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Evaluation of Dormancy Failure *Datura stramonium* Plant Seeds under the Influence of Different Treatments

Salehe Ganjali[*1], Hamideh Khajeh[2], Zahra Gholami[1], Zahra Jomeh-ghasemabadi[1], Bahman Fazeli-Nasab[*3]

[1]*Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Zabol, Iran*
[2]*Agricultural Biotechnology Research Institute, University of Zabol, Zabol, Iran*
[3]*Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran*

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ABSTRACT

The seeds of most medicinal plants have a variety of dormancy. Therefore, it is necessary to know the effective methods of breaking the seed dormancy for their production and cultivation. In this study, to determine the best method of breaking dormancy and increasing the germination percentage of tattoo seeds, various factors have been investigated. Different treatments, scratching with sandpaper, embryo culture, GA treatment (100, 150 and 200 PPM) and sulfuric acid treatment (50, 75 and 100%) with filter paper or on MS medium containing BAP (0.5, 1 and 1.5 mg/l) was applied with untreated samples (control). Embryo culture was performed only on MS medium containing BAP hormone. The analysis of variance showed that all interactions with the studied treatments were significantly different in terms of germination percentage, fresh and dry weight of roots and stems ($P < 0.01$). It was observed that the use of benzyl aminopurine (1.5 mg/l) with gibberellic acid (200 ppm) increased the seed germination percentage compared to the control. The use of benzylaminopurine (1.5 mg/l) and sulfuric acid (50%) significantly increased seed germination compared to the control. It was concluded that the effect of GA with BAP on seed germination was greater than sulfuric acid with BAP.

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1. Introduction

Herbs are an important source of medicines that play an essential role in the health of people around the world (Fazeli-nasab and Fooladvand, 2014; Fooladvand and Fazeli-nasab, 2014). Medicinal plants are commonly used to treat and prevent dangerous human diseases (Fazeli-nasab and Fooladvand, 2016; Valizadeh *et al.*, 2020). These plants or "wild plant species" grow spontaneously in natural or semi-natural ecosystems and can exist independently, and their presence depends on human actions such as selection or reproduction (Calixto, 2000). Dormancy is defined as the lack of germination of healthy seeds under optimal temperature, humidity, and light (Hilhorst *et al.*, 2007). It is characterized by low metabolic activity and insensitivity to growth-promoting signals (Vaistij *et al.*, 2013). Dormancy prevents seeds from germinating under adverse growth conditions (Donohue *et al.*, 2005; Huang *et al.*, 2010; Krasuska *et al.*, 2015).

The environment can directly affect growth, maturity, and dormancy. Environmental conditions may directly affect dormancy by regulating the ability to accumulate dry biomass, or indirectly by limiting the plant's ability to supply seed raw materials (Bewley *et al.*, 2006). The environment can directly affect growth, maturity, and dormancy. Environmental conditions may directly affect dormancy by regulating the dry matter accumulation, or indirectly by limiting the plant's ability to supply seed raw materials (Bewley *et al.*, 2006).

Phytohormones determine the degree of dormancy in growing seeds (Finch-Savage and Leubner-Metzger,

* Corresponding author.
E-mail addresses: Saleheganjali@uoz.ac.ir, bfazeli@uoz.ac.ir

2006). Five classes of dormancy include morphophysiological dormancy, hybrid dormancy, physical dormancy, morphological dormancy, and physiological dormancy, which are characterized by the physiological and morphological condition of the embryo and physical barriers to water absorption by the pericarp or grain cover (Baskin and Baskin, 2014). Usually, the seeds of volunteer species of medicinal plants have more intense dormancy than those of domesticated species. This is one of the main problems in the widespread cultivation of medicinal plants (Hatami *et al.*, 2019).

Datura stramonium L. has been one of the most invasive weeds in the world in recent decades in sugar beet, legume, tobacco, vegetable and summer crops, cotton and soybean fields, and other oilseeds and orchards (Zarin Kaviani *et al.*, 2018). *Datura* has great effects on the eyes, nervous system, heart, blood flow, and body secretions due to its tropane alkaloids, which are hyoscyamine, scopolamine, and atropine (Vaillant *et al.*, 2005).

To identify the mechanism of breaking the seed dormancy, there are various methods such as gibberellic acid treatment, sulfuric acid treatment, scratching, cooling, heating, separating the seed coat, etc. (Bhanuprakash and Umesha, 2015; Fazeli-Nasab *et al.*, 2012; Fazelienasab *et al.*, 2004).

The concentrations of 100 ppm gibberellin and 500 ppm potassium nitrate, when used simultaneously, significantly increase germination in *Datura* (Ghadamyari *et al.*, 2011). The results showed that the germination percentage of *Datura Ferox* seeds at a concentration of 500 ppm gibberellin was 20%. Still, when the seeds were scratched, the germination rate was more than 95% at a concentration of 100 to 500 ppm gibberellin (Schulz *et al.*, 2001). Research has shown that one of the main causes of dormancy in healthy Datura seeds is darkness and lack of light, and another reason is that they are not scratched, and even if these seeds are stored for 24 months in dry conditions, their dormancy will not be removed (Miguel and Soriano, 1974).

Unsuitable environmental conditions for the plant at the time of seed production also affect seed dormancy (Anchez *et al.*, 1981). Also, the lack of sufficient oxygen in the seed, oxygen diffusion, and reduction of its penetration into the seed cause dormancy. The study results showed that if *Datura* seeds are exposed to water vapor at 20 °C for 2 to 3 weeks, the seed dormancy will be eliminated, and it will germinate even in the darkness (Reisman-Berman *et al.*, 1989). The presence of inhibitors of germination inside the seed is another major cause of dormancy (Reisman-Berman *et al.*, 1991).

Due to the importance of *Datura* as an effective medicinal plant, in this study, *Datura* seed germination under different treatments such as abrasion with sandpaper, embryo culture, treatment with gibberellic acid and sulfuric acid, and also untreated samples were studied in vitro to determine the best break dormancy conditions and increase the seed germination percentage.

2. Materials and methods

The experiment was done at the Agricultural Biotechnology Research Institute, university of Zabol, in 2021.

2.1. Plant materials

Datura stramonium L. seeds were prepared by Pakan Bazr Company of Isfahan. Despite the high viability of *Datura* seeds, untreated seeds germinated very little and there was a possibility of factors such as impervious cover, etc. in the inhibition of germination. The seeds were placed in running water for half an hour before treatment, washed with Tween 20 and immersed in 70% v/v alcohol for one minute. The seeds were treated with 7% Hgcl2 for ten minutes and then disinfected with 2% v/v sodium hypochlorite solution for 10 minutes. Finally, after each step, they were completely washed with sterile distilled water (in each step, the seeds were first sanded with sandpaper).

2.2. Preparation of MS culture medium

MS medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.8% agar with a pH of 5.8 was prepared with different concentrations of BAP hormone (0, 0.5, 1, and 1.5).

2.3. Sulfuric acid treatment

For acid treatment, the seeds were placed in containers containing concentrated sulfuric acid at concentrations of 50, 75, and 100% for acid treatment. Then they were washed three times with sterile water twice. Finally, to study germination, the treated seeds were transferred to sterile Petri dishes containing moist

filter paper or MS medium containing BAP hormone and incubated at 25 ± 3 °C. ten sterile seeds, with different treatments (or without treatment), were added to each petri dish containing MS medium or on a moist filter paper.

2.4. Gibberellic acid treatment

In this treatment, the seeds were placed in containers containing concentrated sulfuric acid at 0, 100, 150, and 200 ppm. Then they were washed three times with sterile water twice. Finally, for the germination study, the treated seeds were transferred to sterile Petri dishes containing moist filter paper or MS medium and incubated at 25 ± 3 °C. 10 sterile seeds, with different treatments (or without treatment), were added to each petri dish containing MS medium containing BAP hormone or on a moist filter paper.

2.5. Embryo culture

After surface disinfection of the seeds, a slit was made in the appropriate part of the seeds with a sterile needle. To study germination, in both methods, the treated seeds were transferred to Petri dishes containing moist filter paper and MS medium containing BAP hormone and stored in an incubator at 25 ± 3 °C.

2.6. Measuring the fresh and dry weight of stems and roots

The criterion for germination was at least two millimeters of the root exit. To measure the dry weight of the samples, first, the samples of each treatment were isolated, then washed with distilled water and placed in an oven at 70 °C for 48 hours, and then their dry weight was measured with a digital scale.

2.7. Data analysis

Experiments were conducted through a factorial based on a completely randomized design with three replicates, including 10 seeds per replication. The data analysis was done based on the Duncan test at 1 and 5 probability levels, using SAS 9.4 software.

3. Results

3.1. The effect of gibberellic acid (GA) and BAP hormone on the seed germination of Datura stramonium

The effect of the concentration of Benzylaminopurine + GA on the germination percentage was significant (P<0.01) (Table 1). (Both on filter paper and MS culture medium + BAP). The results showed that the use of Benzylaminopurine + GA increased the germination percentage of seeds compared to the control. The concentration of 1.5 mg/l of Benzylaminopurine and 200ppm of GA significantly increased the germination percentage compared to the control (Fig. 1). The highest germination percentage when the seeds were on the filter paper was related to the concentration of 200 ppm of GA (Fig. 2). The results of the interaction of GA and BAP on the fresh and dry weight of shoots and roots showed a significant difference between different treatments (P<0.01) so that the highest wet and dry weight was observed in the treatment of GA at 50 ppm + BAP 1.5 mg/l (Fig. 3).

Table 1. Analysis of variance (means of squares) of the effects of GA and BAP hormone on *Datura stramonium* seed germination.

Source of variation	Degrees of freedom	Mean squares
GA	3	33.1875000**
BAP	3	62.7430556**
GA ×BAP	9	4.2986111**
Error	32	0.3750000
CV (%)	21.14667	

ns = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01).

Figure 1. Effect of the different treatments on the seed germination of *Datura stramonium*.

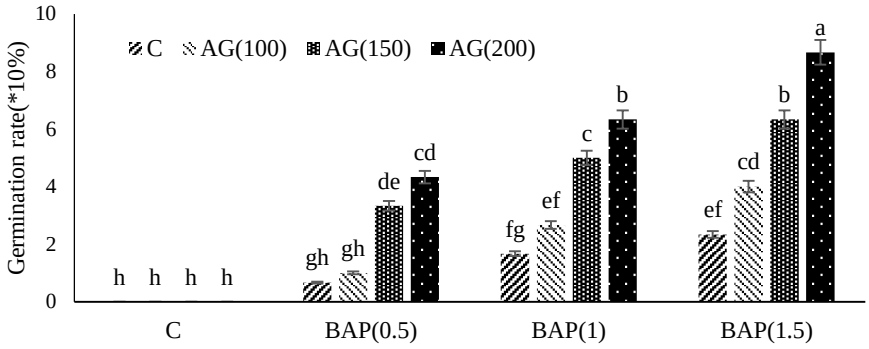

Figure 2. Interaction of GA (100, 150, 200) and BAP (0.5, 1, 1.5 mg/l) on *Datura stramonium* seed germination. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

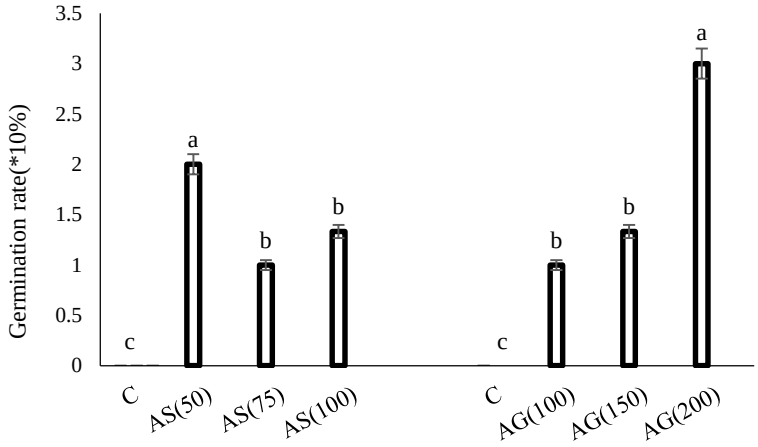

Figure 3. Comparison of means germination effect of GA (100, 150, 200) and Sulfuric acid (50, 75, 100%) on filter paper on *Datura stramonium* seed germination. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

3.2. The effect of sulfuric acid and BAP hormone on the seed germination of Datura stramonium

Benzylaminopurine concentration with sulfuric acid on the germination percentage was significant (P<0.01) (Table 2). (Both on filter paper and MS medium + BAP). Benzylaminopurine + sulfuric acid increased the germination percentage of seeds compared to the control and the concentration of 1.5 mg/l of Benzylaminopurine + sulfuric acid significantly increased the germination by 50% compared to the control (Fig. 4). The highest germination percentage was observed in seeds on filter paper, which was related to 50% sulfuric acid (Fig. 2). The results of the interaction of sulfuric Acid and BAP on the fresh and dry weight of shoots and roots of *Datura stramonium* showed that there is a significant difference between different treatments (P<0.01), so that the highest wet and dry weight was observed in the treatment of 50% sulfuric acid + BAP 1.5 mg/l (Fig. 5, 7 and 8).

Table 2. Analysis of variance (means of squares) of the effects of Sulfuric Acid and BAP hormone on *Datura stramonium* seed germination.

Source of variation	Degrees of freedom	Mean squares
Sulfuric Acid	3	16.2500000**
BAP	3	51.4722222**
Sulfuric Acid ×BAP	9	6.4537037**
Error	32	0.1250000
CV (%)	18.85618	

ns = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01).

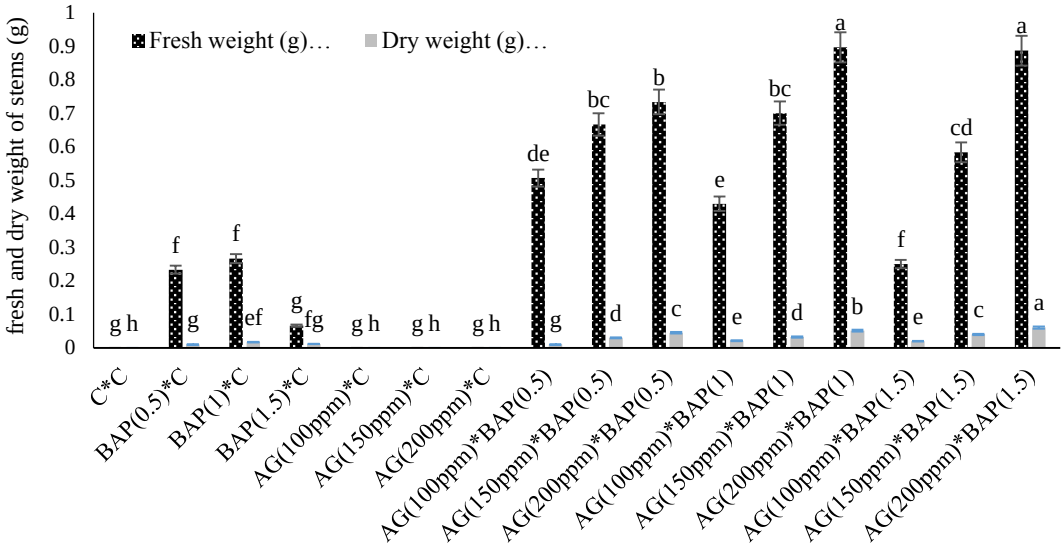

Figure 4. Interaction of GA (100, 150, 200) and BAP (0.5, 1, 1.5 mg / l) on fresh and dry weight of *Datura stramonium* stem. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

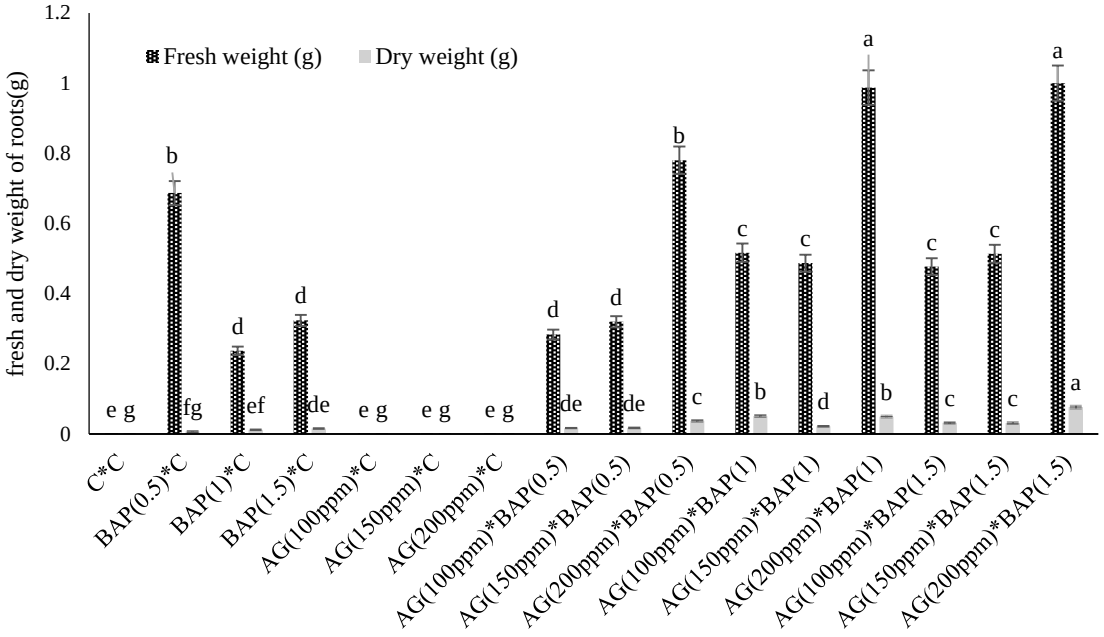

Figure 5. Interaction of GA (100, 150, 200) and BAP (0.5, 1, 1.5 mg / l) on fresh and dry weight of *Datura stramonium* root. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

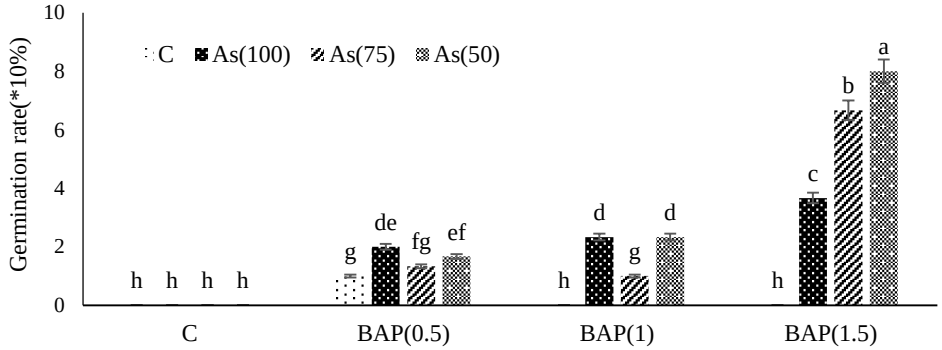

Figure 6. Interaction of Sulfuric acid (50, 75, 100) and BAP (0.5, 1, 1.5 mg / l) on *Datura stramonium* seed germination. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

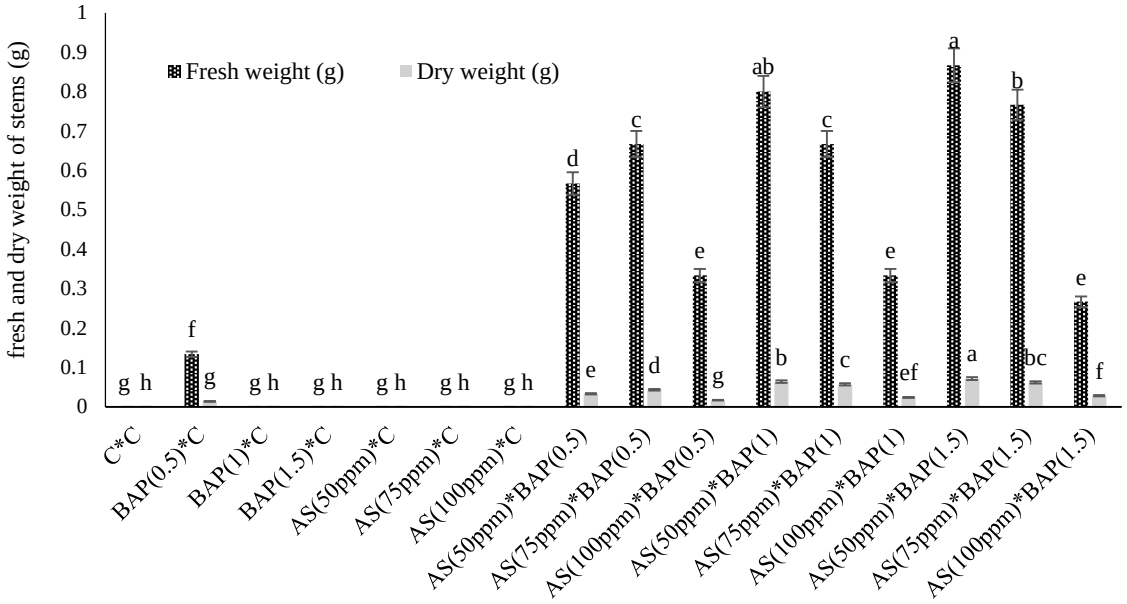

Figure 7. Interaction of Sulfuric acid (50, 75, 100) and BAP (0.5, 1, 1.5 mg/l) on the fresh and dry weight of *Datura stramonium* stem. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

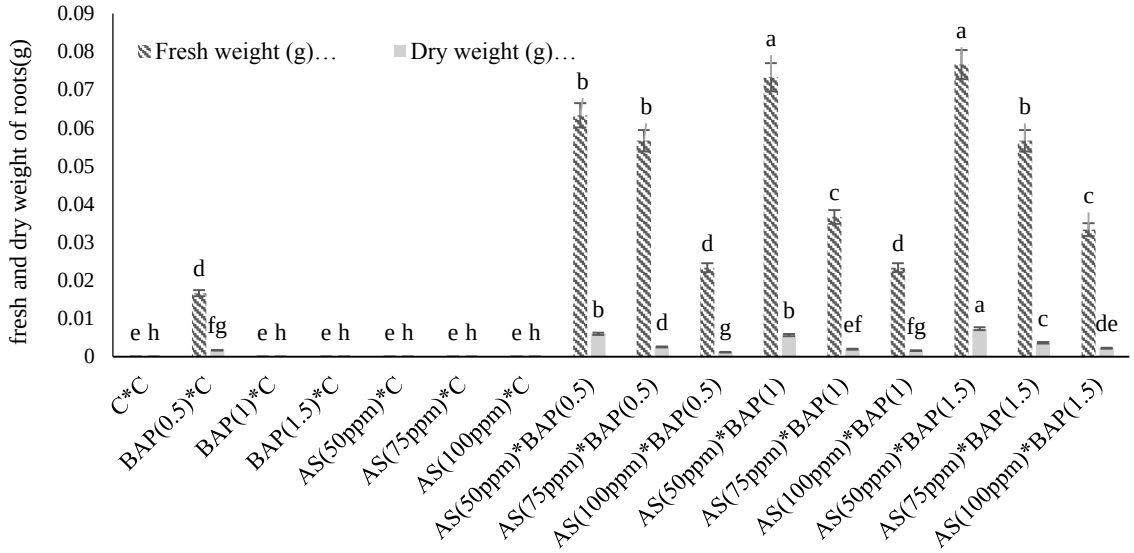

Figure 8. Interaction of Sulfuric acid (50, 75, 100) and BAP (0.5, 1, 1.5 mg/l) on the fresh and dry weight of *Datura stramonium* root. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

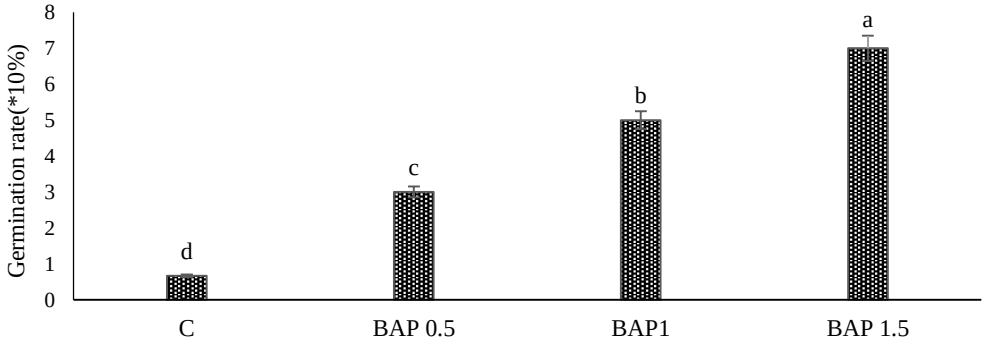

Figure 9. Comparison of means effect of germination through embryo culture on MS medium containing BAP. Each column represents the average of three repeats ± standard error (SE). Different letters above columns indicate significant differences (P< 0.01).

The results of the embryo culture of this plant on different levels of MS culture medium containing BAP showed that the highest germination percentage was observed in MS culture medium containing 1.5 mg/l BAP and the lowest value was observed in MS culture medium without hormone (Fig. 6 and 9).

4. Discussion

Breaking seed dormancy and seed germination is a critical stage in a plant's life cycle, and the strategies adopted at this stage for germination significantly ensure plant survival.

Seed dormancy is a physiological phenomenon that many crops, pastures, and weeds face (Plummer *et al.*, 2001). There are different types of seed dormancy in plants, such as physical dormancy. In this type of dormancy, the embryo is fully developed. It has no problem with germination (Baskin and Baskin, 2014) but there are water-impermeable layers around the seed, which prevent water from entering the seed and prevents seed germination (Baskin and Baskin, 2014; Jangjou and Tavakoli, 2008). This indicates the positive effect of scratching treatment for seed germination. No germination was observed in the control treatment, but germination was observed after scratching the seeds and then treatment with GA and sulfuric acid. According to these results, the lack of germination of Datura seeds is not only related to the hard seed coat but also physiological internal inhibitors, and GA and Sulfuric acid treatments eliminate them or induce germination. Also, the interaction of GA and BAP and sulfuric acid treatments significantly affected the germination of *Datura* seeds. In the best case, 30% for GA + hormone treatment and 21% for sulfuric acid + hormone treatment, seeds succeeded in germination.

The results of previous research showed that gibberellic acid's presence affects the grain's physiological processes by affecting the cell membrane, and grains that have sufficient sources of gibberellin or soluble nitrogen compounds can germinate under suitable conditions (Chakraborty *et al.*, 2003). Gibberellin, in some cases, leads to seed germination by releasing the enzyme alpha-amylase and hydrolyzing starch (Cruz *et al.*, 2007). A study on the germination of *Datura* showed that 38% of the scratched seeds were germinated at a 200 ppm of GA (Ghadamyari *et al.*, 2011). It was also observed in *Heracleum persicum* that when the concentration of GA was higher than 100 ppm, the germination increased significantly compared to the low concentration of GA (Chiwocha *et al.*, 2005).

In *Lallemantia Iberica*, germination is increased when GA is used (Fincher, 1989). However, in cases where scratching has not been done, gibberellic acid treatment does not affect the seed germination. It has also been reported that the use of sulfuric acid and sulfuric acid + BAP is effective in the germination of *Datura* seeds. Scratching and concentrated Sulfuric acid (60 minutes) is an effective treatment for the germination of *Schizolobium amazollicum* seeds (Hartinie and Jualang, 2007). Depending on the plant species, the duration and concentration of acid treatment effective in breaking seed dormancy are varied. Studies have shown that acid treatment with a concentration of 50% is the best concentration in breaking the dormancy of *Datura* plant seeds. In the present study, we did not see high germination at higher concentrations of sulfuric acid. It is concluded that concentrated sulfuric acid, in addition to having an effective effect on thinning the seed coat, also has a harmful impact on the embryo.

Cytokinin (CK) is important in regulating various plant biological processes such as growth and development, seed germination, and the formation of shoots (Fazeli-nasab and Fooladvand, 2016). Cytokines play a key role in stimulating seed stores and using them to provide energy for embryonic growth during germination (Ćosić *et al.*, 2021; Farnsworth, 2000; Mazid *et al.*, 2011; Mohamed *et al.*, 2022; Riyazuddin *et al.*, 2020; Smýkalová *et al.*, 2019). It has been reported that the effect of the BAP hormone on germination is higher than other hormones (Adhikari and Pant, 2019; Damanik *et al.*, 2019; Fadimu *et al.*, 2012; Fazeli-Nasab, 2018). In this study, the interaction effects of GA, sulfuric acid, and scarification on MS medium + BAP medium on the germination of *Datura* seeds were investigated. It was observed that the use of these treatments improves and accelerates the germination process of *Datura* seeds. Because the effect of embryo culture and scalpel scratching was significant compared to the control, it can be concluded that the dormancy of *Datura* seeds is mainly due to the hardness of their shell. On the other hand, GA treatment is also significantly effective in breaking the dormancy of *Datura* seeds. This hormone is effective in breaking dormancy resulting from metabolic barriers. Therefore, *Datura* dormancy is

probably mainly due to mechanical barriers of the shell and, to some extent, is affected by metabolic barriers and the need for cooling.

5. Conclusion

It was observed that scratching is necessary to eliminate the dormancy of *Datura* seeds. Also, the simultaneous use of scratching + GA, sulfuric acid, and Benzylaminopurine improved the germination of the seeds of this plant. The comparison of gibberellic acid with BAP hormone and sulfuric acid with BAP hormone showed that the effect of gibberellic acid with BAP hormone on seed germination was more significant. It was also found that the use of MS medium as a substrate for seed dormancy treatment was superior to filter paper.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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