Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 3, p. 63-71, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Effective of priming techniques in seed germination and seed emergence enhancement in medicinal plant of *Satureja macrantha*

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Article published on September 03, 2014

Key words: Cold, priming, *Satureja macrantha* , osmo and hydro-priming.

Abstract

Medicinal herbs are reserves for natural resources of each country. Iran also are rich of medicinal plant species. More medicinal plants are wild and their seeds have low germination. The experimental was carried out based on factorial (6*2 design in three replication by using of six treatments including: Acid Gibberlic 250) 500, ppm and Potassium nitrate (1 % , 2 %) and cold treatment 4°C in laboratory of seed technology of gene bank in research Institute of forestry and range land on 2011-2012 . The seed germination characteristics including : percent and speed of germination ,rootlet and shootlet length, root to shoot ratio, seed length, fresh weight , vigour index and number of normal seedling were calculated. The analysis of variance showed that there were significance (P≤5%) differences between treatment population and interaction of treatment with populations . Comparing of seed germination characteristics of two populations showed that population of Safarlo with provenance of east Azarbijan had higher seed germination characteristics than Arasbaran population. Comparing of osmo-priming technique and cold treatment with control showed that prime of seed samples with Acid Gibberlic 250ppm and 500ppm was caused to enhancement of seed germination and seed emergence characteristics and vigour for both two populations compare with control and other treatment.

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Introduction

The scientific name of the species is *Satureja macrantha*. This species is shrub plant with a height of 30 to 50 cm. The base of plant have large branches. The right branch of this plant wide, narrow, winding and twisty, rough-coated fluff. It is growing in Iranian-Turanian, with place of stone walls and rocky area at an altitude of 400 to 2650 m from sea level (Jamzad,2010). The geographical distribution of this species in Iran includes the provinces of Azarbaijan, Zanjan and Kurdistan, Hamedan, Kermanshah (Jamzad, 2010).

Dormancy in plants is a way to survive and adapt to the environment. Seed dormancy is a property that plant seeds enables to dormant and persist for germination in unfavorable conditions but those seeds would able to germinate in later (Sarmadnia, 1996). In many plant species, induction materials such as gibberellins and cytokinine would able to break of dormancy.

Priming is a treatment before planting which the seed would be able to absorb of water and permit for metabolic process before germination but without radical emergence (Armin, *et al.*,). Gibberellins can replace light photoblastic and cooling in many seeds such as lettuce, and tobacco (Sarmadnia, 1996).

Evidence proved that using of plant growth regulators would be useful for growth and accelerate of germination and most reports have confirmed the positive results of these materials. Roohi and Jameson (1991) reported that application of auxin improved of pasture grasses (*Boutrloua gracilid*) in the dry conditions because auxin reduces the time between germination and the formation of adventitious roots .

The aim of this experiment was, 1)Identification of barriers of savory seed germination dormancy and characteristics of germination and seedling growth with priming technique including: (gibberellic acid and potassium nitrate) and cold treatments 2)introduce of populations of the species *Satureja macrantha* which have the highest vigour and germination characteristics.

Materials and methods

Place, type of experiments, treatments

This project was carried out as factorial (2*6) by completely randomize design with three replication in laboratory and greenhouse conditions in Research Institute of Forestry and Range land of Iran in 2011- 2012. Name and provenance of populations of *satureja macrantha* on nominated in Table1.This experiment were consist first and second factors, first factors including: 2 population *of satureja macrantha* and second factor were, Gibberlic acid whit 250 ppm and 500 ppm, Potassium nitrate 1% and 2% moist Chilling treatment (3 weeks at 4°C) and Distilled water.

Experiment in laboratory

For the laboratory experiment, The seeds were disinfested with liquid fungicides of vita wax tram 1% for 2 min. 50 seeds were used for each pre-treatment then pre-treated were tested by different treatments including: Moist Chilling at 4°C for 2 weeks, Gibberllic Acid (125 and 250 ppm) as hormone priming, Potassium nitrate (0.5 and 1%) as osmo-priming and distilled water (control) as hydropriming. The seeds were imbibed with 5cc of osmotic solution of Potassium nitrate (KNO_3), solution of Gibberllic Acid (GA_3) and distilled water then those placed Petri dishes (9 cm). These samples were placed in germinator with optimum conditions of with temperature 20±3 and 16 h light and 8 h dark per day. After seedling growth (15 days), the germination characteristics including: germination percent, speed of germination, vigour index, rootlet length, seedling length, fresh and dry weight of seedling were measured.

Table 1. geography specification and studied species population particular.

Species name	Name of Population with provenance	Geography attitude	Geography latitude	Seed thousand weight
Satureja macrantha	Arasbaran	455838	074146	0.03
	Safarlo	125338	203146	0.04

Experiment in greenhouse

For greenhouse experiment, the primed of seed samples were planted and sown in pots (with ratio 1:1:1 of soil, sand and soil leaf were filled) with three replicates of 50 seeds. In the greenhouse experiment, These pots kept in temperature 20-30°C and 10,000 lux of light during the day and the temperature range 5-12°C were at night. Percentage and emergence rate of seeds after 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21th days, were recorded. Seedlings growth was complete for 50 days. The emergence characteristics including: emergence percentage, speed or rate of emergence, length of rootlet and shootlet, seedling length, ratio of rootlet length by shoot let length, vigour index, fresh weight and dry weight of seedlings, ratio of dry weight by fresh weight of seedlings were evaluated during 50th days of experiment. In current research, emergence percentage was calculated according to total number of emerged seedlings in numbering final day (ISTA, 2008),

Speed of germination and emergence were calculated according to Maguire (1962)in laboratory and greenhouse. Vigour index was calculated according to Abdul-Baki and Anderson (1975) that their values obtained from follow equations:

1- Speed of emergence (sprout/day):

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

3- After 50th days of start of the experiment, length of rootlet and shootlet (mm) (that including 5 seedlings per pot on random) was measured according to Lekh and Kairwal (1993). Then seedlings were dried in oven for 24 hours at 80°C and their weight was measured by a Scale with precision 0.001.

vigour index evaluated (Abdol-baki, and Anderson, 1973):

VI: vogour index

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

%Gr: percent germination

MSH: mean seedling length

Result

In laboratory

Analyses of variance showed that there were significant p ≤5% between populations for all traits except rootlet to shoot let ratio. Also Between treatments, there were significance differences for all traits p ≤1% except for number of seedling. Between population and treatment, there were significant differences for all traits p ≤5% but for germination percent and number of normal seedling, there were no significance (Table 2).

Comparison of seed germination characteristics showed that population of Arasbaran had percent germination (97.87%) and population of Safarlo had higher speed of germination (14.32), shoot let (22.3mm), rootlet (25mm), rootlet to shoot let ratio (1.13),seedling let (45.24),fresh weight (13.8), vigour index (44.3) and number of normal seedling (19.9) than other population but the minimum seed germination characteristics including speed of germination (12.61) shoot let (20.9mm), seedling let (41.96 mm) and vigour index (41.1) were related to Arasbaran population (Table 3). The population of Safarlo had higher speed of germination, vigour index and seedling growth than Arasbaran population and for other traits there were no any significant between them.

Table 2. Mean square of seed germination characteristics of population, treatment and interaction between them for *Satureja macrantha* in laboratory condition.

		Mean square							
Name of sources	Df	Percent germination	Speed of germination	Shoot length	Root length	Seedling length	Rootlet /shoot let	Vigour index	Number of normal seedling
Population	1	25*	26.4**	13.6*	152.3**	96.8*	0.1*	90.7*	1*
Treatment	5	24.4**	18.7 **	79.7**	250.3**	327.9**	0.9**	315.5**	0.97ns
Population* treatment	5	6.7ns	3.3*	14.3*	23.03*	53.8*	0.14*	83.9**	0.3ns
Error	22	11.3	0.92	6.3	8.3	18.48	0.04	17.23	0.45
CV		3.4	7.12	11.5	12.5	9.86	19.5	9.73	3.4

ns ,*, **= non significant, significant at 5% and 1% respectively.

Table 3. Mean Comparison seed germination characteristics of two population *Satureja macrantha* laboratory condition.

Popula-tions	Percent germi-nation	Speed of germi-nation (of sprout .day	Rootlet length (mm)	Shoot let length (mm)	Rootlet to shoot let	Seedling length (mm)	Vigour index	Fresh weight (mg)	Number of normal seedling
Safarlo	99.4a	14.32a	25a	22.30a	1.13a	45.24a	44.3a	13.8a	19.90a
Arasbaran	98.87a	12.61b	21.07a	20.9b	1.02a	41.96b	41.1b	13.7a	19.60a

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

Effect of different treatments on seed germination characteristics of the populations of *Satureja macrantha* savory showed that effect of gibberlic acid 500ppm on seed germination characteristics including: percent (100%) and speed of germination (14.94) shoot let (26.89mm), vigour index (48.7), fresh weight (21.7 mg) and number of normal seedling (20) were higher than control and other treatments(Table4). Effect of gibberlic acid 250 ppm on traits of seedling let (50.02 mm), vigour index (50) were higher than (Table4) . Tow treatments of potassium nitrate 1% and 2% had more effect on germination and speed of germination compare with control (Table4). By Chilling treatments, two traits including: rootlet length (30.67mm) and rootlet to shoot let ratio (1.72) were increased by chilling treatment compare with control. The minimum seed germination characteristics including rootlet (13.74mm), shoot let (17.67mm), seedling let (31.4 mm) and vigour index (31.41) were related to potassium nitrate2% treatment compare with other treatments (Table4).

Table 4. Mean Comparison seed emergence characteristics for six treatment in laboratory condition.

Treatment / germination characteristics	control	Gibberlic acid 500ppm	Gibberlic acid 250ppm	Potassium nitrate 1%	Potassium nitrate 2%	Chilling
Percent germination	95b	100a	100a	97.5ab	100a	99.17ab
Speed of germination (sprout /day)	10.58c	14.94a	14.78a	13.9a	14.56a	12.03b
Root length(mm)	28.59a	21.81b	25.08b	17.79c	13.74d	30.67a
Shoot length(mm)	21.07c	26.89a	24.95a	21.35a	17.67d	18.2cd
Rootlet /shoot let	1.37b	0.73d	1.01c	0.84cd	0.79cd	1.72a
Seedling length(mm)	49.66a	48.7a	50.02a	39.14b	31.4c	42.68b
Vigour index	47.29a	48.7a	50.02a	38.37b	31.41c	40.23b
Fresh weight (mg)	13.3c	21.7a	10.58cd	9.8d	10.17cd	17b
Number of normal seedling	19b	20a	20a	19.5ab	20a	19.89b

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

Comparing of mean germination characteristics of two populations macrantha savory showed that population of safarlo with chilling had higher speed of germination (32.7) compare with control(Table 5). By effect of 250ppm treatment, germination characteristic including: shootlet (15.2 mm) and seedling let (51.1 mm) and vigour index as 51(Table 5).These traits have the same trained by 500 ppm treatment. compare with control. Effective of Potassium nitrate 2% on population of Safarlo was considered only on rootlet as 15 compare with control but Potassium nitrate 1%, had more effect on rootlet and seedling let as 15 and 45 compare with contro (Table 5).

Table 5. Mean Comparison seed emergence characteristics between treatments with population in laboratory condition.

Population	Treatments	Speed of germination	Shoot let (mm)	Rootlet (mm)	Rootlet to shoot let ratio	Seedling length	Vigour index
Safarlo	Control	28.7b	23.5abc	11.2cd	1.2bc	50.4a	52.2a
	Chilling	32.7a	20.4cde	14.3ab	1.6a	36.6b	40.7b
	Gibberlic acid 500ppm	24.7b	25.8ab	15.2a	0.97cde	50.5a	50.5a
	Gibberlic acid 250ppm	26.5b	24.6abc	15.2a	1.1cd	51.1a	51.1a
	Potassium nitrate 2%	14.2de	16.9e	14.9a	0.85cd	31b	31c
	Potassium nitrate 1%	23.3bc	22.7bcd	15.1a	1cde	45a	45.95ab
Arasbaran	Control	28.5ab	18.7de	9.9d	1cde	45.95a	45.95ab
	Chilling	28.6ab	16e	9.8d	1.5ab	44.2a	47.2ab
	Gibberlic acid 500ppm	18.9cd	27.97a	14.7a	1.8a	43.9a	44.6ab
	Gibberlic acid 250ppm	23.7bc	25.3ab	14.3ab	0.5f	46.9a	46.9ab
	Potassium nitrate 2%	13.3e	18.5de	14.2ab	0.95cde	48.97a	48.97a
	Potassium nitrate 1%	12.3e	20cde	12.7bc	1cde	45.95a	45.95ab

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

With effect of Gibberlic acid 500ppm on population of Arasbaran, germination characteristics as shootlet (27.97) , rootlet(14.7), seedling length (43.9) vigour index (44.6) were achieved compare with control but by effect of Gibberlic acid 250ppm, only higher shoot let (46.9) was obtained(Table 5). Effect of Potassium nitrate 2% showed that maximum shoot length (48.97) and vigour index (48.97) were recorded but with effect of Potassium nitrate 1%, the seedling length was increased (Table 5). Arasbaran population had lower germination characteristics as speed of germination (13.3, 12.3), shoot let, (18 , 20) , rootlet /shootlet , as (0.95, 1) , by effect of Potassium nitrate 2% and 1%(Table 5).

In greenhouse

Analyses of variance showed that were significant between populations only for emergence percentage and fresh weight p ≤5%. There were significance differences(p≤1%) between treatments for shoot let and seedling length and fresh weight. There were significance differences(p≤1%) between population and treatments for emergence percent, speed of emergence and fresh weight (Table 6).

Table 6. Mean square of seed emergence characteristics of population, treatment and interaction between them for *Satureja macrantha* in greenhouse condition.

Df	Emergence%	Speed of emergence	Shoot length	Root length	Seedling length	Rootlet to shoot let	Fresh weight	Dry weight	DW/ FW
1	336.1*	0.045ns	1.3ns	78.5ns	121.9 ns	0.26ns	486.2**	3.4ns	0.0001ns
5	72.8ns	1.75ns	76.6**	44.7ns	229.6 **	0.14ns	395**	1.5ns	0.0006ns
5	636.1**	3.66 **	16.6ns	51.9ns	110.8ns	0.09ns	499.3**	0.89ns	0.0005ns
22	66.4	0.66	18.4	23.7	57.15	0.114	52.9	1.43	0.0003
	28.48	35.12	22.08	21.9	18.24	28.6	19.64	44.44	24.96

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

Comparison of seed emergence characteristics showed that population of Safarlo had higher shoot let (19.64mm), rootlet (23.64mm), rootlet to shoot let ratio (1.27),seedling (43.28), fresh weight (40.7) and dry weight (3.02),dry weight to fresh weight (0.076) than other population but the minimum seed emergence characteristics including percent emergence (31.67%) and fresh weight (33.35 mg) were related to Arasbaran population (Table7).

Table 7. Mean Comparison seed emergence characteristics of two population *Satureja macrantha* with effect of treatment in greenhouse condition.

Population	Percent emergence	Speed of emer-gence	Rootlet length (mm)	Shoot length (mm)	Rootlet shoot / let	Seedling length (mm)	Vigour index	Fresh weight	Dry weight	Dw/fw
Safarlo	25.56b	1.76a	23.64a	19.64a	1.27a	43.28a	10.83a	40.7a	3.02a	0.076a
Arasbaran	31.67a	1.83a	20.69 a	19.26a	1.09a	39.6a	12.94a	33.35b	2.41a	0.073a

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

Effect of different treatments on seed emergence characteristics of the populations of *Satureja macrantha* savory showed that by effect of gibberlic acid (500ppm), the values of seed emergence characteristics including: rootlet (27.08), shoot let (24.17mm), seedling let (51.25 mm), vigour index (15.06) and effect of gibberlic acid 250ppm for percent (31.67%) and speed of emergence (2.64), fresh weight (52) and dry weight (3.62) were higher than control and other treatments. but the minimum seed emergence characteristics including speed of emergence (1.08), rootlet (19.58mm), seedling let (34.67 mm), fresh weight (31.03 mg) were related to chilling treatment (Table 8).

Table 8. Mean Comparison seed emergence characteristics for six treatment in greenhouse condition.

treatment Emergence characteristics	control	Gibberlic acid 500ppm	Gibberlic acid 250ppm	Potassium nitrate 1%	Potassium nitrate 2%	chilling
Emergence%	24.17a	30a	31.67a	24.17a	30.83a	30.83a
Speed of emergence (sprout /day	1.43b	1.99ab	2.64a	1.67ab	1.99ab	1.08b
Root length(mm)	22.08ab	27.08a	23.17ab	20.59b	20.46b	19.58b
Shoot length(mm)	15.83b	24.17a	23.25a	19.59ab	17.75b	16.1b
Rootlet/shoot let	1.44a	1.13a	1.03a	1.05a	1.18a	1.28a
Seedling length(mm)	37.92bc	51.25a	46.42ab	40.19bc	38.2bc	34.67c
Vigour index	8.94a	15.06a	14.72a	10.3a	11.53a	10.74a
Fresh weight (mg)	33.17bc	32.63bc	52a	32.5bc	40.82b	31.03c
Dry weight(mg)	2.45a	2.35a	3.62a	2.3a	2.62a	2.93a
Dw/w	0.074ab	0.073ab	0.073ab	0.07ab	0.063b	0.093a

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

Comparing of mean seed emergence characteristics of two populations Macrantha savory showed that population of Arasbaran with treatments of gibberelic acid 500ppm had higher emergence characteristics including: percent of emergence (50%), speed of emergence (3.25), fresh weight (10 mg) than other treatments. Population of Safarloo with gibberlic acid 500ppm had lower emergence characteristics including percent of emergence (10%) and fresh weight (2mg) compare with other treatments (Table9).

Table 9. Mean Comparison seed emergence characteristics between treatments with population in greenhouse condition.

Population	Treatments	Emergence percent (%)	Speed of emergence	Fresh weight
Safarlo	Control	30b-d	1.89ab-d	6b-d
	Chilling	26.67b-d	0.39d	5.3b-d
	Gibberlic acid 500ppm	10e	0.73cd	2e
	Gibberlic acid 250ppm	25b-d	2.86a	5b-d
	Potassium nitrate 2%	28.3b-d	2.25ac	5.67b-d
	Potassium nitrate 1%	33.3bc	2.46ab	6.67bc
Arasbaran	Control	18.3c-e	0.98b-d	3.67c-e
	Chilling	35b	1.75ab-d	7b
	Gibberlic acid 500ppm	50a	3.25a	10a
	Gibberlic acid 250ppm	38.3ab	2.4ab	7.67ab
	Potassium nitrate 2%	33.3bc	0.73cd	6.67bc
	Potassium nitrate 1%	15de	0.87cd	3de

Dissimilar letters in each column mean significant difference at the 5% level using Duncan's multiple range test.

Discussion

Mean comparison of the populations in laboratory and greenhouse condition showed that all seed germination and emergence characteristics of Safarlo population were higher than other populations (Table 3 and 7). All seed germination characteristics and seed emergence characteristics were increased with gibberlic acid 500 and 250ppm in both laboratory and greenhouse conditions (Table 4 and 8).

All seed germination characteristics of Safarlo and Arasbaran population were increased by Gibberllic acid 250ppm and population of Safarlo effected with chilling treatment in laboratory condition but in greenhouse experiment, seed emergence characteristics of populations of Arasbaran were increased with effect Gibberllic acid 500ppm compare with control and other treatment (Table 5 and 9). This result was similar with theory of Sarmadniya (1996) who reported that some chemicals are useful to break seed dormancy. However, this can not be generalized to all species. One of the commonly used chemicals, potassium nitrate at a concentration with 0.1 - 0.2 %., the seeds were immersed for different periods depending on the type of the solution and then dried at ambient temperature before pre-seeding. When the seeds dried then germinated in tiny germination paper for 10 or more days.

GA is also typically used in laboratories to break seed dormancy. 100 to 1,000 milligrams per liter of the of the GA prepared then seeds soaked with gibberllic acid solution for 24 to 48 hours, followed by drying at room temperature then primed seed samples were tested for germination. Also other research's showed Combinations of potassium nitrate and gibberellins effects on cell membranes, affect the physiological processes grains and nuts and their result showed compounds with resources gibberellins or nitrogen can be dissolved in suitable conditions, those induced germination easily (Hillhorts, 1995; Jimenez *et al.*, 1993).

Dunand (1992) reported that chilling treatment and gibberellic acid have a significant effect on the target species seed dormancy. The result of this research showed that osmo-priming including : Potassium

nitrate 1 & 2% induced some seed germination and seed emergence characteristics for some population of *Satureja macrantha savory* and this result was similar with result of Dianti and *et al* (2010) who reported that by using of potassium nitrate 2% treatment reduced percent of germination *Agropyron desertoum* seed. Nabei and *et al* (2011) reported Potassium nitrate 2% no significant on rhubarb seeds. Cold treatment on 2˚C and 5 levels (5,10,15,20,25) days on rhubarb plant seeds had significantly increased the percentage and rate of germination. Yang and *et al* (2008) and Copland and McDonald (1995) also treated with potassium nitrate reduction effects noted in other species.

Similarly, the temperature of the species Valerians and chicory seeds improves germination of 28% and 26% compared to control. Hoseinpur-ghazvini and *et al (*2012) treated of the seeds samples of the populations of some species Savory of *(Satureja sahendica, S. bakhtiarica and S. khuzestanica*) with physical, chemical scarification and chilling treatment and they concluded that pre-treatment with chilling increased the percent and speed of germination. To break dormancy plant seed of (*Teucrium Polium*) is a 14-day period with cold temperature at 5˚C which had highly effective for removing of dormancy (Nadjafi *et al*, 2006).

This result was confirmed by Alizadeh and Isvand (2004). They reported that the seed germination characteristics of the of medicinal plant species of *(Anthemis altissima* were increasd with cold treatment. With regard of increasing of gibberlic acid, the same result was obtained by Farajpour *et al* (2010) for the species of Achilla . Also the same result was achieved by Tavily, *et al.*, (2010) for the species of of *Salsola rigida*.

Conclusion

Regarding of the result and discussion it was concluded that the population of Safarlo had higher germination Characteristics in laboratory condition compare with other population. Mean comparison of the population showed that both of two populations had higher seed emergence characteristics. Regarding of seed priming effect on seed germination and seed emergence characteristics of the populations of *Satureja macrantha* savory it was concluded that priming seed with gibberlic acid 500 and 250 ppm more effective than other treatments.

Acknowledgments

We thank the director and researcher deputy of Research Institute of Forest and Rangeland in Iran for their financial supporting of this research work and also from my staff, colleague in Natural Gene Bank Department.

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