.6 MPa). All three ecotypes of Persian cumin had a deep dormancy. The ecotype of Alamut which has been harvested from a cool climate had a deeper seed dormancy than other ecotypes which have been harvested from a relatively warm climate. In all ecotypes, the highest germination rate and the lowest mean germination time were found where their seeds were prechilled for 60 days. The highest response to prechilling was observed in Alamut ecotype. The salinity and drought stresses resulted in a reduction in the rate and a delay in the speed of seed germination in three ecotypes of Persian cumin. The highest sensitive to salinity and drought stresses was observed in Alamut ecotype which its seed was obtained from where soil pH and EC are low. Here, habitat-correlated variation in Persian cumin seed germination response and stress tolerance was demonstrated.

1. Introduction

Persian cumin (*Carum carvi* L.) is a biennial herbaceous plant in the family Apiaceae, native to Europe, West Asia, and North Africa. It produces the seeds containing approximately 8% essential oil composed mainly of carvone [2-Methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one] and limonene [1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene] (Bailer et al., 2001). The demand for Persian cumin's seed which is used as a popular spice in various foods and a carminative for colic is constantly increasing in the global market (Malhotra and Vashishtha, 2007). Recently, the fungicidal activity of extract from Persian cumin have also taken into consideration (Kocic-Tanackov et al., 2014). In Iran, the interest to cultivate this crop have recently been increased in Iran. Due to the fact that this country is located in arid and semi-arid regions of the Earth according to the De Martonne Aridity Index; the moisture stress and high soil-salinity conditions are the most common abiotic stresses in limiting the growth of Persian cumin as well as other crops (Khajeh-Hosseini et al., 2003).

Germination is a critical stage in the life of crops; especially when it happened under drought condition or physiological drought created from salinity condition. The first critical factor for germination, soil moisture, must be suitable for seeds to germinate (Khajeh-Hosseini et al., 2003). It is well-established that the abiotic stresses such as drought and salinity can affect germination in the plant species of family Apiaceae such as rock samphire (*Crithmum maritimum* L.) (Amor et al., 2005; Atia et al., 2009), wild carrot (*Daucus carota* L.), villous deadly carrot (*Thapsia villosa* L.), fennel (*Foeniculum vulgare* Mill.) (Pérez-Fernández et al., 2006), and pennywort (*Centella asiatica* (L.)

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Urban (Devkota and Jha, 2010). In such a situation which the possibility of seedling survival is low during abiotic stresses, germination is prevented by the mechanism of seed dormancy. Persian cumin also follows from this phenomenon (Putievsky, 1998), resulting in a non-uniformity seedling emergence in the fields.

The seed dormancy was classified into two groups: exogenous and endogenous. The former is occurred when the coat of seed is too impermeable for moisture to penetrate; while the latter is occurred when the embryo of seed is not developed due to an imbalance in some hormones (Baskin and Baskin, 1998). In almost all plant species in the family Apiaceae (Baskin and Baskin, 1998) exhibit a deep seed dormancy, relating their undeveloped seed's embryo. Persian cumin also follows from this theorem (Hradilik and Cisarova, 1975; Putievsky, 1980).

To break this type of seed dormancy, the seeds must experience the process of stratification or prechilling (García-Gusano et al., 2004). It is well-established that gibberellic acid can have a role to accelerate this process to move the seeds of Persian cumin from a deep to a non-deep dormancy (Hradilik and Cisarova, 1975). Dormancy-breaking and increased germination affected by prechilling and treating with gibberellic acid in some plant species in the family Apiaceae have already reported such as black zira (*Bunium persicum* (Boiss.) Fedtsch.) (Bonyanpour and Khosh-Khui, 2001), jashir (*Prangos ferulacea* (L.) Lindl.) (Razavi and Hajiboland, 2009), asafoetida (*Ferula assafoetida* L.) (Zare et al., 2011), rough chervil (*Chaerophyllum temulum* L.) (Vandelook et al., 2007), masterwort *(Peucedanum ostruthium* (L.) W.D.J. Koch) (Novak et al., 2011), bluntseed sweetroot (*Osmorhiza depauperata* Phil.) (Walck and Hidayati, 2004), and galbanum (*Ferula gummosa* Boiss.) (Nadjafi et al., 2006; Rouhi et al., 2012).

In this study, the germination behavior of three Persian cumin ecotypes collected from three protected areas in Iran in response to prechilling and treating with gibberellic acid was investigated. Then, the physiological response of prechilled seeds to salinity and drought stresses was investigated. Here, we have documented the role of the parent plant habitat in the emergence of such a response in its offspring to these treatments. Such knowledge can be useful to select, domesticate and/or cultivate the proper ecotypes of Persian cumin.

2. Materials and method

2.1. Seed collection location

Seeds of three Persian cumin ecotypes were collected from three protected areas in Iran during 2009 growing season. The geographical, edaphical, and climatological traits of these protected areas were presented in Table 1. The names of protected areas were contractually considered as the name of ecotypes in our study. The seed morphological traits of these three ecotypes were summarized in Table 2.

2.2. Seed dormancy-breaking

For each ecotype, a total of 70 glass petri dishes comprising of 7 treatments and 10 replications per treatment were maintained in a completely randomized design. The treatments included control, the prechilling of seeds for 20, 40, and 60 days at 5 °C and 10% relative humidity and the inundating of seeds into the solutions of gibberellic acid (GA_3 90%) with concentrations of 0.72, 1.44, and 2.88 mmol GA_3 L^{-1} for 24 h. In the prechilling treatments, 4 g seed of each ecotype was packed separately in a cloth and then placed in the refrigerator. In the gibberellic acid treatments, four g seed of each ecotype was sandwiched separately between two layers of Whatman No. 1 filter paper, inundated into 10 ml-solutions of gibberellic acid, washed with distilled water, and then dried on paper towel at 20 °C. After treating the seeds, 100 seeds of each ecotype were placed in petri dishes on top of a single layer of filter paper. Then, 20 ml distilled water was added to each petri dish. Finally, they were incubated at 20 °C in the dark. Seed germination was daily recorded by visual count at 9:00 a.m. On the basis of International Seed Testing Association (1985) the seeds were defined as germinated when their radicals exceed a length of 2 mm.

Table 2
The seed morphological traits of three Persian cumin ecotypes.

Ecotype	Color	Shape	Length (mm)	Diameter (mm)	1000-seeds weight (g)
Alamut	Mustard	Oval	5.0 ± 0.2	1.1 ± 0.1	2.5 ± 0.1
Khabar	Dark brown	Oval	4.8 ± 0.3	1.2 ± 0.1	2.3 ± 0.1
Tandoureh	Light brown	Oval	4.3 ± 0.3	1.2 ± 0.1	2.1 ± 0.2

Means ± standard error (n = 10).

Germination rate was expressed as percent of germinated seeds over 100 seeds seeded. Mean germination time (MGT) was calculated by the following equation (Mubeen et al., 2011):

$$MGT = \frac{\sum n.\,D}{\sum N} \tag{1}$$

Where, n is the number of germinated seeds on each day of the test; D is the days of incubation from the beginning of the test and N is the total number of seeds germinated on the final day of the test.

2.3. The response to salinity and drought stresses

2.3.1. Seed germination

Before starting the test, the seeds were prechilled for 60 days in the same manner as described above. Two experiments were arranged separately as a completely randomized factorial design to investigate the effect of salinity and drought stress. In the first experiment, three Persian cumin ecotypes (Alamut, Khabar, and Tandoureh) were subjected to four salinity stress levels (0, 2, 4, and 6 dS m^{-1}). In the second experiment, those three ecotypes were subjected to four drought stress levels (0, −0.2, −0.4 and −0.6 MPa). The salinity and drought stresses were induced using NaCl and Polyethylene glycol 6000 (PEG) as described by Michel and Kaufmann (1973), respectively. For control treatment, the distilled water was applied in both experiments. One hundred prechilled seeds were placed in petri dishes on top of a single

Table 1
The geographical, edaphical, and climatological traits of the protected areas at which the seeds of Persian cumin ecotypes were collected in Iran.

Protected area	Geographic coordinates	Soil pH	Soil EC (dS m^{-1})	Climate type	Mean annual temperature (°C)	Mean annual precipitation (mm)
Alamut	Moallem Kalayeh, Qazvin Province 36°26′40″N, 50°35′9″E	7.7 ± 0.3	0.8 ± 0.0	Semi-Arid	5.5 ± 0.7	405 ± 39.5
Khabar	Baft, Kerman Province 28°48′55″N, 56°19′51″E	8.9 ± 0.4	4.0 ± 0.2	Arid	17.0 ± 1.5	180 ± 18.2
Tandoureh	Tagan, North Khorasan Province 37°25′49″N, 58°44′9″E	8.1 ± 0.4	2.2 ± 0.2	Arid	12.0 ± 1.3	210 ± 12.4

Means ± standard error (n = 10). The weather data were obtained from the weather stations in the area of interest and are an average of over the past 10 years. Climate type is according to the De Martonne Aridity Index. The soil samples were analyzed at the Soil Laboratory of Bu-Ali Sina University.