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Original Article

Evaluation of Seed Emergence, Seedling Vigour of Four *Satureja* Species Using Priming Technique and Chilling Treatment

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Abstract

Seeds of four *Satureja* species; *S. laxiflora* (Hayata) Sasaki, *S. macrantha* (Makino) Kudô, *S. sahendica* Bornm. and *S. macrosiphonia* Bornm. were collected from the different regions of Iran. The samples were transferred to seed technology laboratory of natural resource gene bank at Research Institute of Forest and Rangeland (RIFR), Tehran, Iran. The seeds samples were subjected to different treatments including: pre-chilling (4 °C), Gibberlic acid (250 and 500 ppm), Potassium nitrate (2 and 4%) and distilled water (control). The treated seeds were sown in pots using a factorial experiment based on a completely randomized design with three replications in a glass house experiment. The seed emergence characteristics, including: emergence percentage, emergence speed, length of root and shoot, seedling length, root/shoot length ratio, vigor index, seedling fresh and dry weight, dry/fresh weight were evaluated during 45 days. The results showed that *S. machrantha* had higher mean values of seed emergence characteristics than to the other three species. The effect of treatment was significant on seed emergence characteristics of the species, it was concluded that priming technique using Gibberlic acid was more effective than the other priming techniques.

Keywords: Emergence, *Satureja laxiflora, S. macrantha, S. sahendica, S. macrosiphonia*, Vigour and Priming

Introduction

Priming is a treatment before seed sowing and it is a process that the seed would be able to absorb water and permit for metabolic process before radical emergence [1]. One of the main problem of medicinal plant is their lower seed vigour which it would be solved by using of priming technique.

The goal of seed priming technique is improving seed efficiency under special condition. Practical usages of priming technique, is increasing percent and rate of germination and emergence in special environment for improving of seed vigour and seedling growth [2]. The priming technique has many benefits as dormancy breaking, reduction of germination time, increasing of germination uniformity, high seedling performance, simultaneity flowering and highly competitive with weeds [3]. The imbibing of the seeds in water is the simple priming technique before germination processing. Immersing of drying seeds on the solutions containing of low osmotic materials like polyethylene glycol (PEG), sorbitol, manitol, then re-drying of the seeds is nominated as osmo-priming. Any osmotic materials which have negative osmotic potential would have able to cause more water absorption by the seeds. Any osmotic materials which have negative osmotic potential would have able to cause more water absorption by the seeds. While the metabolic process would be started before germination but the germination and radical emergence would be inhibited [4,5]. The primed seeds which cultivated in fields, have high emergence percentage and also more emergence uniformity. The researcher attributed of high vigor and seedling performance the primed seeds to the

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more amylase and lipase enzyme activity in the germination process. These two enzyme activities would be increased by priming technique [6].

Gibberellic can replace light photoblastic and cooling in many seeds such as lettuce, and tobacco [7]. Evidence proved that using of plant growth regulators would be useful for growth and enhancement of seed emergence. There are some reports have confirmed the positive results of these materials.

Satureja sahendica is distributed in many parts of Iran including the provinces of Azarbaijan, Zanjan, Kurdistan, Kerman shah. Its habitats is rocky slopes- rocky in altitude of 1300 to 2500 m above sea level in Iranian-Turanian region [8]. *Satureja macrantha*, is growing in Iranian-Turanian, with place of stone and rocky area at an altitude of 400 to 2650 m above sea level [8]. The geographical distribution of this species in Iran includes the provinces of Azarbaijan, Zanjan and Kurdistan, Hamedan, Kermanshah [8]. *Satureja laxiflora* is an annual plants, is growing in the highland and calcareous rocks habitats at altitude of 500 to 1850 m above sea level. The geographical distribution of this species in Iran includes: Qazvin, Azerbaijan [8]. *Satureja macrosiphonia* is habituated in rocky slopes in the Iran-Turanian region at an altitude of 1450 m above sea level. This plant is endemic of Iran. It is distributed in the mountain of Kohdasht and kohpelkeh in Lorestan, Iran [8].

In some countries like England, plants of savory are consumed as a spice. Savory has purview that increase hypertension and medicate cough. The species of savory genus have many carvacrol which used in the pharmacy industry special in dentistry. This plant is anti bloat and it would be used for removal swoon and stomach pain [9].

One of the problems in maintenance of seed resources in gene banks, is seed aging and reduce seed germination during storage. Sometimes germination drastically reduced, therefore, to revive and increase germination and seedling growth, need some treatments for breaking dormancy. Priming technique is also one of the methods for enhancement and uniformity of germination of the deteriorated seed [10]. The aim of this research was to determine the effects of different seed priming treatments (osmo-priming, hormonal priming, hydro-priming) and pre cooling treatment to enhance seed emergence parameters and of seedlings in 4 species of savory.

Material and Methods

The seeds of four species of *Satureja* (*S. laxiflora* (Hayata) Sasaki, *S. macrantha* (Makino) Kudô, *S. sahendica* Bornm. and *S. macrosiphonia* Bornm.) were investigated in the greenhouse. Prior to experiments, seeds were disinfected with liquid fungicides of Vitawax tiram 1% for 5 min. Treatment were moist pre-chilling (four weeks at 4 °C), hormonal priming: Gibberlic acid (250 and 500 ppm) and Potassium Nitrate (2% & 4%) and distiled water (control). The primed seeds were dried at room temperature (24 °C) for 24h. Then, the primed seeds were sown in 2000 ml pots (with ratio 1:1:1 of soil, sand and compost) with three replications and kept at greenhouse temperature of 30-20 °C and 10000 lux of light during the day and 12-5 °C were at night. The irrigation of the pots took place by interval time.

Percentage and seed emergence rate were recorded after 3, 6, 9, 12, 15, 18 and 21 days. Seedlings growth were completed in 45 days. Then root and shoot lengths, seedling length, vigor index, seedling fresh weight and dry weight were evaluated during the experiment.

According Maguire, [11], the speeds of emergence (GS) were calculated by following equation:

$$G.S = \frac{\sum n}{\sum n(n * DN)} * 100$$

Where:

n is the number of seeds germinated on day Dn,

Dn is the number of days from sowing, corresponding to n,

The vigour index was measured as Abdul-Baki and Anderson formula, [12]. As following equation

$$Vi = \frac{\%Gr \text{ x } MSH}{100}$$

Where:

VI = Gibberlic Index

%G r = Emergence Percentage

MSH = Mean Seedling Height

The data were analyzed by factorial experiment based on completely randomized design with three replications. Data was analyzed of variance using the software of Minitab14 and SAS9 and mean comparisons were made using Duncan method.

Results

The results of analysis of variance for germination characteristics (Table 1) revealed a significant difference (p≤1%) between specie, treatment and specie by treatment intraction for all traits except for seedling dry weight.

Main Effect of Species

Mean comparison between species showed higher seedling emergence values (28.6%), speed of emergence (1.80 seeds per day), vigor index (11.9), seedling fresh weight (37 mg) and number of normal seedling (5.7 out of 10) were obtained in *S. macrantha*. Similarly, the higher values of seedling length (43.6mm) and seedling fresh weight (3.3 mg) were belonged to *S. laxiflora* (Table 2). In contrast, the lowest values of all of the traits except the speed of emergence and seedling fresh weight were observed in *S. sahendica*. For the latter traits the lowest values were obtained in *S. macrosiphonia* (Table 2).

Main Effect of Priming Treatments

Mean comparison between priming treatments showed that the higher values for all of the traits were obtained in the Gibberlic acid of 250ppm followed by Gibberlic acid of 500ppm that were significantly higher than other treatments and control. For the other treatments, there were no significant differences with the control except for cold treatment that had the lowest value of speed of emergence and ranked as the same group with control (Table 3).

Species by Treatment Interaction Effects

Species × treatments interaction effects were significant for all of traits except seedling dry weight (p<0.01) (Table 1), indicating that species had different responses to priming treatments (Table 4). Comparison between treatment for each species showed that the higher values of seedling length (59 mm) and vigor index (21) were obtained in *S. laxiflora* using Gibberlic acid of 500 ppm, that were significantly higher than control and other treatments. The lower values of seedling length (32mm) and vigour index (3) were obtained in *S. laxiflora*, that subjected to cold treatment. In *S. macrantha*, the higher seed length (51mm) and vigour index (15) were related to Gibberlic acid 500 ppm compare to other treatments.

For seedling fresh and dry weight the higher values of 44mg and 5mg, respectively, were obtained in *S. laxiflora* using Gibberlic acid of 500ppm, followed by Gibberlic acid of 250ppm for seedling fresh weight (52 mg) in *S. macrantha* which were significantly higher than control and other treatments. In contrast, the lower value of seedling fresh weight (25.7mg) was obtained in *S. laxiflora* using Potassium nitrate1%. Similarly, the lower values of seedling fresh weight (33 and 31mg) were obtained in *S. macrantha* in control and cold treatments, respectively. There was no significance difference between treatments for seedling dry weight in *S. macrantha* (Table 4).

For *S. sahendica* the higher values of seedling fresh weigh (35 mg) and seedlings dry weight (4.1mg) were obtained using Gibberlic acid of 500ppm and cold treatment compare to other treatments and control. In *S. macrosiphonia* the higher value of fresh weight (29.2mg) was related to cold treatment.

For number of normal seedlings, the higher value of (7.3 and 7 seedlings out of 10) were obtained in *S. laxiflora* using Gibberlic acid of 500ppm and 250 ppm than that for control and other treatments, respectively. In this species, the lower value of normal seedling (1.7 seedlings) was related to cold treatment. There was no significance difference between treatments for number of normal seedlings in *S. macrantha* (Table 4).

Table 1 Analysis of variance of the studied traits in four *Satureja* species subjected to 6 priming treatments using factorial experiment in greenhouse condition

Source of variation	DF	MS						
		Seedling Emergence (%)	Speed of Emergence Per day	Seedling Length (mm)	Vigor index	Fresh weight (mg)	Dry Weight (mg)	No. of normal seedlings
species	3	1334.7 **	6.13 **	561.9 **	328.8 **	1216.3 **	9.54 ^{ns}	53.4 **
treatment	5	568.1**	5.34 **	936.7 **	290.4 **	338.8 **	2.60 **	24.0 **
Species* treatment	15	309.8 **	1.42 **	158.3 **	82.7 **	202.5 **	1.74 ^{ns}	12.5 **
Error	115	49.07	0.31	54.06	15.9	51.74	1..52	1.99
CV		34.12	45.04	18.1	44.7	22.39	43.54	34.77

*, **= respectively significance 5% and 1%

Table 2 Mean comparison of seed emergence characteristics between four *Satureja* average over treatments in greenhouse condition

Name of species	Seedling Emergence (%)	Speed of Emergence Per day	Seedling Length (mm)	Vigor index	Fresh weight (mg)	Dry Weight (mg)	No of normal seedlings
S. laxiflora	20.6 b	1.20 b	43.6 a	10.2 a	a 34.3	3.3 a	4.1 b
S. macrantha	28.6 a	1.80 a	41.4 ab	11.9 a	37.0 a	2.7 b	5.7 a
S. sahendica	15.0 c	0.88 c	38.1 b	5.9 b	29.6 b	2.4 b	3.0 c
S. macrosiphonia	20.8 b	1.17 b	34.5 c	b 7.1	21.3 c	2.5 b	4.2 b

Means of each column followed by the same letters had no significant differences (P#0.05) based on DMRT method.

Table 3 Mean comparison of seed emergence characteristics between six treatments average over species in greenhouse condition

Treatments	Seedling Emergence (%)	Speed of Emergence Per day	Seedling Length (mm)	Vigor index	Fresh weight (mg)	Dry Weight (mg)	No of normal seedlings
Control	16.40 b	0.91 c	36.05 b	5.83 b	27.32 b	2.44 b	3.23 b
Cold	21.28 a	0.70 c	35.28 b	8.40 b	29.67 b	2.88 ab	4.28 ab
1% KNo3	19.50 b	1.32 b	34.95 b	6.92 b	27.00 b	2.40 b	3.90 b
2% KNo3	18.55 b	1.24 b	34.15 b	7.13 b	32.75 a	2.65 ab	3.73 b
Ga250 (ppm)	23.07 a	1.75 a	43.67 a	11.45 a	33.65 a	2.93 a	5.10 a
Ga500 (ppm)	17.89 b	1.60 a	33.80 b	12.79 a	32.75 a	3.05 a	5.25 a

Means of each column followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Table 4 Mean Comparison of species x treatment interaction effect for seed emergence characteristics in six priming treatments of four *Satureja* species in greenhouse condition

Name of Species	Treatment	Seedling Emergence (%)	Speed of Emergence Per day	Seedling Length (mm)	Vigor index	Fresh weight (mg)	Dry Weight (mg)	No of normal seedlings
S. laxiflora	Control	14.2 c	0.7 b	40.2 c	5.6 bc	33.2 bc	3.2 b	2.6 bc
	Cold	8.3 c	0.1 c	22.2 d	3.1 c	30.3 cd	2.5 b	1.7 c
	Ga500 (ppm)	30.2 a	2.5 a	5.0 a	20.8 a	43.6 a	4.8 a	7.3 a
	Ga250 (ppm)	35.0 a	2.1 a	51.9 a	17.9 a	33.3 bc	2.9 b	7.0 a
	2% KNo3	17.9 b	1.1 b	40.7c	8.7 b	38.8 ab	3.3 b	3.6 b
	1% KNo3	11.7 bc	0.8 b	37.6 cd	4.7 bc	25.7 d	3.1 b	2.3 bc
S. macrantha	Control	24.2 a	1.4 b	37.9 c	8.9 a	33.2 bc	2.5 a	4.8 a
	Cold	30.8 a	1.1 b	34.7 c	10.7 a	31.0 c	2.9 a	6.2 a
	Ga500 (ppm)	30.0a	1.9 ab	51.3 a	15.1 a	32.6 bc	2.4 a	6.0 a
	Ga250 (ppm)	21.6 a	2.6 a	46.4 ab	14.7 a	52.0 a	3.6 a	6.3 a
	2% KNo3	30.8 a	1.9 ab	38.2 bc	11.5 a	40.8 b	2.6 a	6.2 a
	1% KNo3	24.2 a	1.7 ab	40.2 bc	10.3 a	32.5 bc	2.3 a	4.8 a
S. sahendica	Control	8.90 c	0.74 cd	35.9 a	3.20 cd	27.6 b	1.8 b	1.8 c
	Cold	36.0 a	1.40 a	41.4 a	15.2 a	28.16 b	4.1 a	7.2 a
	Ga500 (ppm)	5.0 d	0.03 e	39.0 a	1.95 d	35.0 a	2.3 b	1.0 d
	Ga250 (ppm)	8.8 c	0.70 d	40.28 a	3.6 cd	30.2 ab	1.6 b	1.8 c
	2% KNo3	12.2 c	0.94 bc	35.0 a	4.39 c	26.0 b	2.1 b	2.4 c
	1% KNo3	18.8 b	1.10 b	37.0 a	6.89 b	30.5 ab	2.4 b	3.8 b
S.macrosiphonia	Control	18.3 b	0.79 ab	30.2 ab	5.6 bc	15.2 b	2.17 a	3.7 ab
	Cold	10.0 b	0.18 b	42.8 a	4.6 bc	29.2 a	2.0 a	2.0 b
	Ga500 (ppm)	33.3 a	1.90 a	39.9 ab	13.3 a	19.8 ab	2.7 a	6.7 a
	Ga250 (ppm)	26.7 ab	1.60 a	36.1 ab	9.6 ab	19.0 a	3.6 a	5.3 ab
	2% KNo3	13.3 b	0.95 ab	22.7 ab	3.94 c	25.4 ab	2.6 a	2.7 b
	1% KNo3	23.3 ab	1.60 a	25.0 b	5.8 bc	19.3 ab	1.8 a	4.7 ab

Means of each column followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Discussion

Greenhouse evaluation of seed emergence characteristics among four species of *satureja* showed that *S. macrantha* had higher values of seed emergence characteristics than other three species except of dry weight. The lower values of seed emergence and speed of emergence were related to the *S. sahendica* using Gibberlic acid (500ppm). The values of seed emergence and speed of emergence and vigor index were increased in all of species except *S. sahendica*, compare with control and other treatments. Also mean of all of the characteristics were higher using hormonal treatments as both of Gibberlic acid (250 and 500ppm), in all of species except *S. sahendica*, compare with control and other treatments. This result was confirmed by Tavily and Sabery [13]. They reported that Gibberlic acid had significantly increased seed emergence and seedling length of *Salsola rigida*.

Application of some treatments like NO_3K 1% and 2%, showed that some traits as seed emergence, vigor index, dry weight and number of normal seedlings of the species of *S. macrantha* were higher than that the control and other treatments. Also, using cold treatments, seed emergence, vigor index, dry weight and number of normal seedlings of the species of *S. macrantha*, were increased. Seed emergence, speed emergence, vigor index, dry weight and number of normal seedlings of *S. sahendica* were increased compared to control and other treatments. This results are similar with results of other researchers that reported, using of pre-chilling treatment, Gibberlic acid and Potassium Nitrate that increased the root and shoot length (seedling length) and Gibberlic index [14-16]. Also, these result was confirmed by Alizadeh and Isvand, [17] who reported that the seed germination characteristics of the of medicinal plant species *(Anthemis altissima* Guss. ex Nyman*)* were increased with cold treatment. The same conclusion in *Achilla* species was obtained by Farajpour [18].

Conclusion

We can summarize the obtained results in following:

1) It was concluded that seed germination characteristics of *Satureja macrantha* were higher than the other three species.

2) Both Gibberlic acids (250 and 500 ppm) were more effective on seed emergence characteristics compared with control

3) Abdication of Gibberlic acid on seedling characteristics on three species *S. laxiflora*, *S. macrantha* and *S. macrosiphonia* and the effect of cold treatment on seed emergence characteristics of *S. sahendica* clarified the best priming method for enhancement of seed and seedling performance.

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