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Original Article

Effect of Different Treatments on Breaking Dormancy of *Teucrium chamaedrys* L. Seed

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Abstract

In order to find the most effective method to overcome seed dormancy in *Teucrium chamaedrys* L. medicinal plant species the effect of some chemical and physical breaking methods including: cold stratification (5 °C), scarification with sandpaper, stratification with needle, concentrated sulfuric acid, gibberellic acid and combination of these methods were investigated. Treated seeds along with control were sown in the germinator at 25±2 °C for 45 days according to a randomized complete block design with three replications. Results showed that all seed treatments increased seed dormancy, however, the highest germination percentage and lowest mean germination time obtained when seeds were treated with sulfuric acid followed by chilling and gibberellic acid, respectively. Sulfuric acid increased the permeability of the seed coat and the effect of gibberellic acid was enhanced by cold treatment. Results showed that *T. chamaedrys* seeds have both physical and physiological dormancy.

Key words: Germination, MGT, Seed dormancy, *Teucrium chamaedrys*

Introduction

Seed dormancy is of challenges facing the development and cultivation of medicinal plants so that seeds of such plants are not able to germinate even with the presence of the suitable conditions. Studies have shown that generally two types of seed dormancy including physical or external dormancy (due to impermeable seed coat to oxygen or water) and physiological or internal dormancy (due to some physiological conditions) delay seed germination in medicinal plants [1,2]. Some reported conventional methods for overcoming external seed dormancy are: scarification, hot water, dry heat, alternately cold-heat, freezing and re-thawing, acids or other chemicals and light. Also, chilling and sometimes hormones and chemical materials are the most common methods of breaking the internal seed dormancy [3].

Teucrium chamaedrys L. belonging to the *Lamiaceae* family has hard shell seeds. This plant has been used as a medicinal herb for more than two thousand years [4,5]. This herb has been used in the treatment of liver cancer, diabetes, gastric disturbances, inflammation and rheumatism [6]. It has also antibacterial, antioxidant and refrigerant activity [7]. It contains a variety of compounds including flavonoids [8,9].

There is an increment in human demand on medicinal plants, though the wild habitats are not sufficient to supply adequate plant material. Therefore there is an increasing need to cultivate the medicinal plants. As most of the medicinal plants have problem with their germination in agricultural lands then some effort have been done to improve their germination performance.

During an investigation, Shakeri-Almoshiri *et al*, [10] reported that only 2.2% of newly collected

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Teucrium Polium L. seeds germinated while their embryo survival power was more than 80%. They concluded that the seeds of this plant had physical dormancy.

Razavi [11] reported the long time chilling as a solution to overcome seed dormancy problem among the Apiaceae family while, treatment with gibberellic acid hormone had no effect on seed germination.

Nadjafi *et al*, [12] demonstrated that various concentrations of gibberellic acid increased the seed germination in *Ferula gummosa* Boiss. and *T. polium*. They also concluded that the hard seed coat in *T. polium* acts as a barrier against germination so that the germination percentage reached to 31% after treatment with sulfuric acid. Also, Benvenuti *et al*, [13] reported the significant effect of chilling, gibberellic acid, hormone and sodium hypochlorite on breaking seed dormancy in *Teucrium macrum* Boiss. & Hausskn.

As there is lack of information on seed germination requirements of *T. chamaedrys,* this research was conducted to study seed dormancy mechanisms as well as evaluation of various physical and chemical methods to increase seed germination in *T. chamaedrys*.

Material and Methods

Seeds were prepared from Isfahan Pakan Bazr Company. Viability test was done with 2,3,5-triphenyl tetrazolium chloride according to ISTA rules [14)]. For this propose, seeds were punched gently by sharp forceps to make staining solution easily penetrate the embryo, soaked with 1% 2,3,5-triphenyl tetrazolium chloride, sealed, and incubated at 30 °C for 18 hr. Seeds that were viable stain red, and seeds that were dead do not stain. About two third of the seeds completely stained. Then the mature seeds were selected and sterilized by 1% sodium hypochlorite for 5 minutes followed by washing with distilled water. The experiment was conducted at Seed Laboratory of Shahed University, Tehran, Iran in 2012. The experimental design was as completely randomized design with three replications. Laboratory germination tests were performed according to methods of the ISTA [14]. Seeds were considered germinated when radicles emerged at least 2mm. Parameters related to germination, such as maximum germination (GP) and mean germination time (MGT) were calculated using the software package Seed Calculator V 3.0 (Plant Research International, Wageningen, The Netherlands).

Treatments used in this study included physical and chemical methods and also combination of these techniques is shown in Table 1. Seed with no treatment were used as control. Treated and untreated seeds were placed in germinator at 25 ± 2 °C for 45 days. Combined methods showed lower MGT and higher germination percentage rather than physical and chemical methods.

Statistical analysis

Data were transformed to follow normal distribution by using Box-Cox transformation and were statistically analyzed by analysis of variance (ANOVA) using SAS software. Probability of significant differences among treatments by Duncan test ($p<0.01$) were used to compare means within and among treatments.

Table 1 Seed breaking dormancy treatments

Method	Abbreviation	Condition	Time
Chemical	SA10	Sulfuric Acid Solution (96%)	10 minutes
	SA15		15 minutes
	SA20		20 minutes
	SA30		30 minutes
Physical	S10	Stratification at 5 °C	10 days
	S20		20 days
	S30		30 days
	P	Puncturing with sterilized needle	
Chemical+ Physical	SC	Scarification with sand paper	
	SA30 → GA	Sulfuric Acid Solution (96%)→Gibberelic Acid Solution (1500 ppm)	30 minutes→three days
	SA30 → S	Sulfuric Acid Solution (96%) → Stratification at 5 °C	30 minutes→ three days
	SA30 → S → GA	Sulfuric Acid Solution (96%)→Stratification at 5 °C → Gibberelic Acid Solution (1500 ppm)	30 minutes→ three days → three days
	SA30 → GA → S	Sulfuric Acid Solution (96%) → Gibberelic Acid Solution (1500 ppm)→ Stratification at 5 °C	30 minutes→ three days → three days

Results

A. Effect of chemical and physical treatments

Germination percentage affected by chemical and physical seed breaking dormancy treatments. However, these treatments had no effect on MGT. Germination percentage and MGT affected by combined seed breaking dormancy treatments. Our preliminary tests on the germination of freshly harvested seeds of *T. chamaedrys* revealed, only 2% of seeds germination, indicating a kind of seed dormancy. Results of the analysis of variance showed that the variations due to treatments were significant so that all treatments significantly increased seed germination (Fig. 1).

Seed treatment for 30 min with sulfuric acid (SA30) significantly increased the germination percentage (46%) (Fig. 1). The lowest germination percentage in treated seeds (24%) was recorded for scarification with sand paper (SC) which was significantly higher than control (Fig. 1). However, the seeds which were treated for 10, 15 and 20 min with sulfuric acid (SA10, SA15 and SA20) as well as stratification (S10, S20 and S30) and scarification (SC and P) did not show any difference in germination percentage although they showed significant increment in germination percentage in compare to control.

B. Effect of combined (chemical and physical) seed dormancy breaking methods

As Studies have shown that internal inhibitors have important role in germination of those dormant seeds that require low temperature for germinating thus the effect of the combination of treatments sulfuric acid, gibberellic acid and chilling on the germination percentage and MGT were studied. Results showed that combination of physical and chemical methods applied in this study had significant effect on germination percentage and mean germination time. Based on the results of the mean comparison, a significant difference was found for germination percentage for all levels of the combined treatments (Fig. 2).

The highest germination percentage with 53% was belonging to combined treatment of sulfuric acid (30 minutes), chilling (three days) and gibberellic acid (three days). Also, this combined treatment showed the lowest MGT (Fig. 2). The results showed that cold treatment after sulfuric acid had more effect on germination percentage and MGT than gibberellic acid after sulfuric acid (Fig. 2).

C. Orthogonal contrast among different methods

As it is shown in Table 2, the result of orthogonal contrast was significant for MGT and germination percentage in combined methods in contrast to other methods (chemical and physical).

Means with the same letter(s) are not significantly different at $P < 0.01$ level. SA10= sulfuric acid for 10 min, SA15= sulfuric acid for 15min, SA20= sulfuric acid for 20 min, SA30= sulfuric acid for 30 min, S10= stratification in 5 °C for 10 days, S20= stratification in 5 °C for 20 days, S30= stratification in 5 °C for 30 days, P= Puncturing with sterilized needle, SC= Scarification with sand paper.

Table 2 Orthogonal comparison among different seed dormancy breaking

Dependent Variable: MGT					
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Physical methods VS other methods	1	21.44	21.44	3.62	0.07
Chemical methods VS other methods	1	0.09	0.09	0.01	0.91
Combined methods VS other methods	1	26.75	26.75	4.51	0.04
Chemical methods VS physical methods	1	5.02	5.02	0.85	0.37
Dependent Variable: Germination Percentage					
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Physical methods VS other methods	1	58.22	58.22	1.15	0.29
Chemical methods VS other methods	1	9.19	9.19	0.18	0.67
Combined methods VS other methods	1	122.65	122.65	2.43	0.05
Chemical methods VS physical methods	1	4.45	4.45	0.09	0.77

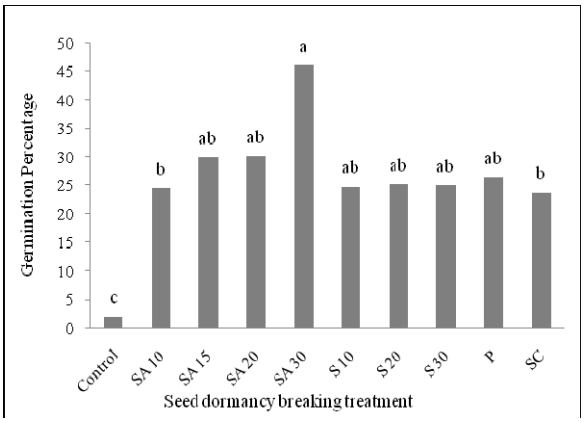

Fig. 1 *T. chamaedrys* seed germination after different individual seed treatment

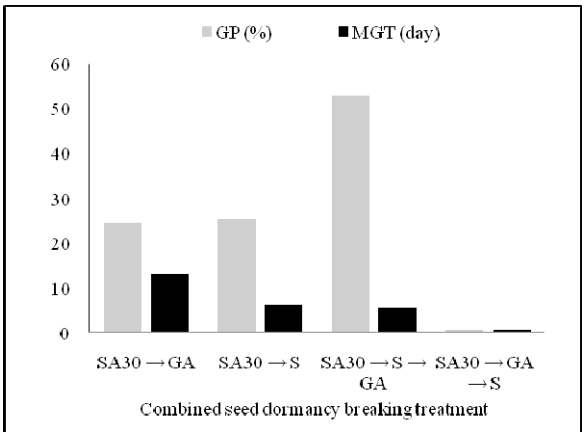

Fig. 2 *T. chamaedrys* seed germination after combined (chemical and physical) seed treatment

Means with different letter(s) are t significantly different at $P < 0.01$ level. SA30 → GA= 30 minutes sulfuric acid solution (96%)→ three days gibberelic acid solution (1500 ppm), SA30 → S = 30 minutes sulfuric acid solution (96%)→ three days Stratification at 5 °C, SA30 → S → GA= 30 minutes sulfuric acid solution (96%)→ three days stratification at 5 °C → three days gibberelic acid solution (1500 ppm), SA30 → GA → S= 30 minutes sulfuric acid solution (96%) → three days gibberelic acid solution (1500 ppm) → three days stratification at 5 °C

Discussion

Although the use of sulfuric acid, temperature, scarification and stratification similarly increased germination percentage, however, seeds of this plant probably had also a physiological dormancy since a high percentage of the seeds were not germinated yet. Since, the physical and chemical treatments increased germination percentage, it is suggested that there was an external dormancy which was probably due to the impervious seed coat. The dormancy in *T. chamaedrys* could be similar to the dormancy of seeds in *T. macrum* and *T. polium*. However, the use of the sulfuric acid for 30 minutes was more effective than other methods. This result revealed that the seed coat was a serious obstacle against the penetration of water and gas.

Increment of germination percentage due to the cold treatment showed that the germination had an internal barrier either. Studies have shown that internal inhibitors have important role in germination of those dormant seeds that require low temperature for germinating [15]. Taylor and Wareing [16] and Najdafi, *et al*. [12] found that the chilling raises cytokinin and gibberellin hormones in the seed leading to increase germination and root growth.

Results derived from the effect of chilling on germination were in agreement with reports of Dvazdah Emami and Shah Mansouri [17], Hidayati *et al*. [18] and Widrlechner and Kovach [19]. In present study, the effect of temperature on germination was notable so that, weakening the seed coat by sulfuric acid followed by cold temperature for 3 days seed germination percentage was higher than those seeds treated by gibberellic acid. Also, applying gibberellic acid increased germination percentage after cold treatment while gibberellic acid had not effect on germination percentage by itself. Similarly, Widrlechner and Kovach [19] reported that the effect of gibberellic acid treatment on germination was enhanced when seeds were primarily exposed to cold temperature for two to three weeks.

As reported by Iglesias and Babiano [20], gibberellic acid hormone plays an important role in the induction of germination through controlling seed dormancy. However, results of this study showed the cold's stimulatory effect on gibberellic acid activity in the germination of *T. chamaedrys* seeds. Increased seed germination by using combination of treatments showed that the seeds of this plant have both physiological and physical dormancy.

Conclusion

Results of this study revealed that seed dormancy in *T. chamaedrys* was of both physical and physiological kinds. Applying individual application of all seed dormancy breaking treatments including cold stratification, puncturing, scarification and acid sulfuric treatment had the

same promoting significant effect on germination percentage and MGT of *T. chamaedrys* seed germination. Combined treatment comprising sulfuric acid, cold stratification and gibberellic acid had the most significant effect on germination percentage. Although this treatment highly increased the germination in compare to other, however it is believe that seed puncturing is the simple and economically sound technique to overcome *T. chamaedrys* seed dormancy.

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