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Full Length Research Paper

Effect of drought stress on germination and seedling growth of *Salvia* species

Jaafar Abdollahi[1]*, Seyed Hassan Tafaroji[2], Mohsen Ebrahimi[3], Hossein Ali Ramshini[3], Ahamad Reza Dehghani Tafti[1], Mehdi Mortazavian[3], Mostafa Eftekhari[1], Amir Hossein Dezfoulian[1] and Asma Bagheri[1]

[1]Department of Agriculture, Yasooj Branch, Islamic Azad University, Yasooj, Iran.
[2]Department of Agricultural, Payame Noor University (PNU), Beyram, Iran.
[3]Department of Agronomy and Plant Breeding Sciences, College of Abouraihan, University of Tehran, Iran.

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Medicinal plants play a key role in the human health and have a huge share in the global economy. Cultivation of *Salvia* species is increasing mainly due to high commercial worth of this plant in food, medicine, perfumery, and cosmetic industries. The aims of this research were: (1) to determine drought tolerant *Salvia* spp. and (2) examining drought stress effects on germination and seedling growth, separately. Seeds of 36 *Salvia* accessions (15 species) collected from different regions of Iran were primarily treated by soaking and gibberellic acid was used to break the seed dormancy. Afterwards, germination rate, percentage and early seedling growth were measured in two separate tests with four osmotic potentials: 0 (control), -0.3, -0.6, and -0.9 MPa using polyethylene glycol (PEG, 6000). The principal-component analysis (PCA) summarized all traits into two components ("germination" and "seedling growth") in all osmotic treatments. There was significant correlation between germination traits and among seedling growth traits in -0.3 and -0.6 MPa, but correlation between the traits of two components was not significant. Tolerance of genotypes to drought stress in germination and seedling stage showed a high diversity. Tolerant accessions for both components are suitable for cultivation in controlled conditions, but tolerant accessions only for "seedling growth" component, probably would be appropriate for distribution in their habitats for conservation purposes. However, the accessions which simply germinate at water stress conditions, but are susceptible at seedling stage, will die and lose their seed bank in the soil.

Key words: Germination, medicinal plant, osmotic stress, polyethylene glycol (PEG), *Salvia*, seedling growth.

INTRODUCTION

Nowadays in the world, especially with recent changes such as reduction of irrigation, development of sustainable agricultures is possible by the introduction of new crops (Olesen and Bindi, 2002; Bisio et al., 2010). To achieve this aim the cultivation of aromatic and medicinal species can be worthwhile, as most of them show dehydration tolerance (Auge et al., 2003).

Lots of *Salvia* species are, frequently, utilized in food, medicine, and perfumery industries for their useful essential oils (Ulubelen and Topcu, 1998; Ozcan et al., 2003; Goren et al., 2006).

Numerous studies have indicated possession of characteristics, such as antioxidant, antiviral, and antimicrobial activities in some species of this genus (Sivropoulous et al., 1997; Javidnia et al., 2002).

*Corresponding author. E-mail: abdollaij@ut.ac.ir. Tel: +98-939-0833480. Fax: +982923040811.

Abbreviations: ANOVA, Analysis of variance; **P,** error probability level; **r,** coefficient of correlation (Pearson-r); **n,** number; **MPa,** megapascals; **PCA,** principal component analysis; **PEG,** polyethylene glycol; **GA3,** gibberellic acid.

Table 1. Accessions and their collection regions of *Salvia* spp. applied in this study.

S/N	Species	Region
1	*S. nemorosa*	Ardabil
2	*S. nemorosa*	Khansar
3	*S. sclarea*	Kordestan
4	*S. virgata*	Esfahan
5	*S. aethiopis*	Markazi
6	*S. sharifii*	Beyram
7	*S. eremophila*	Kerman
8	*S. macrosiphon*	Bushehr
9	*S. macrosiphon*	Golpayegan
10	*S. macrosiphon*	Yazd
11	*S. nemorosa*	Borujen
12	*S. sclarea*	Dena
13	*S. sclarea*	Shiraz
14	*S. sclarea*	Yazd
15	*S. spinosa*	Lorestan
16	*S. verticillata*	Ardabil
17	*S. verticillata*	Khoi
18	*S. aethiopis*	Ardabil
19	*S. choloroleuca*	Semnan
20	*S. limbata*	Ilam
21	*S. limbata*	Lorestan
22	*S. limbata*	Miandoab
23	*S. reuterana*	Esfahan
24	*S. sclarea*	Semnan
25	*S. spinosa*	Hamedan
26	*S. syriaca*	Fars
27	*S. syriaca*	Ilam
28	*S. verticillata*	Tehran
29	*S. aethiopis*	Sari
30	*S. chloroleuca*	Ilam
31	*S. sclarea*	Khoramabad
32	*S. sharifii*	Beyram
33	*S. spinosa*	Kashan
34	*S. sharifii*	Hormozgan
35	*S. macrosiphon*	Bushehr

In total of 70 species and 40% endemism, *Salvia* has a consequential center of diversity in Iran. It exhibits an interesting range of morphological variation which is as great as, if not more than, anywhere in the whole world (Rechinger, 1982). Such diversity of medical plants is valuable in plant breeding programs.

Water limitation is the most important hazard affecting plant physiology and development (Boyer, 1982). Furthermore, plants affected by water-stress possibly will expose more to other biotic or abiotic stresses, such as pathogen attack, chilling or air pollution, which limits plant productivity. Seed germination and seedling growth may be significantly reduced by the decreasing rate of water absorption in drought condition (Almansouri et al., 2001; Murillo-Amador et al., 2002; Patanè et al., 2009). For the majority of medicinal plants, drought tolerance remains mainly undetermined.

An effect comparable with drought stress is created by adding polyethylene glycol (PEG) to the culture medium under *in vitro* conditions (Carpita et al., 1979; Pandey and Agarwal, 1998). PEG has, frequently, been employed to induce osmotic stress throughout drought tolerance studies (Hohl and Peter, 1991; Lu and Neumann, 1998). PEG, in comparison with other osmotica (that is, potassium salts), is generally more applied, since it has no toxicity (Bradford, 1986; Kaya et al., 2006).

The objectives of this study were: (1) to determine tolerant *Salvia* spp. and accessions against drought stress induced by PEG 6000, and (2) to assay drought stress effects separately on the traits of germination and seedling growth, and to compare these results.

MATERIALS AND METHODS

Seeds of 36 *Salvia* accessions containing 15 species from different regions of Iran were used (Table 1). In general, new harvested seeds have natural dormancy, requiring cold treatment and a period for ripening and maturity to germinate (Hashemi and Estilai, 1994; Budvytyte, 2001). Seeds were matured during one year storage in darkness at -4°C.

Seeds were surface sterilized in 4% sodium hypochlorite solution containing a few drops of the surfactant Tween for 12 min to eliminate fungus contamination on seed coat and were then rinsed three times with sterile distilled water before soaking. Seeds were placed in silk fabrics, separately, and then were set in plastic cups to be soaked by tap water. After 24 h soaking, seeds were air dried on draining papers for 24 h. Seeds were surface sterilized again by the same method after drying.

Seeds were treated by 150 mg/L gibberellic acid (GA3) to breakage seed dormancy and enhance germination, and also, PEG 6000 was used to induce osmotic stress. Treatments included control, -0.3, -0.6, and -0.9 MPa for PEG 6000 (Michel and Kaufmann, 1973). To predict accurate difference between the effect of PEG on germination and seedling traits, two separate experiments were conducted.

In germination test (test 1), seeds were placed on Whatman No. 3 filter paper in Petri dishes containing 0 (control), -0.3, -0.6, and -0.9 MPa induced by PEG 6000 supplemented with 150 mg/L GA3. For each accession, seeds were allocated to four replicate Petri dishes, each containing 100 seeds in a completely randomized design. Seeds were incubated for 16 days in a germination chamber in the following growth condition, 14/10 h light/dark cycle at 20 ± 1°C. All Petri dishes were sealed with parafilm to prevent desiccation. To ensure no systematic influences due to position of Petri dish placement in the chamber, re-randomization of Petri dishes was done every other day (Yang et al., 1999). Seeds with at least 2 mm radicle emergence were recorded daily as ‘germinated’.

To examine seedling growth in drought conditions (test 2), 3000 seeds of each accession after a day soaking, drying and sterilization were placed on filter paper discs in large glass Petri dishes containing distilled water supplemented by 150 mg/L GA3 to germinate. As soon as seed radicles appeared, they were transferred to the Petri dishes containing four treatments of PEG. Synchronous germinated seeds from each accession were transferred into the separate Petri dish to neutralize the effect of time on seedling traits. Length, fresh weight and dry weight of

Table 2. The effect of osmotic stress treatments on germination and seedling traits (*F*-value).

ANOVA	Plumule L	Radicle L	Plumule FW	Plumule DW	Radicle FW	Radicle DW	Ger. Rate	Ger. Per.
Accessions	98**	207**	279**	119**	310**	142**	100**	39**
Treat	5619**	4479**	6559**	2287**	4889**	2292**	1557**	1517**
Accession × Treat	33**	44**	55**	21**	86**	41**	16**	894**

F-values from the one-way ANOVA for the 36 accession studied in this work (**P < 0.01); L, length; FW, fresh weight; DW, dry weight; Ger. Rate, germination rate; Ger. Per., germination percentage.

Table 3. The justification amount of changes by eigenvalues and proportions of the first two components in three osmotic treatments.

Treatment	Component	Eigenvalue	Proportion	Cumulative
-0.3 MPa	1	4.7524	0.594	0.594
	2	1.7651	0.221	0.815
-0.6 MPa	1	4.792	0.599	0.599
	2	1.891	0.236	0.835
-0.9 MPa	1	4.266	0.533	0.533
	2	1.861	0.233	0.766

shoots and roots were recorded at day 10.

Seed germination and mean germination time of accessions were recorded in trial 1. Five seedlings of each treatment in trial 2 were selected randomly and primary lengths and weights (fresh and dried) of radicle and plumule were measured for each experimental situation during germination tests on the 10th day of translocation to osmotic treatments.

Data for seed germination percentage were arcsine transformed. One-way analysis of variance (ANOVA) in SAS (SAS Institute Inc., Version 9, 2002) was carried out for each accession, separately to identify treatment differences. Data for all traits were standardized by the following equation:

$$SD = (TDC-TDS)/TDC \quad (1)$$

where SD is the standardized data for each trait, TDC is the trait data in control and TDS is the trait data in stress condition (-0.3, -0.6 or -0.9).

The correlation structure of germination and seedling growth traits in both trials for three osmotic treatments was investigated by principal component analysis (PCA) on standardized variable values. The PCA was carried out for dimension reduction of data. Germination and seedling related traits were used in this analysis. Minitab (Minitab Inc., State College, PA, USA) was used for PCA. Pearson correlation method was applied to the measured traits among all osmotic levels.

RESULTS

ANOVA of each accession showed highly significant differences (P≤0.01) among all measured traits under drought stress levels (Table 2). The largest diversity among accessions was observed in -0.3 MPa between the osmotic treatments, so that it was possible to separate accessions, accurately, with different range of tolerance to drought stress. The amplitude of this diversity was lower in the treatment of -0.6 MPa and it was negligible in -0.9 MPa. However, there was no interaction effect between osmotic treatments and accessions, in other words, the traits values decreased in all accessions by increasing stress severity.

PCA was applied in three treatments. In osmotic treatment of -0.3 MPa, the first and second components explained over 81% of variations. This amount was about 83% in -0.6 MPa and about 76% in -0.9 MPa (Table 3). The first component, in all treatments mainly was affected by the length and weight of plumule and radicle. Since these traits are related to germination growth features, this component is referred to as "seedling growth" component (Table 4). The second component in all treatments, also largely coincided with the variation of germination rate and percentage, so it was called "germination" component (Table 4).

The magnitude of evaluated trait effects on the first and second components were approximately the same, therefore, the osmotic stress response of accessions in these treatments were similar (Table 4).

Figures 1, 2 and 3 show the score plot of PCA in three levels of treatment for accessions. The accessions of *Salvia nemorosa* Ardabil and Khansar, *Salvia virgata* Esfahan, *Salvia eremophila* Kerman, *Salvia macrosiphon Yazd*, *Salvia spinosa* Lorestan, *Salvia verticillata* Ardabil, *Salvia chloroleuca* Semnan and *Salvia sharifii* Hormozgan were tolerant in both "germination" and

Table 4. Principal component loadings for three osmotic treatments on the two principal components.

Variable	-0.3 MPa		-0.6 MPa		-0.9 MPa	
	PC1	PC2	PC1	PC2	PC1	PC2
Ger. Per.	0.081	0.693	-0.146	0.674	-0.176	0.659
Plumule L	0.396	-0.036	-0.401	0.084	-0.391	0.164
Radicle L	0.412	0.03	-0.403	-0.162	-0.399	-0.181
Plumule FW	0.407	-0.087	-0.406	0.032	-0.397	-0.022
Plumule DW	0.414	-0.099	-0.418	-0.084	-0.412	-0.047
Radicle FW	0.404	-0.039	-0.395	-0.205	-0.39	-0.24
Radicle DW	0.403	-0.012	-0.37	-0.169	-0.381	-0.254
Ger. Rate	0.06	0.706	-0.15	0.658	-0.18	0.618

PC, Principal component; L, length; FW, fresh weight; DW, dry weight; Ger. Rate, germination rate; Ger. Per., germination percentage.

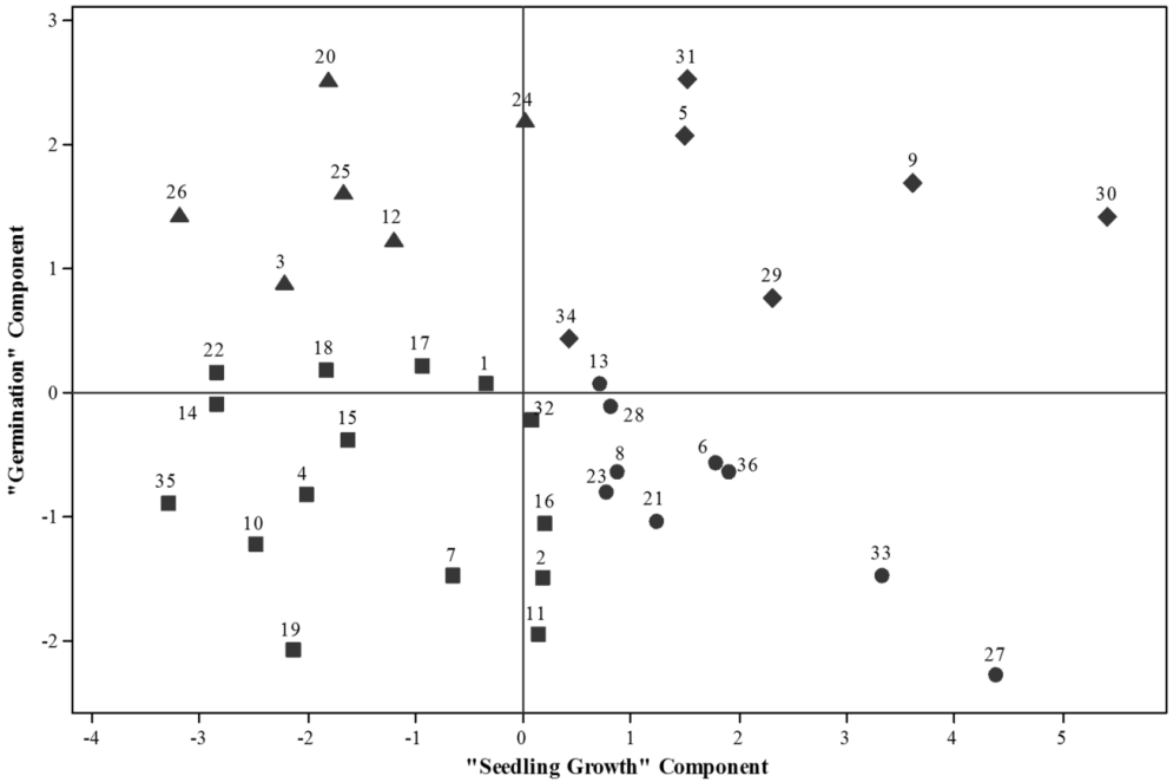

Figure 1. Distribution of accessions in PCA ordination space along component 1 (seedling growth) and 2 (germination) performed on the results from the osmotic treatment of -0.3 MPa (to detect of species numbers as shown in Table 1). ■ Tolerant in both components; ◆ Susceptible in both components; ● Tolerant in "Germination" component and susceptible for "Seedling Growth" component; ▲ Tolerant in "Seedling Growth" component and susceptible for "Germination" component.

"seedling growth" in -0.3 and -0.6 MPa treatments. However, in -0.9 MPa treatment, except the accessions of *S. nemorosa* Khansar and Borujen, *S. verticillata* Khoi, *S. spinosa* Hamedan and *S. sharifii* Hormozgan which were resistant considering both "germination" and "seedling growth" traits, all other accessions were susceptible at least for one of these traits.

The accessions of *Salvia sclarea* Kordestan and Yazd, *Salvia limbata* Ilam and *Salvia aethiopis* Ardabil were susceptible to "germination" component, but tolerant in "seedling growth" component. There was significant and positive correlation between germination rate and percentage in -0.3 and -0.6 MPa. The correlation among all the seedling growth traits in these two osmotic

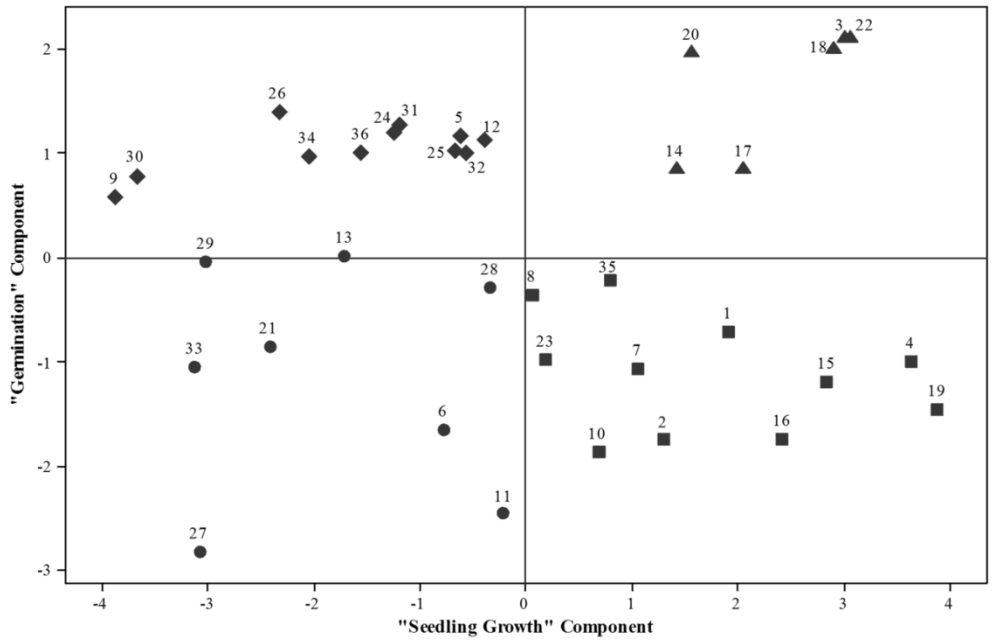

Figure 2. Distribution of accessions in PCA ordination space along component 1 (seedling growth) and 2 (germination) performed on the results from the osmotic treatment of -0.6 MPa (to detect of species numbersas shown in Table 1). ■ Tolerant in both components; ◆ Susceptible in both components; ● Tolerant in "Germination" component and susceptible for "Seedling Growth" component; ▲ Tolerant in "Seedling Growth" component and susceptible for "Germination" component.

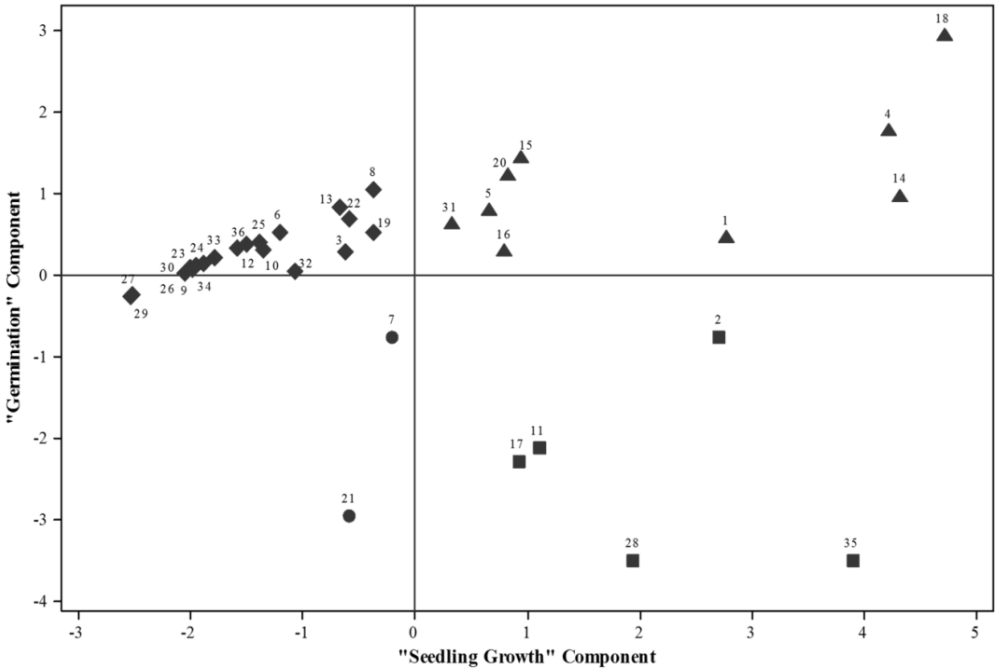

Figure 3. Distribution of accessions in PCA ordination space along component 1 (seedling growth) and 2 (germination) performed on the results from the osmotic treatment of -0.9 MPa (to detect of species numbers as shown in Table 1). ■ Tolerant in both components; ◆ Susceptible in both components; ● Tolerant in "Germination" component and susceptible for "Seedling Growth" component; ▲ Tolerant in "Seedling Growth" component and susceptible for "Germination" component.

Table 5. Pearson correlation between standardized evaluated traits in -0.3 MPa (n = 36).

Correlation	Plumule L	Radicle L	Plumule FW	Plumule DW	Radicle FW	Radicle DW	Ger. Rate
Ger. Per.	o.17^{ns}	0.14^{ns}	0.09^{ns}	0.08^{ns}	0.04^{ns}	0.04^{ns}	0.78**
Plumule L	-	0.63**	0.93**	0.91**	0.55**	0.56**	0.05^{ns}
Radicle L	-	-	0.66**	0.68**	0.90**	0.90**	0.16^{ns}
Plumule F W	-	-	-	0.94**	0.61**	0.60**	0^{ns}
Plumule F W	-	-	-	-	0.65**	0.64**	0.01^{ns}
Radicle F W	-	-	-	-	-	0.99**	0.09^{ns}
Radicle D W	-	-	-	-	-	-	0.12^{ns}

ns, Non significant; **P-value < 0.01; L, length; FW, fresh weight; DW, dry weight; Ger. Rate, germination rate; Ger. Per., germination percentage.

Table 6. Pearson correlation between standardized evaluated traits in -0.6 MPa (n = 36).

Correlation	Plumule L	Radicle L	Plumule FW	Plumule DW	Radicle FW	Radicle DW	Ger. Rate
Ger. Per.	0.35^{ns}	0.09^{ns}	0.28^{ns}	0.16^{ns}	0.05^{ns}	0.10^{ns}	0.94**
Plumule L	-	0.67**	0.89**	0.86**	0.61**	0.51**	0.31^{ns}
Radicle L	-	-	0.71**	0.75**	0.84**	0.79**	0.13^{ns}
Plumule F W	-	-	-	0.93**	0.60**	0.52**	0.25^{ns}
Plumule F W	-	-	-	-	0.72**	0.62**	0.14^{ns}
Radicle FW	-	-	-	-	-	0.95**	0.11^{ns}
Radicle DW	-	-	-	-	-	-	0.15^{ns}

ns, Non significant; **P-value < 0.01; L, length; FW, fresh weight; DW, dry weight; Ger. Rate, germination rate; Ger. Per., germination percentage.

Table 7. Pearson correlation between standardized evaluated traits in -0.9 MPa (n = 36).

Correlation	Plumule L	Radicle L	Plumule FW	Plumule DW	Radicle FW	Radicle DW	Ger. rate
Ger. Per.	0.34^{ns}	0.92**	0.90**	0.19^{ns}	0.19^{ns}	0.07^{ns}	0.94**
Plumule L	-	0.36^{ns}	0.44^{ns}	0.80**	0.82**	0.21^{ns}	0.29^{ns}
Radicle L	-	-	0.98**	0.17^{ns}	0.19^{ns}	0.15^{ns}	0.19^{ns}
Plumule FW	-	-	-	0.25^{ns}	0.27^{ns}	0.12^{ns}	0.10^{ns}
Plumule DW	-	-	-	-	0.98**	0.26^{ns}	0.12^{ns}
Radicle FW	-	-	-	-	-	0.27^{ns}	0.19^{ns}
Radicle DW	-	-	-	-	-	-	0.18^{ns}

ns, Non significant; **P-value < 0.01; L, length; FW, fresh weight; DW, dry weight; Ger. Rate, germination rate; Ger. Per., germination percentage.

treatments was also significantly high. While, there was no significant correlation between "germination" and "seedling growth" traits (Tables 5 and 6). However, non-significant correlation was observed between germination rate or percentage and any of the seedling growth traits. These findings showed that the effect of drought on "germination" traits was independent of its effect on "seedling growth" ones.

The osmotic treatment of -0.9 MPa exerted such a severe effect on both germination and seedling growth traits, such that some correlation between them changed (Table 7).

DISCUSSION

Salvia spp. are usually used in two ways: (1) cultivation in controlled conditions for commercial aims and (2) distribution of seeds in their habitats to increase the canopy and support their seed bank in the soil for conservation purposes.

In this experiment, by PCA analysis in which all the traits data were summarized, accessions were categorized into four groups: (1) tolerant accessions for both components, (2) tolerant accessions only for "germination" component, (3) tolerant accessions only

for "seedling growth" component, and (4) the accessions which were susceptible for both components

The accessions which germinate and grow well in stress condition are more suitable to cultivate. Therefore, Group 1 is suitable for this aim. The accessions involved in Group 3 are not suitable for cultivation, because they would germinate unevenly and are not agriculturally acceptable. For this reason, their tolerance to osmotic stress in seedling stage is unimportant.

In natural conditions, Group 3 accessions would be successful at dry climate, because their seeds only germinate after appropriate precipitation, and generally, they are not exposed to water stress at germination stage. However, they are commonly exposed to osmotic stress in seedling growth and establishment. From this point of view these accessions, naturally, must be evolved to refuse to germinate on stress condition, but grow well after germination in this condition. The accessions of Group 2 that simply germinate at water stress condition but are susceptible at seedling stage will die easily after germination and lose their seed bank in the soil, and then they are unsuitable for growing in natural conditions.

It can be concluded that if the experiments are done separately to assay the response of accessions to osmotic stress considering germination and seedling traits, we would be able to recognize, precisely, tolerant accessions. Otherwise, the severe decrease in germination rate and percentage would affect the measurements of seedling traits and the correlation between two separate components, would consequently be significantly positive. Thus, it could not be an unbiased result.

In wild conditions the traits of germination rate and percentage are not appropriate criteria to show drought tolerance of medicinal plants, such as *Salvia* accessio, instead, the traits related to seedling growth are more important to assay the tolerance of species to drought. Therefore, *S. nemorosa* Ardabil and Khansar, *S. virgata* Esfahan, *S. eremophila* Kerman, *S. macrosiphon* Yazd, *S. spinosa* Lorestan, *S. verticillata* Ardabil, *S. chloroleuca* Semnan ns, and *S. sharifii* Hormozgan are suitable accessions to be cultivated in controlled conditions for commercial aims. However, these accessions are not as suitable as *S. nemorosa* Khansar and Borujen, *S. verticillata* Khoi, *S. spinosa* Hamedan, and *S. sharifii* Hormozgan for distribution of seeds in their habitats to increase the canopy and support their seed bank in the soil for conservation purposes.

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