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Methods for overcoming seed dormancy in jimsonweed (*Datura stramonium* L.)

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ABSTRACT

This study aimed to examine dormancy-breaking of jimsonweed seeds. Seeds were collected from Hamedan. They were subjected to different treatments: (a) concentrated sulfuric acid for 1, 1.5, and 2 min; (b) hot water at 80°C and 90°C for 5 and 10 min; (c) mechanical scarification with sandpaper; (d) light exposure for 10, 20, and 30 days; and (e) fluctuating temperature (5–15°C). The highest germination (90%) was for seeds scarified with sandpaper, but it did not differ significantly from that of seeds scarified with sulfuric acid for 1.5 min. Hot water treatment increased germination percentage but it was lower than sandpaper and acid treatments. Superior treatment affected radicle and plumule length, vigor index, mean germination time, and seedling length. Lower α- and β-amylase activities were detected in dormant seeds, and these enzymes' activity increased significantly in superior treatment. It seems that scarification by sandpaper or sulphuric acid for 1.5 min is a general requirement for breaking dormancy of jimsonweed seeds. So, they are recommended.

Keywords: α-amylase, β-amylase, germination, sandpaper.

روش‌هایی جهت غلبه بر شکست خواب بذر تاتوره (*Datura stramonium* L.)

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چکیده

هدف از این مطالعه بررسی شکست خواب بذرهای تاتوره بود. بذرها از همدان جمع آوری شد و تحت تاثیر تیمارهای مختلف شامل: الف) اسید سولفوریک غلیظ به‌مدت یک و یک و نیم دقیقه، ب) آب داغ با دمای ۸۰ و ۹۰ درجه سانتی‌گراد به‌مدت ۵ و ۱۰ دقیقه، ج) خراشدهی مکانیکی با کاغذ سمباده، د) تیمار نوری به‌مدت ۱۰، ۲۰ و ۳۰ دقیقه و ه) نوسان دمایی (۱۵-۵ درجه سانتی‌گراد) قرار گرفت. بیشترین جوانه‌زنی (۹۰٪) به تیمار کاغذ سمباده تعلق داشت هرچند که تفاوت معنی‌داری با تیمار اسید سولفوریک به‌مدت یک و نیم دقیقه نداشت. آب داغ، درصد جوانه‌زنی بذور را افزایش داد اما مقادیر کمتری را نسبت به کاغذ سمباده و اسید سولفوریک نشان داد. طول ریشه‌چه، طول ساقه‌چه، شاخص بنیه، متوسط زمان جوانه‌زنی و طول گیاهچه تحت تأثیر تیمار برتر قرار گرفتند. کمترین فعالیت آنزیم‌های آلفا و بتا آمیلاز در بذور خواب مشاهده شد و فعالیت آنزیم‌های مذکور تحت تاثیر تیمار برتر قرار گرفت. به نظر می‌رسد خراشدهی با کاغذ سمباده و اسید سولفوریک به‌مدت یک و نیم دقیقه جهت شکست خواب بذرهای تاتوره لازم است بنابراین کاربرد آن‌ها توصیه می‌شود.

واژه‌های کلیدی: آلفا آمیلاز، بتا آمیلاز، جوانه‌زنی، کاغذ سمباده.

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Introduction

Jimsonweed (*Datura stramonium* L.) is native to North America, but was soon spread to the old world [Reisman *et al*., 1989]. Today, it grows wild in all the world's warm and moderate regions, where it is found along roadsides and at dung-rich livestock enclosures [Arana *et al*., 2006; Veblen, 2012]. In Europe, it is found as a weed on wastelands and in garbage dumps [Arana *et al*., 2006]. People who discover it growing in their gardens and are worried about its toxicity, have been advised to dig it up or have it otherwise removed [Oudhia and Tripathi, 1998]. In traditional Ayurvedic medicine in India, jimsonweed has long been used for curing asthma symptoms. The active agent is atropine. The Chinese also used it in this manner, as a form of anesthesia during surgery. Its seeds can lie dormant underground for years and germinate when the soil is disturbed [Brown and Bridglall, 1989; Veblen, 2012]. Hardseededness, which is prevalent in many species of a number of families, is one form of dormancy and is caused by both environmental and genetic factors [Copeland and McDonald, 2001]. One or more water-impermeable layers of palisade cells in the seed or fruit coat cause physical dormancy [Baskin and Baskin, 2004]. Morrison *et al*. (1998) have presented evidence that, in some taxa of Fabaceae, dormancy break by heating may occur through the disruption of the seed coat in a region(s) other than the strophiole (lens). Sixtus *et al*. (2003) found that sulfuric acid and sandpaper treatments increased germination of *Ulex europaeus* seeds, while hot water treatment did not affect seed germination. Farashah *et al*. (2011) noted that scarification of oregano seed enhanced germination. It shows that an impermeable covering layer restricts seed germination. Physical dormancy is a big problem for cultivating jimsonweed as a medicinal plant and controlling the damage to crop plants as a weed; therefore, the aim of this study was to assess the effect of different seed dormancy-breaking treatments on seed germination traits and α- and β-amylase activities in jimsonweed seedlings.

Materials and methods

Site description, plant material, and measured traits

This study was done at the Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University. Jimsonweed seeds were collected from Hamedan province. The measured seed germination traits were: final germination percentage (FGP), mean germination time (MGT), abnormal germination, plumule length, radicle length, seedling length, vigor index, and α-and β-amylase activities.

Seed treatments

Details of various treatments applied to break the seed dormancy and improve germination of jimsonweed are presented in Table 1.

Germination tests

Before keeping the seeds for germination, the seeds were surface-sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 3 min and washing thoroughly with sterilized water. After performing the dormancy-breaking treatments, seeds were germinated between two layers of Watman No.1 filter paper [ISTA, 1996] with 10ml of water in Petri dishes (10cm diameter). Petri dishes containing seeds were placed in polythene bags to avoid loss of water. Seeds were incubated at 20 ± 1° C in the dark for 25 days [ISTA, 1996]. The criterion for germination was when the emerging radicles were 2mm long. Germination percentage was registered every day for 21 days. Mean germination time (MGT) was calculated by the following equation (Schelin *et al*., 2003).

$$MGT = \sum (fini)/N$$

fi: day during germination period (between 0 and 21st day); ni: number of germinated seeds per day; N: sum of germinated seeds. The seed vigor index (VI) was calculated as following (Sepehri & Rouhi, 2016).

$$VI = {}^{Ls \times Pg}/_{100}$$

Where Ls is the mean of seedling length (mm) and Pg is percentage of germination.

Amylase enzyme extraction and assays

After the starting of germination in each treatment, amylase enzymes were extracted and calculated according to the method of Kishorekumar *et al*. (2007) and Tárrago & Nicolás (1976).

Statistical analysis

The experiment was laid out in a completely randomized design. Four replications and 100 seeds per replicate were used. Before analysis of variance, data for germination and abnormal germination percentage were subjected to arcsine transformation. Statistical analysis was carried out using SAS 9.1 software. Mean comparison was performed using Duncan's test at the 5% level of probability.

Results

Analysis of variance showed that the effect of treatments on measured parameters were significant (Table 2).

Final germination percentage

There were significant differences ($p<0.05$) among the methods used for breaking dormancy of jimsonweed seed. Among the five methods used, the highest germination (90%) was for seeds scarified with sandpaper, although >75% germination was also achieved after sulfuric acid treatment for 1 min and 1.5 min (Table 3). The longest duration of acid treatment (2 min) resulted in numerous abnormal seedlings, about 28% (Table 3). Submersing seeds in hot water at 80°C and 90°C for 5 and 10 min caused significant increases in germination percentage compared to the control group (Table 3), but these were lower than the germination after sandpaper and sulfuric acid treatments. In addition, there was an increase in the percentage of abnormal seedlings when the time was increased to 10 min at 90° C (Table 3). Treating seeds with light (in all durations) and fluctuating temperature did not have positive effects on germination percentage of jimsonweed seed compared to the control group (Table 3).

Table 1. Details of used treatments to break dormancy of jimsonweed seed

Treatments	Concentration/Duration	Method	Remarks
Light exposure	10, 20 , 30 days	Light emitting green safe lamp with energy in the 500 to 700 nm irradiation of 660 nm	
Acid scarification 98 % (v/v)	1, 1.5, 2 minutes	Using concentrated sulfuric acid (98 % v/v)	Washed with distilled water thoroughly
Scarification by hot water	5 and 10 minutes	Using distilled hot water with a temperature of 80 and 90 °C	
Mechanical scarification	Until seeds were scarified	Using sandpaper	
Fluctuating temperature	5-10 °C	Seeds placed in growth chamber for 21 days, 12 hr at 5 °C then 12 hr at 20 °C under dark condition	

Table 2. The ANOVA table of dormancy breaking treatments on germination traits of jimsonweed

S.O.V	df	Mean Squares								
		FGP	MGT	AG	PL	RL	SL	VI	α-amylase	β -amylase
Treatment	12	4085.66**	152.85**	334.14**	2099.64**	2276.92**	8650.40**	10612.18**	0.72**	0.75**
Error	39	6.38	0.96	2.37	3.49	1.73	5.15	10.19	0.0078	0.0042
CV	-	8.56	4.85	20.30	5.33	3.80	3.36	9.55	14.64	10.55

ns,**,*: non-significant and significant of 1 and 5 percent of probability, respectively

S.O.V: Source of Variation, df: degree of freedom, CV: Coefficient of Variation, FGP: Final Germination Percentage, MGT: Mean Germination Time, AG: Abnormal Germination, PL: Plumule Length, RL: Radicle Length, SL: Seedling Length, VI: Vigor Index.

Table 3. Effects of treatments on jimsonweed seed germination traits

Dormancy breaking treatments	FGP (%)	MGT (day)	AG (%)	PL (mm)	RL (mm)	SL (mm)	VI	α-amylase (units mg^{-1}prtein)	β -amylase (units mg^{-1}prtein)
Control	5.00 f	23.51 c	0 a	1.10 e	9.30 e	10.40 f	0.52 e	0.12 e	0.15 e
Light exposure									
10 (days)	6.00 f	24.45 c	0 a	31.60 c	27.10 c	58.70 c	3.52 e	0.13 e	0.14 e
20 (days)	5.00 f	24.40 c	0 a	31.50 c	26.90 c	58.40 c	2.92 e	0.12 e	0.15 e
30 (days)	6.00 f	23.55 c	0 a	28.00 d	24.20 d	52.20 e	3.13 e	0.13 e	0.15 e
Acid scarification 98 % (v/v)									
Sulfuric acid (1 min)	75.00 c	9.60 a	8 b	61.25 b	65.30 b	126.55 b	94.91 c	0.96 b	1.02 b
Sulfuric acid (1.5 min)	85.00 b	9.82 a	9 b	74.85 a	78.88 a	153.73 a	130.67 b	1.03 b	1.01 b
Sulfuric acid (2 min)	23.00 e	18.31 b	28 c	31.70 c	27.00 c	58.70 c	13.50 d	0.85 c	0.87 c
Scarification by hot water									
80 ℃ + 5 min	25.00 de	23.11 c	8b	30.50 cd	26.00 cd	56.50 cd	14.12 d	0.81 c	0.79 c
80 ℃ + 10 min	22.00 e	24.52 c	7 b	31.60 c	27.00 c	58.60 c	12.89 d	0.80 c	0.81 c
90 ℃ + 5 min	28.00 d	24.11 c	7 b	28.00 d	24.00 c	52.00 e	14.56 d	0.81 c	0.83 c
90 ℃ + 10 min	7.00 f	23.52 c	25 c	27.90 d	25.80 cd	53.70 de	3.76 e	0.64 d	0.61 d
Mechanical scarification	90.00 a	9.90 a	7 b	75.1 a	80.1 a	155.20 a	139.68 a	1.35 a	1.42 a
Fluctuating temperature	7.00 f	24.11 c	0 a	2.55 e	9.50 e	12.05 f	0.84 e	0.15 e	0.13 e

Data that do not share the same letters differ significantly at $P < 0.05$ level. Final germination percentage (FGP), mean germination time (MGT), abnormal germination (AG), plumule length (PL), Radicle length (RL), seedling length (SL) and vigour index (VI).

Mean germination time

The mean germination time was significantly lower in mechanical scarification treatment (sandpaper), but was not significantly different with acid scarification for 1.5 min (Table 3). The MGT values after hot water treatment at 80°C and 90°C for 5 and 10 min, respectively, were not significantly decreased when compared to the control group. Seed treatment with light (in all durations) and fluctuating temperature did not have positive effects on mean germination time of jimsonweed seed compared to the control group (Table 3).

Abnormal germination

All the treatments except light exposure and fluctuating temperature treatments resulted in abnormal germination (Table 3).

Plumule length

The longest plumule was observed in the sandpaper treatment, but it was not different with sulfuric acid for 1.5 min (Table 3). After the mentioned treatments, scarification with acid for 1 min was ranked second. Regarding Table 3, plumule length was not increased in response to light exposure, hot water, and fluctuating temperature treatments; hence, these treatments were

not detected as efficient treatments and did not have significant differences with the control group.

Radicle length

The results of the experiment on this trait suggested that the highest radicle length was detected in the sandpaper treatment (Table 3). Significant difference was not observed with sulfuric acid treatment for 1.5 min. Also, other results were similar to obtained results of plumule length.

Seedling length

This trait is made up of plumule and radicle lengths; hence, results were similar to the mentioned traits (Table 3). Acid scarification for 1 min and 2 min, which ranked second after the above treatments, had positive effect on this trait.

Vigor index

The highest vigor index was obtained in the mechanical scarification that did have significant difference ($p < 0.05$) with other treatments (Table 3). Acid scarification for 1.5 min and 1 min ranked second and third after and had positive effect on this trait. After mechanical scarification, chemical scarification improved vigor index in comparison to other treatments. The lowest value of this trait was detected in fluctuating temperature and light treatments. These treatments did not have significant differences with the control group (Table 3). All levels of hot water except 90° C for 10 min had similar effect and did not have significant differences but they were better than the control group (Table 3).

α-amylase and β-amylase activities

Study of α-amylase and β-amylase activities in breaking dormancy treatments and the control group showed that enzyme activity in dormant seeds was very low (Table 3) and did not have significant difference with light exposure in all durations. However, enzyme activity was increased in germinating seeds of jimsonweed dormancy after breaking treatment with sandpaper, sulfuric acid, and hot water respectively. The most activities of the abovementioned enzymes were observed in sandpaper, acid, and hot water, respectively.

Discussion

Five methods for breaking seed dormancy assessment were used in this study: sulfuric acid, hot water, and mechanical scarification, as well as fluctuating temperature and light exposure. Except for the light exposure and fluctuating temperature treatments, all of the seed treatments for breaking dormancy had positive effects in comparison to the control group (Table 3). Positive effects of sandpaper and sulfuric acid on seed dormancy breaking were supported by previous findings [Aleiro, 2004; Farhoudi *et al*., 2007; Schwienbacher *et al*., 2011] and the effect of these treatments were stronger than the other treatments. Farhoudi *et al*. (2007) found that scarification of Madder seed with concentrated sulfuric acid (98%) decreased MGT, E1st, E50%, and increased germination percentage. Schwienbacher *et al*. (2011) reported that scarification with sandpaper resulted in immediate water uptake in *Anthyllis apicola* and *Trifolium pallescens*. An increase in abnormal seedlings after scarification with concentrated sulfuric acid for long time has also been reported for *Rubia tinctorum* [Farhoudi *et al*., 2007] and in *Adonis vernalis* [Rouhi *et al*., 2013]. Presumably, applying acid for a long time destroys seed structure and tissues because of acid penetration in them. Baskin and Baskin (2004) stated that physical dormancy (PY) is caused by

one or more water-impermeable layers of palisade cells in the seed or fruit coat. Jayasuriya *et al*. (2009) noted that physical dormancy can be overcome by various artificial methods like manual scarification, acid scarification, etc. and the effectiveness of dormancy-breaking treatment may differ from species to species. After the scarification of most hardseeded species such as jimsonweed, they can germinate easily. Alvarado and Bradford (2005) showed that, with increasing loss of dormancy, the time to germination decreased while the percentage of germination increased progressively. In this experiment, hot water treatment did not have strong effects on germination traits. Farhoudi *et al*. (2007) showed that hot water is a suitable treatment for breaking dormancy of Madder seed. Neither fluctuating temperature nor exposure to light had any effect on seed germination or seedling growth compared to the control group. Investigating enzyme activities in germinating seeds indicates that α- and β-amylase play important roles in jimsonweed seed germination. Biswas *et al*. (1978) reported that dormant seeds of large crabgrass contained very little or no activity of α-amylase, whereas broken dormancy seeds showed appreciable activity. Farashah *et al*. (2011) noted that the activity of α-amylase and β-1,3-glucanase in oregano (*Origanum vulgare*) germinated seeds were higher than dormant seeds. In this research, the marked improvement in germination, which follows sandpaper as mechanical scarification and sulfuric acid as chemical scarification treatments of jimsonweed, indicated that these were appropriate treatments to break dormancy caused by hardseededness.

Conclusions

The results showed sulphuric acid treatment in long duration (2 min) and hot water treatment at 90°C for 10 min result in increased abnormal germination; so, they are not recommended for breaking dormancy of this species. Finally, it seems that scarification by sandpaper or sulphuric acid for 1.5 min is a general requirement for breaking dormancy of jimsonweed seeds.

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