

## Dormancy-breaking Seed Treatments for *Datura stramonium* L.

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### Abstract

**Purpose:** The study aimed to overcome the seed dormancy of *Datura stramonium* L. by chemical scarification, pre-chilling, and different gibberellic acid (GA<sub>3</sub>) levels.

**Materials and Methods:** The seeds were prechilled at 4°C and 10°C for 7 days and treated with sulfuric acid (97-98% H<sub>2</sub>SO<sub>4</sub>) for 15 minutes, and increasing doses of GA<sub>3</sub> (250, 500, and 1000 ppm). Distilled water for hydration and untreated seeds served as a control.

**Results:** The results showed that no germination was observed in the control and prechilled seeds of *Datura stramonium* L. The dormancy rate was 100% and the seed dormancy resulted from both hard seed coat and physical dormancy. Mean germination time and germination index of control seeds could not be calculated because no seed germination occurred. Although the chemical scarification showed a beneficial effect for breaking dormancy, the gibberellic acid applications were more effective seed treatments for increasing germination compared to sulfuric acid. The germination percentage increased from 0.0% to 44.5% in the seeds scarified and treated with 1000 ppm GA<sub>3</sub>, while the germination percentage of primed seeds with 1000 ppm GA<sub>3</sub> was 68.5%. Mean germination time was lessened by GA<sub>3</sub> doses and scarification. Furthermore, the germination index was promoted by the applications of GA<sub>3</sub>, especially in the seeds without scarification. The higher germination index was obtained from control seeds under all levels of GA<sub>3</sub>. The highest shoot length was measured at the highest level of GA<sub>3</sub> with 14.6 cm.

**Conclusion:** Seed dormancy can be effectively broken by the application of 1000 ppm GA<sub>3</sub> without chemical scarification.

**Keywords:** Jimson weed, GA<sub>3</sub>, Scarification, Germination.

### *Datura stramonium* L.'da Tohum Uygulamaları ile Dormansinin Kırılması

#### Öz

**Amaç:** Bu çalışmada, *Datura stramonium* L.'un tohumlarında dormansiyi kırmak amacıyla yapılan ön üşütme ve sülfürik asit uygulaması ile giberellik asit (GA<sub>3</sub>) dozlarının etkilerinin incelenmesi amaçlanmıştır.

**Materyal ve Yöntem:** Tohumlara 4°C ve 10°C sıcaklıklarda 7 gün ön üşütme, sülfürik asitte (%97-98'lik H<sub>2</sub>SO<sub>4</sub>) 15 dakika aşındırma ve artan dozlarda giberellik asit (250, 500 ve 1000 ppm GA<sub>3</sub>) uygulanmıştır. Tohumlar hidrasyon için distile su ile muamele edilmiş ve işlem görmemiş tohumlar kontrol olarak kullanılmıştır.

**Araştırma Bulguları:** Kontrol ve ön üşütme yapılan *D. stramonium* tohumlarında çimlenme olmadığı belirlenmiştir. Tohumlarda dormansinin hem sert kabuktan hem de fizyolojik etkilerden kaynaklandığı ve dormansi oranının %100 olduğu belirlenmiştir. Kontrol tohumlarında ortalama çimlenme süresi ve çimlenme indeksi, çimlenme olmadığı için hesaplanamamıştır. Dormansiyi kırmada kimyasal aşındırma faydalı bir etki gösterse de, giberellik asit uygulaması sülfürik aside kıyasla çimlenmeyi arttırmada daha etkili bulunmuştur. Aşındırılmış ve 1000 ppm GA<sub>3</sub> uygulanmış tohumların çimlenme yüzdesi %0.0'dan %44.5'e artarken, 1000 ppm GA<sub>3</sub> uygulanan tohumların çimlenme oranı %68.5 olmuştur. GA<sub>3</sub> dozları ve kimyasal aşındırma uygulaması ortalama çimlenme süresini kısaltmıştır. Ayrıca, aşındırma yapılmayan tohumların çimlenme indeksi GA<sub>3</sub> uygulaması ile artmıştır. Tüm GA<sub>3</sub> dozları,

kontrol tohumlarından daha yüksek çimlenme indeksi vermiştir. En yüksek sürgün uzunluğu 14.6 cm ile en yüksek GA<sub>3</sub> seviyesinde ölçülmüştür.

**Sonuç:** Kimyasal aşındırma olmadan 1000 ppm GA<sub>3</sub> uygulaması ile tohum dormansinin etkili bir şekilde kırılacağı sonucuna varılmıştır.

**Anahtar Kelimeler:** Boru çiçeği, GA<sub>3</sub>, Aşındırma, Çimlenme

## Introduction

Jimson weed, *Datura stramonium* L., is an annual plant from the *Solanaceae* family and originates from North America (Reisman- Beirman et al., 1989; Veblen, 2012). It is widely available in all regions of Turkey and is generally an aggressive invasive weed (Weaver and Warwick, 1984) decreasing the yield and quality of sugar beet. It is rarely grown as an ornamental plant for trumpet-shaped fragrant flowers in gardens. However, jimson weed is a significant plant used for traditional medicine due to its containing atropine alkaloid, which treats asthma symptoms and surgery operations as an analgesic (Mousavi et al., 2019). For this reason, it should be cultivated to obtain standard alkaloid content when it is used for pharmaceuticals.

One of the major obstacles for the cultivation of *D. stramonium* L. is the seed dormancy inhibiting uniform germination, emergence, stand establishment, and ultimately regular maturation and harvest. The optimum temperature for germination of *D. stramonium* ranges between 25-30°C and it can survive for several months in soils as dormant (Kenanoğlu et al., 2019) because of the hard seed coat (Veblen, 2012; Bisht et al., 2019). The hard seed coat was impermeable covering a layer restricting germination (Baskin and Baskin, 2004). However, lower  $\alpha$  and  $\beta$  amylase activities were determined in dormant seeds of *D. stramonium* L. (Surki et al., 2017). To circumvent this phenomenon, several seed treatments have been demonstrated in *Datura* ssp. up to now. Heating (Surki et al., 2017), chemical scarification (Mu et al., 2011; Moosavi et al., 2021), light (Brown and Bridglall, 1987), chilling (Dorado et al., 2009), and cold scarification (Bisht et al., 2019) have been successfully applied to enhance germination in *Datura* species. Besides, the germination percentage in *D. stramonium* was enhanced by the application of 100 ppm gibberellin and 500 ppm potassium nitrate treatments

(Ghadamyari et al., 2011). The dose of 500 ppm GA<sub>3</sub> increased the germination percentage in *Datura ferox* L. (Sanchez et al., 1966), and pre-treatment with gibberellic acid and potassium nitrate up to 2000 ppm reached the maximum germination of 63% (Mousavi et al., 2019). Furthermore, a reduction in seed dormancy of jimson weed by chemical scarification was announced by Surki et al. (2017). So the present study focused on determining a useful combination between chemical scarification and gibberellic acid concentrations for breaking dormancy in *D. stramonium* L.

## Materials and Methods

### Seed collection and preparation

This study was carried out at the Department of Field Crops, Faculty of Agriculture, Eskişehir Osmangazi University, Turkey. The mature pods (dry, brown, and cracked from the top of the fruit) of *Datura stramonium* L. were collected from the plants naturally grown in the natural habitats on the campus in late September 2021. The seeds were threshed from the pods and kept in room condition until used. One thousand seed weight and seed moisture content of the collected seeds were determined as 6.28 g and 6.7% just after threshing, respectively.

### Seed treatments

The seeds were divided into two groups, control, and chemical scarification. For scarification, the seeds were subjected to sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) with a purity of 97-98% for 15 minutes to thin and soften the seed coat. After the treatment, the seeds were thoroughly rinsed with tap water three times and then washed with distilled water. Afterward, the control and scarified seeds were primed with 250, 500, and 1000 ppm GA<sub>3</sub> solutions by soaking in respective levels and in distilled water (attained as hydration) for 16 hours at 25 °C in the dark. After the incubation period, the seeds were surface-dried with paper towels and were left to dry back to the beginning seed weight at room conditions. The seeds without seed treatments were used as a control. The moisture of both primed and control seeds was equaled at room conditions for 2 days. For prechilling, the seeds were incubated on the wetted filter papers in Petri dishes at 4°C and 10°C for seven days.

### Germination test

Four replicates of fifty seeds from each treatment, a total of 200 seeds, were germinated among three moistened filter papers wetted with 7 mL of distilled water with fungicide (Thiram, 80%). To avoid

moisture loss, each rolled paper was put into a sealed plastic bag during the germination period. The germination test was performed at  $25 \pm 1$  °C in the dark for 21 days using a thermostatically controlled incubator as described by the rules of ISTA (2003). Radicle elongation with a minimum of 2 mm was considered as germination criteria. At the end of the experiment, germination percentage, mean germination time, and germination index were evaluated for seed dormancy. Germinated seeds were counted every 24 h for 21 d and the mean germination time (MGT) was calculated for the speed of germination according to ISTA (2003). The germination index (GI) was also determined by the following formula (Salehzade et al., 2009).

GI = Number of germinated seeds/days of first count + . . . + Number of germinated seeds/ days of the final count.

Shoot length was measured from randomly selected five seedlings in each replicate after 21 days.

The experiment design was a two-factor factorial (2×5) arranged in a completely randomized design; with 4 replicates and a total of 200 seeds for each treatment. The data were analyzed by the JMP 13.0 statistical package program.

## Results and Discussion

No seed germination was observed in pre-chilled seeds at both 4°C and 10°C, so data were not shown. Analysis of variance and mean values of the investigated parameters were shown in Table 1. Chemical scarification, gibberellic acid doses, and their interactions were significant. The germination percentage, mean germination time, germination index, and shoot length were higher in non-scarified seeds than that in scarified seeds. Increasing GA<sub>3</sub> levels improved germination percentage, germination index, shoot length, and mean germination time. The highest germination was observed in 1000 ppm GA<sub>3</sub>.

A significant two-way interaction of the investigated traits was illustrated in Figure 1. No seed germination was obtained in non-scarified seeds. This means that there was severe dormancy in *D. stramonium* L. Chemical scarification promoted germination, while the germination percentage did not reach over 12.0% in similarity with hydration; suggesting that seed

dormancy resulted mainly from a hard seed coat rather than an impermeable coat. Because lower  $\alpha$  and  $\beta$  amylase activities existed in dormant seeds of *D. stramonium* (Surki et al., 2017) and it can be easily argued that hydration may induce  $\alpha$  and  $\beta$  amylase activities of the seeds and slightly enhanced germination. Also, extended hydration duration may be useful for increasing germination. Germination percentage was considerably improved by scarified and control seeds when gibberellic acid doses were increased. The maximum germination of 68.5% was achieved in non-scarified seeds with the application of 1000 ppm GA<sub>3</sub>. All levels of GA<sub>3</sub> gave higher germination than hydration and control seeds, but the beneficial effect of GA<sub>3</sub> was more prominent in non-scarified seeds than in chemical scarification.

Mean germination time could not be calculated in seeds without any treatments because none of the seeds germinated. On the other hand, scarification induced germination of *Datura* seeds, and mean germination time could be determined. Increased GA<sub>3</sub> levels reduced the requirement of time for germination and the minimum mean germination time was achieved at 1000 ppm GA<sub>3</sub>. Both chemical scarification and gibberellic acid levels provided an increase in the germination index. Because of insufficient germination in control seeds, the germination index could not be computed. Increasing GA<sub>3</sub> doses resulted in promoting germination index and the highest value of 4.58 was observed in non-scarified seeds treated with 1000 ppm GA<sub>3</sub>. Similar results were reported by Surki et al. (2017) and (Bisht et al., 2019), who found a valuable improvement in mean germination time when the seeds were scarified.

The shoot length was promoted by increasing GA<sub>3</sub> levels. Only gibberellic acid application without scarification enhanced the shoot length. GA<sub>3</sub> levels of 500 and 1000 ppm produced the longest shoots with 14.6 cm. Compared to scarification, gibberellic acid was more effective to increase shoot length. Similarly, these findings were confirmed by Surki et al. (2017), who determined that seedling length was promoted by sulfuric acid scarification for 1 or 1.5 min and an increase in plumule and radicle length of *D. stramonium* L. by the application of GA<sub>3</sub> was identified (Mousavi et al., 2019).

Table 1. Germination performance and shoot length of *Datura stramonium* L. treated with chemical scarification and GA<sub>3</sub> doses

Factor	Germination percentage (%)	Mean germination time (day)	Germination index	Shoot length (cm)
<b>Scarification (A)</b>				
No scarification	30.8 <sup>a</sup>	11.5 <sup>a</sup>	1.89 <sup>a</sup>	7.74 <sup>a†</sup>
H <sub>2</sub> SO <sub>4</sub>	17.6 <sup>b</sup>	8.8 <sup>b</sup>	0.90 <sup>b</sup>	5.80 <sup>b</sup>
<b>GA<sub>3</sub> dose (B)</b>				
Control	6.0 <sup>e</sup>	10.7 <sup>ab</sup>	0.04 <sup>c</sup>	2.60 <sup>c</sup>
Hydration	12.0 <sup>d</sup>	11.7 <sup>a</sup>	0.33 <sup>c</sup>	2.16 <sup>c</sup>
250 ppm	25.8 <sup>c</sup>	10.0 <sup>bc</sup>	1.35 <sup>b</sup>	7.19 <sup>b</sup>
500 ppm	21.0 <sup>b</sup>	9.2 <sup>bc</sup>	1.45 <sup>b</sup>	10.91 <sup>a</sup>
1000 ppm	56.3 <sup>a</sup>	9.3 <sup>c</sup>	3.85 <sup>a</sup>	10.99 <sup>a</sup>
<i>Analysis of Variance</i>				
A	**	**	**	**
B	**	**	**	**
A×B	**	**	**	**

†: Means followed by the same letter are not significant at p<0.05. \*\*: significant at p<0.01%.

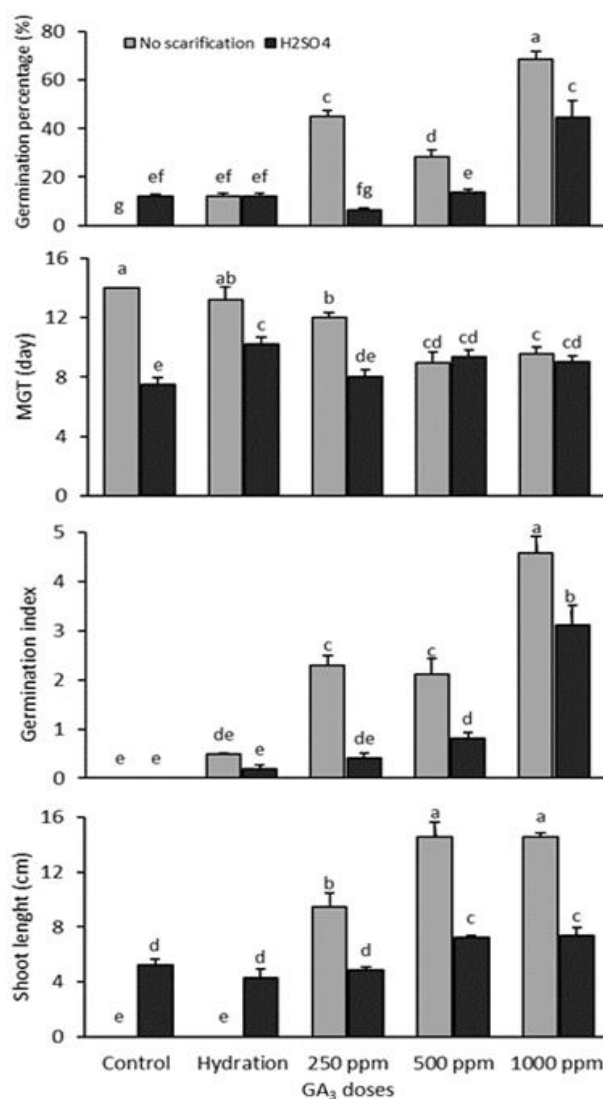


Figure 1. The interaction of different gibberellic acid (GA<sub>3</sub>) doses and chemical scarification on germination percentage, mean germination time, germination index, and shoot length of *Datura stramonium* L. Bars and letters on each column denote standard error (SE) and significance level, respectively.

## Conclusion

Our results showed that *D. stramonium* L. suffered from germination disability and hardly ever seeds germinated under optimum conditions. The use of GA<sub>3</sub> is required for breaking dormancy in *D. stramonium* L. and increasing germination percentage, germination index, and shoot length. It was concluded that seed dormancy in *D. stramonium* L. results from physiological dormancy along with a hard seed coat and it can be overcome by the application of 1000 ppm GA<sub>3</sub>. It should be advised that a longer priming time may be treated in further research.

## Conflict of interest

There is no conflict of interest between the authors.

## Authors' Contributions

MDK: contributed to the stages of planning the research, supplying the necessary materials for the research, establishing and conducting germination experiment, seed treatments, obtaining data and making statistical analyzes.

PH: seed collection, threshing, cleaning of the seed materials required for the research, and the establishment and conduct of the germination experiment.

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