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Study of Two Treatments on the Germination of *Valeriana officinalis* L. Seeds in Two Growth Media

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Abstract: Seed dormancy is a common phenomenon in many medicinal plants. In such plants, seed germination occurs under specific environmental signals or factors. In order to evaluate the effects of some treatments on seed dormancy and germination of *Valeriana officinalis*, two different growth media, water with agar and Murashige and Skoog (MS) medium, were used. The stages of breaking seed dormancy were incorporated with application of gibberellic acid (GA_3) in 400, 800 and 1600 part per million (ppm) concentrations for time periods of 72, 144 and 216 h at 4°C; and sulfuric acid (H_2SO_4) (10 and 15%) for periods of 10, 15 and 20 min. Seed germination significantly increased in high concentration of GA_3 (800 ppm). The best result in H_2SO_4 treatments obtained at 10% concentration for 15 min. Also the percentage of germination and days to germination was higher and faster in water with agar medium than MS medium. In the present study, the obtained results suggested that the seeds of *valeriana officinalis* have exogenous and endogenous dormancy. GA_3 and H_2SO_4 pretreatments in the certain concentrations and time periods stimulated seed germination.

Key words: Germination · MS medium · *Valeriana officinalis* · Water + agar medium

INTRODUCTION

Plants within the *Valerianaceae* family have a long tradition in folk medicine [1]. The roots and rhizomes of *Valeriana officinalis* L. (commonly called valerian) are used as mild sedatives [2]. During seed germination process a part of the embryo, usually the radicle extends to penetrate the structures that surround it and this process is followed by adequate water and oxygen at a suitable temperature. Dormancy is defined as a state of seed that does not permit germination, although conditions for germination (temperature, water and oxygen) may be provided. Thus dormancy effectively delays germination. Two types of seed dormancy have been recognized: coat-imposed dormancy and embryo dormancy [3]. The required conditions for breaking dormancy and allowing subsequent germination are often very different from those that are necessary for growth or survival of the autotrophic life stage of a plant. Timing of seed germination can be critical for the survival of natural plant populations and dormancy mechanisms play a major role in this time. Various methods have been used by seed scientists and technologists to break seed dormancy. Stratification plays an important role as a stimulator that helps to break dormancy [3-5]. In order to accelerate this method, it can be combined with some treatments such as chemical substances and mechanical seed coat removal [6-7]. For breaking physical dormancy in many species, lots of seeds are sometimes scarified by chemical treatments such as sulfuric acid [8-11]. Many investigators have studied effects of exogenous growth regulators on seed germination. Gibberellins eliminated the chilling requirements of peach and apple seeds and increased their germination [6-12]. The beginning of the embryo dormancy is associated with accumulation of growth inhibitors such as abscisic acid (ABA), while the breaking of dormancy is accompanied with a shift in the balance of growth regulators towards growth promoters such as gibberellic acid (GA_3) that overcome the effect of growth inhibitors [13]. The objective of this study was to determine the effects of chemical scarificators on breaking seed dormancy in *Valeriana officinalis* and to compare the role of water+ agar and MS media as two different substrates.

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MATERIALS AND METHODS

This study was carried out in the Biotechnology and Science Institute of Urmia University. The seeds of *V. officinalis* were collected from a field (Isfahan province, Iran) at late August 2011. The seeds were thoroughly washed under running tap water for 15 min and surface sterilized by immersing in 70% (v/v) ethanol for 1 min and in 2% (v/v) solution of sodium hypochlorite (commercial bleach) for 10 min. Finally, sterilized seeds were immediately rinsed with sterile distilled water for 10 min to wash out the sterilization agents before placing in glass vessels containing 7.5 g/l water-agar and MS for germination. The cultures were maintained in a growth chamber at 24 ± 2°C with a photoperiod of 16 h light and 8 h dark under light intensity of 40 ìmol / ms.

Medium and Culture Conditions: MS medium supplemented with vitamins, macro and micro elements, 3% sucrose and 0.7% agar [14]. The pH was adjusted to 5.9 with KOH (0.1 N) or Hcl (1 N) before autoclaving. The other medium used in this study included only water and 7.5% agar. Both media were autoclaved at 121°C for 20 min.

Treatment with Acid Scarification: The seeds were scarified with commercial grade sulfuric acid (10, 15 and 20%) for 10, 15 and 20 min. After washing, the seeds were placed in Petri dish (100 × 15 mm) with water + agar and MS growth medium at a rate of 30 seeds per dish. The Petri dishes were wrapped with a strip of Para film around the edge to prevent evaporation. The experiment was arranged in a randomized split plot design with four replicates. The Petri dishes were placed in a chamber at 22 ± 2°C with 70% of humidity under dark condition to enhance seed germination. Germination was recorded in daily observations when the white radicles (1 cm length root) emerged from the seeds. A number of germinated seeds in each dish were counted every day and recorded daily on a prepared data sheet for about six weeks. At the end of the test (40 days after sowing the seeds in growth medium), seedlings were evaluated and final observations were recorded as germination percentage.

Treatment with Gibberellic Acid (Ga_3): After washing, the seeds were surface-disinfected with 70% (v/v) ethanol for 1 min, treated with 2% (v/v) sodium hypochlorite solution (NaOCl) for 15 min, thoroughly rinsed with sterile distilled water for 4 times and were blot-dried inside a laminar hood. A set of treatments with GA_3 (400, 800 and 1600 part per million (ppm) for time periods of 72, 144 and 216 h at 4°C were tested (Table 3 and 4). The germination experiments carried out using four replications of 30 seeds per each treatment.

Statistical Analysis: Factorial analysis of variance (ANOVA) was performed on the data based on completely randomized design (CRD) with four replicates. The data were analyzed using the linear model procedure of the SAS statistical package (SAS Institute Inc. Cary, NC) and significant differences between the mean values was determined using Fisher's least significant differences (FLSD) at a 1% or 5% probability level.

RESULTS AND DISCUSSION

In the present study the obtained results showed that there were significant differences ($P<0.01$) between used methods for stimulating of seed germination (Table 1 and 2). Untreated (control) seeds did not germinate in MS growth medium (Table 4 and 6). Although germination rate in untreated seeds (control) was 34.81% in water+agar. Moreover, the germination rate improved in water+ agar when seeds treated with GA_3 and sulfuric acid. The response to external GA_3 was dependent on GA_3 concentrations and a significant difference ($P<0.01$) was observed among treated seeds with various concentrations of GA_3 (Table 1). It has been tested in *Alibizia* species, which three concentrations of gibberellic acid promoted germination rate [16]. Our experiments showed that the germination percentage was higher in 800 ppm than 400 and 1600 ppm of GA_3 in both MS and water + agar media (Table 3 and 4). Decreasing germination in 1600 ppm as compared with 800 ppm GA_3 (Table 3 and 4) indicates that in addition to effect of gibberellic acid with different concentrations on breaking seed dormancy, it may has a negative effect as a weak acid on embryo[15]. The effect of GA_3 (400 ppm for 8 h) on the germination of *V. officinalis* seeds has previously been reported [17]. Some studies have also shown that the results of application of GA_3 to break dormancy or induce germination may be widely different between species and within species [16]. Endogenous dormancy (embryo dormancy) is thought to be due to the presence of inhibitors, especially ABA (abscisic acid).

Table 1: Analysis of variance for the GA_3 treatments in relation to percent of the germination (PG) and days to first germination (DFG).

Source	df	Mean square	
		PG	DFG
Medium	1	5680.35**	1.52**
Treatment	3	4523.84**	2.13**
Time	2	4.14n.s	0.12n.s
m*tr	3	457.20**	3.65**
m*ti	2	1.52n.s	0.08n.s
tr*ti	6	12.44*	0.06n.s
m*tr*ti	6	4.27n.s	0.04n.s
Error	-	2.32	0.02
C.V	-	1.52	0.15

D.F, Degree of freedom; C.V, coefficient of variance; *, P<0.05; **, P<0.01; n.s, non- significance; m, medium; tr, treatment and ti, Time.

Table 2: Analysis of variance for the sulfuric acid treatments in relation to percent of the germination (PG) and days to first germination (DFG).

Source	DF	Mean square	
		PG	FDG
Medium	1	2534.42**	2.10**
Treatment	2	3894.99**	4.17**
Time	2	1616.85**	0.91**
m*tr	2	1152.18**	5.16**
m*ti	2	5.12n.s	0.01n.s
tr*ti	4	513.06**	4.79**
m*tr*ti	4	19.69*	0.15**
Error	-	2.73	0.02
C.V	-	1.65	0.14

D. F, Degree of freedom; C.V, coefficient of variance; *, P<0.05; **, P<0.01; n.s, non- significance; m, medium; tr, treatment and ti,Time.

Table 3: Comparison of average of the effect of treatments on percent of the germination, days to first germination and days to 50% germination in relation to GA_3 treatments in water + agar medium.

Treatments	GA3 concenteation	Percent germination	Days to first germination	Days to 50% germination
T1:control	0	34.81g	5.48b	0.49f
T1:72	400ppm	58.85f	4.84c	4.84c
	800ppm	81.84b	3.80f	4.16e
	1600ppm	75.51c	4.16e	4.45d
T2:144h	400ppm	65.20e	4.84c	4.84c
	800ppm	87.20a	3.80f	4.12e
	1600ppm	73.52cd	3.80f	4.84c
T3:216h	400ppm	66.85e	6.45a	6.50a
	800ppm	73.90c	4.16e	5.13b
	1600ppm	71.18d	4.45d	5.15b

Values with different letters within a column differ significantly at 5% level of significance.

Table 4: Comparison of average of the effect of treatments on percent of the germination, days to first germination and days to 50% germination in relation to GA treatments in MS medium.

Treatments	GA3 concenteation	Percent germination	Days to first germination	Days to 50% germination
T1:control	0	0.49d	0.49g	0.49e
T1:72	400ppm	46.49c	5.15c	0.49e
	800ppm	51.50a	3.80f	6.8c
	1600ppm	50.16ab	4.45e	7.18b
T2:144h	400ppm	49.83ab	5.15c	7.15b
	800ppm	52.15a	6.50a	6.81c
	1600ppm	51.83a	4.84d	8.18a
T3:216h	400ppm	49.83ab	5.86b	7.18b
	800ppm	50.51ab	5.15c	5.86d
	1600ppm	47.84bc	5.15c	0.49e

Values with different letters within a column differ significantly at 5% level of significance.

The loss of embryo dormancy is often associated with a sharp drop in the ratio of ABA to GA_3 [18]. Other traits such as days to first germination and days to 50% germination were also affected by GA_3 treatments. In our experiment the highest germination rate among GA_3 treatments was 81.84% using 800 ppm for 72 h in water agar medium that was the best result and the lowest rate was 46.49% using 400 ppm for 72 h in MS medium (Table 3 and 4). Also the best germination percentage with acid treatments obtained in 10% for 15 min (Table 5 and 6). In this case, both treatment time and acid concentration had prominent role on seed germination. Decreased germination rate at longer time period and higher concentration of sulfuric acid may be due to damage of the seed embryo. Therefore no seed germination was observed in 20% concentration and in

Table 5: Comparison of average of the effect of treatments on percent of the germination, days to first germination and days to 50% germination in relation to sulfuric acid treatments in water + agar medium.

Treatment	H_2S0_4 concenteration	percent germination	days to first germinatio n	days to 50% germination
T_0:Control	0	34.80^{d}	5.84^{c}	0.49^{c}
T_1:10min	10%	62.52^{b}	4.84^{d}	8.11^{a}
	15%	38.14^{c}	5.86^{c}	0.49^{c}
T_2:15min	10%	73.18^{a}	4.84^{d}	5.31^{b}
	15%	31.50^{e}	7.51^{b}	0.49^{c}
T_3:20min	10%	40.82^{c}	7.84^{a}	5.21^{b}
	15%	0.49^{f}	0.49^{e}	0.49^{c}

Values with different letters within a column differ significantly at 5% level of significance.

Table 6: Comparison of average of the effect of treatments on percent of the germination, days to first germination and days to 50% germination in relation to sulfuric acid treatments in MS medium.

Treatments	H_2S0_4 concenteration	percent germination	days to first germination	days to 50% germination
T_0:Control	0	0.49^{e}	0.49^{f}	0.49^{c}
T_1:10min	10%	57.49^{b}	5.15^{d}	71.32^{a}
	15%	34.15^{c}	7.84^{c}	0.49^{c}
T_2:15min	10%	66.53^{a}	4.16^{e}	7.11^{b}
	15%	21.15^{d}	10.11^{b}	0.49^{c}
T_3:20min	10%	23.37^{d}	11.49^{a}	0.49^{c}
	15%	0.49^{e}	0.49^{f}	0.49^{c}

Values with different letters within a column differ significantly at 5% level of significance.

other concentrations for 20 min. Ren and Tao (2004) reported that sulfuric acid treatments significantly promoted overall germination in *Calligonum* species [19]. In sulfuric acid treatments the highest and the lowest germination rate in term of time were similar in MS and water + agar. So we assumed that these results may be due to a physical dormancy. The physical dormancy is attributed to the presence of physical factors such as seed coat. The effect of sulfuric acid on promotion of seed germination relates to the highly desiccant effect of the acid on the seed coat, allowing easier water uptake and oxygen diffusion [20]. The results also indicated that in the seeds of *V. officinalis* the percentage and Mean germination time were higher in water + agar than the MS medium. It seems that the minerals within the MS medium and probably pH (5.9) has acted as an effective barrier and reduced the percent of seed germination. Although the MS medium is suitable for the seed germination of most plant species, in this project the *V. officinalis* did not show considerable germinaton stimuli in MS growth medium as compared with water + agar. GA_3 treatments were more effective than sulfuric acid for seed germination of *V. officinalis* L. These results indicated that there were physiological and physical dormancies in the seeds of *V. officinalis* and GA_3 treatments were partially effective on breaking the above mentioned dormancies and promoting the seed germination.

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