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Original Article

Investigating Propagation of Different Native Species of *Perovskia* spp.

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ABSTRACT

Perovskia spp. from the Lamiaceae family mostly grows as a wild plant the mountain areas with dry and cold climates. *Perovskia* is a beautiful plant, which can be used for urban landscaping, due to water shortage crises in the world. However, its propagation has several problems as seed dormancy in sexual propagation and low rooting percentage of cuttings in vegetative propagation. Therefore, this project was carried out to collect endemic species of *Perovski*, investigate their propagation, and use them in urban landscaping. *Perovskia* seeds in different species (*P. atriplicifolia, P. abrotanoides, P. artemisioides*) were exposed to cold treatment (5 °C) for different durations (3, 4 and 5 months) and seeds quality parameters (germination percentage, germination rate, seedling vigour index and seedling length) were measured. On the basis of the results, highest amount of germination percentage (98.66%), germination rate (10.23 g/d), seedling vigour index (6.93) and seedling length (7.35 cm) was recorded for *P. atriplicifolia* with 5 months' cold treatment. Also, vegetative propagation of all studied species was investigated. Cuttings were treated with IBA at different levels (0, 300 and 500 ppm) and cultivated in autumn and spring. Different parameters (rooting percentage, speed of rooting and roots length) were recorded. Results indicated that highest rooting percentage (88%), speed of rooting (3.70r/d) and roots length (5.73 cm) were observed in *P. atriplicifolia*, which their cuttings were treated with 500 ppm IBA and cultivated at spring. Finally, by solving propagation problems of *Perovskia*, we can suggest it as a suitable plant for urban landscaping.

INTRODUCTION

Perovskia spp. belonging to *Lamiaceae* family is a wild flowering plant, it is native to the hills of southwestern and central Asia. It is successful over a wide range of climate and soil conditions. It has an upright habit, typically reaching 0.5–1.2m tall (1.6–3.9 ft), with square stems and grey-green leaves that yield a distinctive odour when crushed. It is best known for its flowers. Its flowering season extends from mid-summer to late October, with blue to violet blossoms arranged into showy, branched panicles.

Perovskia is frequently propagated by cuttings. Because its woody crown is resistant to division, softwood cuttings are taken from shoots near the base, generally in late spring [8]. Hardwood cuttings selected in mid-to-late summer also provide a viable propagation technique [8]. Dumitraşcu, [5], investigated rooting capacity of *Perovskia atriplicifolia* and concluded that Rhizopon AA 100 mg/L treatment on sand + perlite rooting substrate showed best results regarding rooting parameters. The plant is also grown from seed in cultivation. Such seeds require exposure too cold for 30–160 days to germinate [19] and seed-raised specimens may not preserve the characteristics of named cultivars [8]. Therefore, in this research *Perovskia*

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endemic species propagation was studied, to see their differences.

MATERIAL AND METHODS

Collection of germplasm: Perovskia plants (Fig. 1) collected from nature of Isfahan (P. artemisioides), khorasan (P. abrotanoides) and Markazi (P. atriplicifolia) provinces in Iran, with 140 mm annual rain and 25 °C annual temperature (Table 1.). Experimental site: The experiment was laid out in Mahallat city (33.54 °N, 50.28 °E) and 1750 meter above sea level.

Seed Propagation Experiment

P. atriplicifolia

P. artemisioides

P. abrotanoides

Fig. 1 Perovskia plants in the research field of Ornamental Plants Research Center

Seeds of Perovskia spp. have a low germination rate due to their dormancy. Therefore, seeds were treated at 5 °C for 3, 4 and 5 months. Fresh seed germination, without any cold treatment, was considered as a control. After 3, 4 and 5 months, 50 seeds from each species were separated and placed on filter paper in each petri-dish. For the germination test, petri-dishes were placed in the germinator with 25 ± 2 °C, 13.5 µMol (photons)/s/m2 (12 hr light, 12 hr dark) and 60% relative humidity for 15 days. Seeds germination parameters were recorded. Germination percentage, speed of germination (Maguire, 1962), seedling vigour (Agrawal, 2003), shoot and rootlet length was recorded based on the following formulas. The experiment was laid out in the form of a factorial completely randomized design with three replications.

Germination Rate (g/d) = $\sum Ni/ Di$

Which: Ni= Number of seeds germinated in Di

Di= Days after germination

(g/d) = germination/day

Seedling vigour index = (germination % × seedling lenght) ÷ 100

A factorial experiment was conducted with two factors. The first factor was Perovskia species (P. atriplicifolia, P. abrotanoides, P. artemisioides) and the second factor was cold treatment (0, 3, 4 and 5months), based on Randomized Complete Block Design (RCBD) with three replications. Collected data were analyzed by SAS software and mean values were calculated by Duncan's multiple range test. Also, figures were created by excel software.

Semi-hardwood cuttings of Perovskia spp. were prepared in two seasons autumn and spring. Cuttings were prepared, with a length of 10 cm and 5mm width. The leaves of cuttings were removed and the base of them was 0.5 mm freshly trimmed. The end part of the cuttings was immersed in different concentrations of IBA solution (0, 300 & 500 mg/l), for one minute.

Cutting Propagation Experiment

Then cuttings were cultivated in the wet sand, in glasshouse with constant condition (with 25 ± 2 oC, 18.5 µMol (photons)/s/m2 (12 hr light, 12 hr dark) and 60% relative humidity) and recorded their different parameters as rooting percentage, rooting rate and roots length.

Table 1 Place of collection and characteristics of Perovskia plants

Perovskia spp.	Collection place	Altitude and latitude	Plants characteristics				
			Height	Diameter	No. of fl. stem	Start of fl.	Fl. duration
P. artemisioides	Isfahan	51 º66´- 32 º67´	111.66 cm	97.6 cm	17.6	22th May	6 months
P. abrotanoides	khorasan	59 º21´- 32 º86´	105.3 cm	74.6 cm	15.6	30th April	6 months
P. atriplicifolia	Markazi	49 º68´- 34º08´	135.3 cm	101.3 cm	19.3	25th April	7 months

Experiment was laid out in the form of factorial completely randomized design with three replications. Each replication contained thirty cuttings.

Rooting Rate = $\sum N_i / D_i$

Which: N_i = Number of cuttings rooted in D_i

D_i = Days after rooting

A split factorial experiment was conducted with three factors. The first factor was *Perovskia* species (*P. atriplicifolia*, *P. abrotanoides*, *P. artemisioides*), the second factor was season of cutting (spring and autumn) and the third factor was IBA treatment (0, 250 and 500 ppm), based on Randomized Complete Block Design (RCBD) with three replications. Collected data were analyzed by SAS software and mean values were calculated by Duncan's multiple range test. Also, figures were created by excel software.

RESULTS AND DISCUSSION

Results of this research showed the positive effects of cold treatment for breaking dormancy and improving seeds quality germination parameters, which is in agreement with Cetinbas and Koyuncu, 2006; Chen *et al*., 2007; Tewari *et al*., 2011; Pipinis *et al*., 2012.

Germination percentage: Analysis of variance indicated that, different species and durations of cold treatment in *Perovskia* plants had significant effect on the amount of germination percentage at ($P < 0.01$) of significance (Table2). Highest amount of germination percentage was observed in *P. atriplicifolia* after 5 months of cold treatment and lowest amount in *P. abrotanoides* without any cold treatment (Fig. 2, 3).

Germination rate: Analysis of variance indicated that, interaction effect of different species and durations of cold treatment in *Perovskia* plants had significant effect on the speed of germination at ($P < 0.01$) of significance (Table. 2). Highest amount of germination speed was observed in *P. atriplicifolia* and lowest amount in *P. abrotanoides* without any cold treatment (Fig. 4).

Seedling vigour: Analysis of variance indicated that, different species and durations of cold treatment in *Perovskia* plants had significant effect on the amount of seedling vigour at ($P < 0.01$) of significance (Table. 2). Highest amount of seedling vigour was observed in *P. atriplicifolia* and lowest amount in *P. abrotanoides* without any cold treatment (Fig. 5).

Seedling length: Analysis of variance indicated that, interaction effect of different species and durations of cold treatment in *Perovskia* plants had significant effect on the seedling length at 1% level of significance (Table. 2). Highest amount of seedling length was observed in *P. atriplicifolia* and lowest amount in *P. abrotanoides* without any cold treatment (Fig. 6).

Fig. 2 Seeds of *perovskia* plants

Cold treatment at the range of 1-10 °C is useful for breaking dormancy [3]. Our results support this idea and cold treatment at 5 °C improved seeds germination. Which can be due to the increase of GA in the cold condition [9]. GA causes cell expansion by stimulating related genes [7]. So, gibberellic acid increases the growth potential in the embryo [18, 13, 6]. Therefore, GA causes breaking dormancy and an increase in seed germination. Also, our results were in agreement with Liu *et al*., [14], who showed that storage condition affects seeds germination, as in warmer conditions germination decreases and in colder conditions germination increases. Brits *et al*. [4], studied different of seed storage in ambient conditions and cold storage. They concluded that seeds, which stored at low temperatures maintained a higher vigour in compare with ambient condition.

Seeds need special condition for the germination. When seeds are in dormancy period cannot germinate, even all situation like air, humidity and temperature be ideal (Yang *et al*., 2007). Therefore, chemical and mechanical treatments can help for breaking the dormancy and fastening the germination (Gusano *et al*., 2004; Du Zhou *et al*., 2003). For estimating effect of breaking dormancy treatment is collecting seeds in the same year [15], which has been done in our work.

Rooting percentage: Analysis of variance showed that effect of different species, season of cutting and IBA treatment levels had a significant effect on rooting percentage at ($P< 0.01$) of significance (Table 3). In addition, the interaction effect of season × IBA levels and species × season × IBA levels, was significant at ($P< 0.01$) and ($P< 0.05$), respectively. The highest amount of rooting percentage was observed in *P. atriplicifolia* and *P. artemisioides*, which propagated in the spring treated with IBA 500 ppm. Lowest amount of rooting percentage recorded for *P. abrotanoides* and *P. artemisioides*, which propagated in autumn and did not treat with hormone (Fig. 7, 8).

Rooting speed: Analysis of variance showed that effect of different species, season of cutting and IBA treatment levels had a significant effect on rooting percentage at ($P< 0.01$) of significance. In addition, an interaction effect of season × species and season × IBA levels on rooting speed, was significant at ($P< 0.01$). In addition, an interaction effect of different species × season × IBA levels was significant at ($P< 0.05$) (Table 3). The highest amount of rooting speed was observed in *P. atriplicifolia* and *P. artemisioides*, which propagated in the spring treated with IBA 500 ppm. The lowest amount of rooting percentage was recorded for *P. abrotanoides* and *P. artemisioides*, which propagated in autumn and did not treat with hormones (Fig. 9).

Root length: Analysis of variance in recorded data showed that the effect of different species, season of cutting and IBA treatment levels had a significant effect on root length ($P< 0.01$) of significance (Table 3). The highest amount of root length was observed in *P. atriplicifolia* and *P. artemisioides*, which propagated in the spring treated with IBA 500 ppm. The lowest amount of root length recorded for *P. abrotanoides* and *P. artemisioides*, which propagated in autumn and did not treat with hormone (Fig. 10).

Propagation of plants by cutting is still a simple, cheap and fast method for propagators. Also, produces plants with high uniformity with the parents and no genetic change happens [10]. By propagating *Perovskia* plants by cutting we can get a high yield with homogenous plants.

Fig. 7 Cuttings of *Perovskia* plants

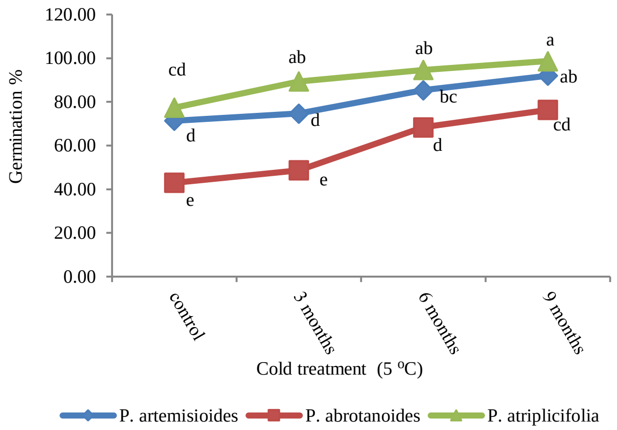

Fig. 3 Effect of cold treatment on seed germination

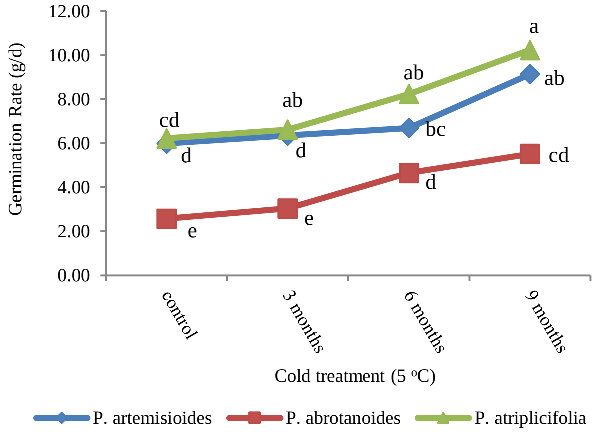

Fig.4 Effect of cold treatment on germination speed

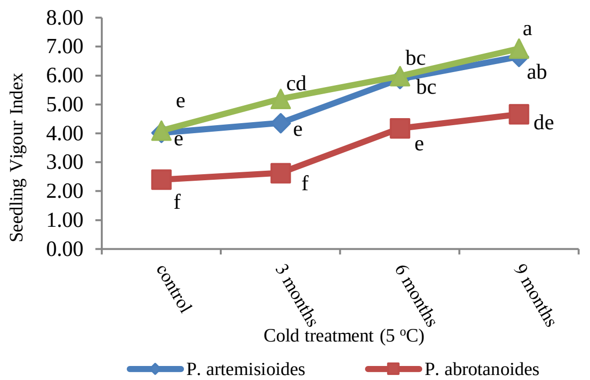

Fig. 5 Effect of cold treatment on seedling vigour index

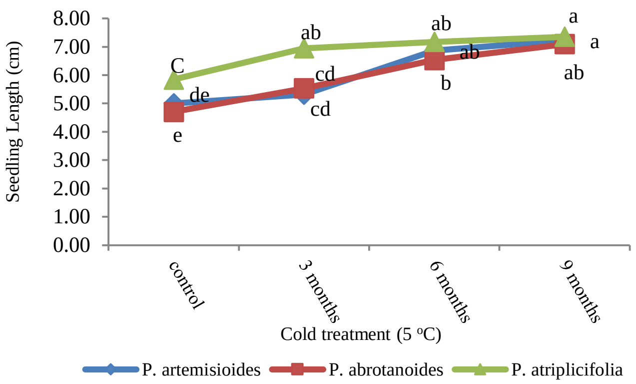

Fig. 6 Effect of cold treatment on seedling length

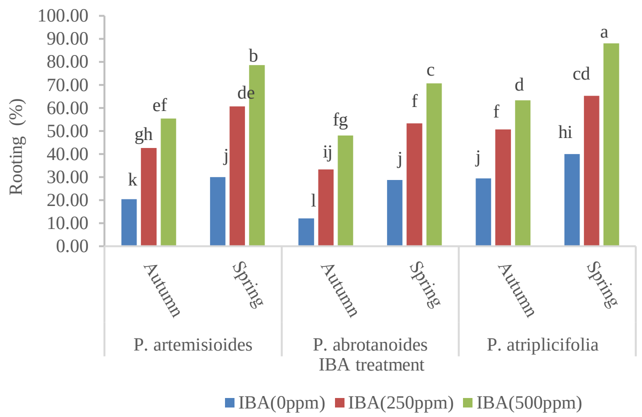

Fig. 8 Effect of cold treatment on rooting percentage

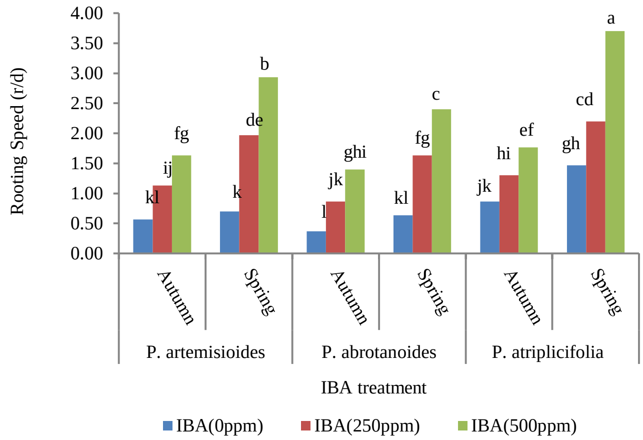

Fig. 9 Effect of cold treatment on rooting speed

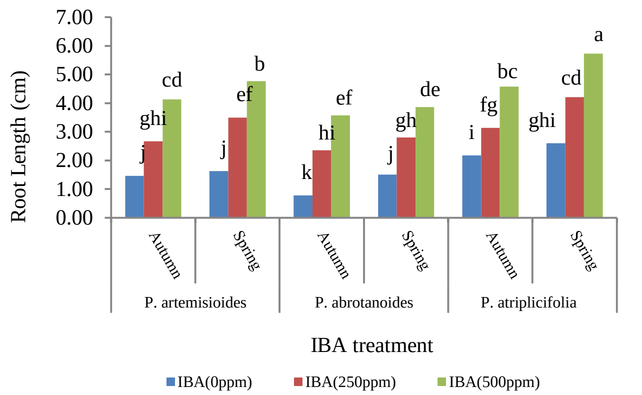

Fig. 10 Effect of cold treatment on root length

*Similar letters show a non-significant difference at the 5% level of significance on the base of Duncan's multiple range test.

Also, because of time required for breaking seeds dormancy, cutting can be preferred for propagation. According to Hartman *et al*., (1997), providing growth regulators helps in the rooting of the cuttings. Even effects in the root length, as non-treated cuttings had lower results.

Table 2 Effect of different species and cold treatments on seed quality parameters in *Perovskia* plants

Source	df	G (%)	G. R. (g/d)	S. V. I	S.L. (cm)
Species	2	3025.86 ***	50.62 ***	15.11 ***	2.59 ***
Cold treatment	3	1158.32 ***	20.50 ***	12.45 ***	7.67 ***
Species*cold	6	61.49 ns	0.61*	0.15 ns	0.39 *
Block	2	17.02 ns	0.42 ns	0.39 ns	0.38 *
Error	22	31.75	0.21	0.19	0.12
Total	25	-	-	-	-
CV %	-	7.35	7.34	9.32	5.50

ns, * & ** means non-significance and significance at 5% and 1%, respectively

G: Germination, G.R.: Germination Rate, S.V.I.: Seedling Vigour Index, S.L.: Seedling Length

Table 3 Effect of different species, cutting season and IBA treatment on rooting parameters of *Perovskia* plants

Source	df	Rooting (%)	Rooting speed (r/d)	Root length (cm)
season	1	4284.46 ***	9.96 ***	5.53 ***
Species	2	1029.79 ***	2.02 ***	7.15 ***
IBA treatment	2	7516.35 ***	10.65 ***	33.98 ***
Season × Species	2	13.12 ns	0.28 ***	0.21 ns
Season × IBA	2	141.90 **	1.30 ***	0.14 ns
Species × IBA	4	7.29 ns	0.07 *	0.07 ns
Season × Species*IBA	4	10.40 ns	0.07 *	0.17 ns
Block	2	1.68 ns	0.03 ns	0.13 ns
Block × Season	2	61.90*	0.13 *	0.000001 ns
Error	32	17.29	0.02	0.09
Total	53	-	-	-
CV%	-	8.60	10.69	10.01

ns, * &** means non-significance and significant at 5% and 1%, respectively

On the other hand, in the same physiological condition of stock plants, seasonal timing is another factor that affects the root production of cuttings. The results of the present study showed that the season of cutting significantly influenced the rooting of *Perovskia* spp. the different levels of IBA hormone also increased rooting parameters significantly ($P< 0.01$), which is in agreement with [2,16,17,20,]. The branch cutting prepared in spring had better rooting in comparison with autumn. Also, different levels of IBA had different effects. The best level of IBA concentration was 500 ppm in all the seasons and all different species. Cuttings root formation is affected by IBA, which affects the hydrolysis of polysaccharides. Therefore, the content of physiologically active sugar increases and energy is needed for meristematic tissue and root formation, as reported by Husen and Pal [12] in *Tectona grandis* and Husen, [11] in *Dalbergia sissoo*. Totally, all the IBA concentrations caused more rooting in comparison with the control in all the species, which can be due to the effect of auxins on increasing cell division, cell enlargement, carbohydrates hydrolysis, proteins synthesis and metabolites accumulation [26]. Singh *et al*., [24], reported the highest rooting in bougainvillaea in 3000 mgL^{-1} and the lowest number of rooted cuttings belonged to the control. In this research, with increasing IBA concentration, better root growth was observed, which might be due to better absorption and transportation of nutrients from the soil [21]. In this research, the results were similar to many researchers [23,23,25], which reported an increase in root length of cuttings by using IBA. Amri *et al*., [1] also, reported cuttings of *Dalbergia melanoxylon* treated with IBA produced higher root numbers and root length in comparison with untreated cuttings.

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