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ORIGINAL ARTICLE

Evaluation of Physicochemical Methods for Dormancy Breakage and Germination of *Datura stramonium* Seeds

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KEYWORDS

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ABSTRACT: *Datura stramonium* L. broadly performed in plant virology study has a significant seed germination difficulty due to its seed dormancy. This study was evaluated to find a simple seedling germination method intended for *D. stramonium* seeds as a traditional healthy detector plant. A factorial experiment organized as completely randomized design (CRD) in triplicate, determining results regarding seedling scarification, gibberellins (GA_3) along with potassium nitrate (KNO_3) at different concentrations from 0 to 2000 ppm. The particular germination rate along with radicle and plumule length scarified seed had been significantly affected using concentrations of GA_3 as well as KNO_3. After five days of treatment, radicle and plumule length and the percentage of germination were recorded, uncovering the real key function involving mechanical seed scarification method as being a requirement for germination of *D. stramonium*. The highest germination rate occurred at a concentration of 100 ppm GA_3 and 500 ppm for KNO_3 (63%).

INTRODUCTION

Datura stramonium from Solanaceae (Nightshade family) is an alkaloid-containing plant. The seeds of the plant are similar to tomato seeds; they are in brown colour, flat disks about 1/8 inches in diameter [1].

Potassium is an important macro-component needed in huge quantities for normal plant growth [2]. Gibberellic acid included GA_3, GA_4 and GA_7 has become shown and helped to seed dormancy behavior have been broken into several types as well as increase seed germination with several genera [3, 4]. GA_3 is an organic growth hormone which is an integral part of a variety of plant hormones named gibberellins. This hormone stimulates cellular section and improvement of bloom and their size [5].

Seed dormancy is an essential evolutionary alteration by plants that supply a mechanism for plants productive regeneration and also survive [6]. Besides, seed dormancy and inability germinative cause some problems in the proliferation of plants and plant protection. Several studies have focused on the elimination of seed dormancy in plants, the use of different treatments (or synergistic effect) including plant hormones, sulfuric acid, methanol, potassium nitrate, boiling water, chilling, leaching [7, 8]. However, plant species exhibit different reactions to some treatments. These treatments mostly require special facilities and materials which are more difficult to handle and are time-consuming. Therefore, achieving a quick and easy way to eliminate seed dormancy of plant species like

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Datura seems necessary to produce healthy seedlings. *Datura* sp. can easily find as a weed in cotton and soybean field. Nowadays many researches have been focused on biology and control of this plant in the fields. *Datura* spp. is also one of the most important medicinal plants which the leaves and seeds are used to make painkiller in the pharmaceutical industry. In epidemiological studies, extracts from *D. stramonium* were used to control citrus nematode (*Tylechulus semipenetrans*) [9], *Meloidogyne* spp. [10] and also for groundnut leaf rust (*Puccinia arachidis*) [11]. *Datura* sp. is used for the detection of the virus in plant molecular virology studies.

The main problem for germination of *Datura* sp. is seed dormancy. Regarding previous report addition of gibberellin had a positive effect on seeds of *Ferula ovina* [12]. In research by Alboresi *et al*., potassium nitrate had a role in seed dormancy in Arabidopsis [13]. In another study, the effect of gibberellic acid, potassium nitrate and scarification could break seed dormancy in *Prunus avium* [14]. The use of compounds such as gibberellin and potassium nitrate always did not show positive results to overcome dormancy and also did not increase seed germination and some other techniques such as scarification and stratification showed more effective results. The hard shell around seeds in Solanaceae family is another reason for seed dormancy in *D. stramonium* and *D. ferox* which prevents water absorption by the skin even in the final stages of germination and roots [15, 16]. On the other hands, innate seed dormancy is caused by some conditions within the seed that inhibited germination. Sometimes dormancy occurs due to the lack of oxygen or low influence and diffusion of oxygen in the seed. Another research showed that to breakage seed dormancy in *D. ferox*, water vapour for 3-4 weeks at 20 °C caused changing the endogenous inhibitor levels and provoke germination even when incubated in darkness condition [17, 18]. Also due to the latest reports the presence of inhibitors of germination in seeds which caused seed dormancy [15]. According to the previous research, using 500 ppm of gibberellic acid cause increasing 20% of germination, although using scarification increased 95% of germination from 100 to 500 ppm [19]. In another experiment, the results of using of gibberellin were similar with exposure *D. ferox* seeds to the far- red-light (FR) which promoted germination by increasing the growth of the embryo which caused endosperm softening [16, 20]. As mentioned by in latest research, gibberellin had a positive effect on seed dormancy in *D. stramonium* but another component such as potassium nitrate, sodium azide, sulfuric acid and boiling water did not affect seed germination and in some cases caused increased inhibitory due to impenetrable shell of the seed [21]. Regarding last research cyclic seasonal changes in seed dormancy were discovered in buried seeds of *D. ferox*, not in *D. stramonium* due to dormancy is alleviated during the wintertime and germinability is maximal in early spring in *D. ferox* [22]. That is a reason *D. stramonium* was selected for further research on breaking dormancy and also due to the easy access of potassium nitrate and gibberellin hormone and also the possibility of scarification of seeds in the laboratory.

Through this research, we expected to determine the effect of simultaneous treatments to overcome seed dormancy and increasing seed germination of *D. stramonium* and also to provide a simple method for the production of Datura in greenhouse condition.

MATERIALS AND METHODS

D. stramonium L. seeds were collected from around the city of Karaj in winter 2010 and uniformly were mixed. A uniform random sample of the mixture was transferred to the laboratory. To prevent infection during the experiment, the seeds were sterilized in 3% sodium hypochlorite (NaOCl) and soak for 15 min more, washed twice with sterile distilled water. Thirty treated seeds (used scarification) and thirty healthy seeds in each of the solutions contain gibberellins and potassium nitrate separately for 24 hours. The treated seeds were then kept in petri dish on Whatman 40 mm filter paper and were incubated at 20 °C for 12 hours of daylight and 12 hours of darkness [23, 24].

In this study, the effect of 5 different concentrations of gibberellin (0, 100, 500, 1000 and 2000 ppm) and 5 different concentrations of potassium nitrate (0, 100, 500,

1000 and 2000 ppm) were determined on two kinds of treatments; seed scarification (treated seeds) and healthy seeds as control group. This study was conducted using a factorial experiment in a completely randomized design (CRD) with three replications. Since none of the healthy seeds did not show germination; for statistical calculations, we took them into two factors based on a factorial experiment in treated seeds. On the fifth day after treatment, the number of germination, the percentage of germination, root and shoot length were recorded.

Statistical analysis

All the experiments were carried out in triplicate and statistical analysis of the data was performed by analysis of variance, using MSTATC software. A probability value of difference $p \leq 0.05$ was considered to donate a statistical significance.

RESULTS

The main effects of different concentrations of two chemical treatments namely gibberellin and potassium nitrate, and interaction between them on germination, length of shoot and root showed significant value. While potassium nitrate had no significant on allometry ratios (root-shoot ratio) but gibberellin had significant (Table 1).

Table 1. ANOVA for treated seeds affected by different concentrations of gibberellins and potassium nitrate

Source of variation	df	Germination (%)	Plumule length (cm)	Radicle length (cm)	Allometry ratios
Gibberellin	4	985^{**}	5.5^{**}	2.8^{**}	0.177^{**}
Potassium nitrate	4	1145^{**}	2.1^{**}	3.14^{**}	0.0146^{ns}
Gibberellin+Potassium nitrate	16	429^{**}	1.1^{**}	1.48^{**}	0.07^{**}
Error	50	18.6	0.16	0.15	0.0237
CV	-	14	15	16.7	12

*p<0.05, **p<0.01; ns: Non-significant

The use of gibberellin with potassium nitrate showed significant on germination, plumule length, radicle length and allometry ratios in comparison with the effect of each of those alone (Table 1). The highest germination of *D. stramonium* happened at a concentration of 100 ppm gibberellin and 500 ppm for potassium nitrate (63%) and the lowest germination occurred in distilled water treatment without the use of gibberellins and potassium nitrate (0%) that there was no germination found. However, increasing the amount of potassium nitrate up to 500 ppm with gibberellin was significantly increased germination percentage of *D. stramonium*, whereas the reduction in germination was due to increase in the concentration higher than 500 ppm. Gibberellin at high dose (2000 ppm) showed the highest germination rate of 38% and the lowest level of germination was obtained in the non-treated group (Figure 1).

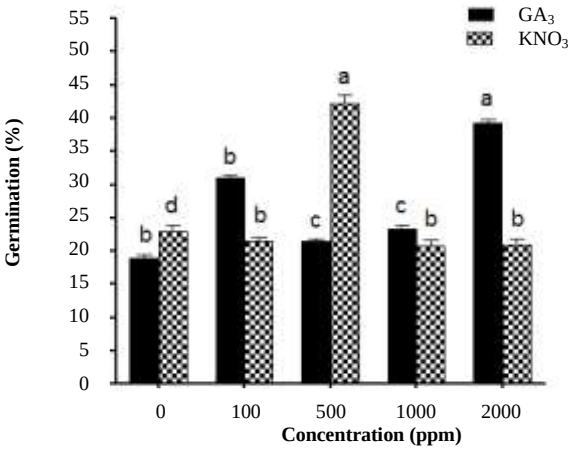

Figure 1. Mean of germination percentage of *D. stramonium* (scarified seed) after their treatment with different concentration of GA_3 and KNO_3.

The highest length of plumule and radicle at concentrations of 1000 and 2000 ppm gibberellins were 3.2 and 2.7 cm, respectively, while the lowest length of plumule at both concentrations of 0 and 100 ppm were about 2 cm. The lowest length of radicle at concentrations of 0, 100 and 500 ppm was about 2 cm. The ratio of plumule to radicle in all concentrations of gibberellins was equal to each other except at concentration 0 ppm.

From all concentrations of potassium nitrate, a concentration of 500 ppm caused to the emergence of plumule about 44% in *D. stramonium* seeds and rate of germination was between 24 to 26% (Figure 1). Changes of plumule length at all concentrations of potassium nitrate were statistically the same except at concentration of 0 ppm. The highest length of the radicle was achieved at doses of 500, 1000 and 2000 ppm of potassium nitrate and the lowest at a dose of 0 ppm (Figure 3). The ratio of plumule to radicle or allometry ratios between different concentrations of potassium nitrate was statistically significant (Table 2).

Table 2. Comparison of the mean measured traits under the influence of different concentrations of gibberellin and potassium nitrate in *D. stramonium*

GA_3 (mg/L)	Potassium nitrate	Germination (%)	Plumule length (cm)	Radicle length (cm)	Allometry ratios
0	0	0	0	0	0
	100	9.3^{k}	3^{bcd}	2.50^{bc}	1.20^{abc}
	500	20^{i}	2.50^{def}	2.70^{abc}	0.96^{cd}
	1000	16^{j}	2.30^{efg}	2.80^{abc}	0.85^{d}
	2000	17^{j}	2^{gh}	2.53^{abc}	0.83^{d}
100	0	26^{g}	1.5^{h}	1e	1.92^{ab}
	100	50^{c}	2.53^{cde}	3.06^{ab}	0.84^{d}
	500	63^{a}	2^{gh}	1.50^{de}	1.40^{abcd}
	1000	20^{i}	2.30^{efg}	2.1^{cd}	1.14^{bcd}
	2000	16^{j}	2.06^{fgh}	2.03^{cd}	1.14^{bcd}
500	0	16^{j}	1.96^{gh}	1^{e}	2^{a}
	100	53.6^{b}	2^{gh}	1.23^{e}	1.71^{abc}
	500	40.3^{d}	2.50^{def}	2.03^{cd}	1.26^{abcd}
	1000	23^{h}	2.83^{bcd}	3^{ab}	0.94^{cd}
	2000	33.3^{f}	3.53^{ab}	2.50^{bc}	1.41^{abcd}
1000	0	40.5^{d}	3.20^{abc}	2.70^{abc}	1.18^{abcd}
	11	43^{d}	3.30^{abc}	2.60^{abc}	1.27^{abcd}
	500	24^{gh}	3.53^{ab}	3^{ab}	1.18^{abcd}
	1000	32^{f}	3.20^{abc}	2.50^{bc}	1.32^{abcd}
	2000	23.7^{gh}	3^{bcd}	3.30^{a}	0.92^{cd}

	0	40.6[d]	3.10[abc]	3[ab]	1.06[bcd]
	100	22.5[h]	2.56[cde]	2.03[cd]	1.37[abcd]
2000	500	26[g]	3.83[a]	3[ab]	1.33[abcd]
	1000	37.3[e]	3.53[ab]	3.13[ab]	1.15[abcd]
	2000	36[e]	3[bcd]	2.50[bc]	1.25[abcd]

The highest length of plumule was achieved at a concentration higher than 500 ppm gibberellin but in all concentration of potassium nitrate was observed (3.1 to 3.8 cm). The lowest length of plumule was found at the lower concentration of 500 ppm in all concentrations of potassium nitrate (Figure 2).

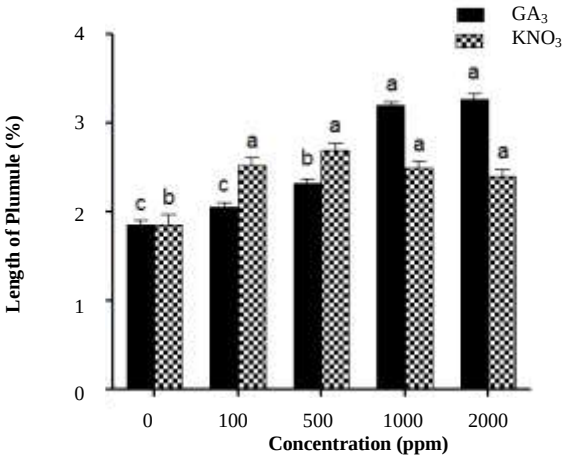

Figure 2. The effect of different concentrations of GA_3 and KNO_3 on the length of plumule

The highest length of radical (2.5 to 3.3 cm) was obtained at concentration 0 and above 500 ppm gibberellin with all concentrations of potassium nitrates (Figure 3).

The maximum allometry ratios or plumule to radicle length ratio (1.15 to 1.4) was observed at concentration 100 ppm and above of gibberellin included all concentrations of potassium nitrate (Figure 3). One day after treatment, the scarified seeds with different concentrations of gibberellin and potassium nitrate began to germinate; for non-scarified seeds, any different concentrations of gibberellin and potassium nitrate did not affect seedling emergence, then for statistical calculations were not included.

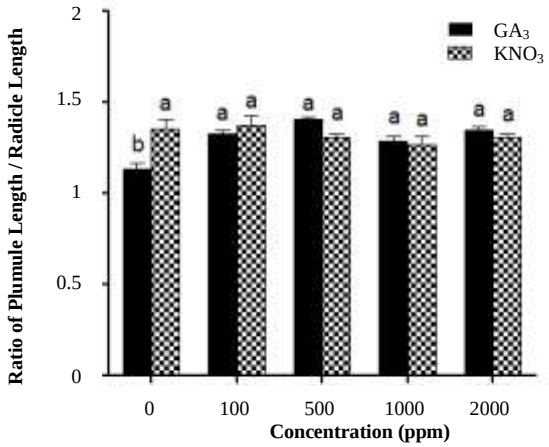

Figure 3. The effect of different concentrations of GA_3 and KNO_3 on the plumule to radicle length ratio

DISCUSSION

Datura seed coat is a solid barrier against the influence of foreign substances into the seed and also out of growth-inhibiting substances from the seed. The same results were found in *Tilia platyphyllos* [10], *Rubia tinctorum* [25]. On the other hand, no germination was observed in the control group, but the use of GA_3 and KNO_3 promoted dormancy break in treated seeds. However, another study exhibited that gibberellic acid did not affect on seed dormancy of *Rubia tinctorum* but may cause an increase in root and shoots during germination. In contrast, scarification exhibited an increased rate of germination (83%). The same result was obtained by Khaninejad *et al*. on breaking the dormancy of Caper (*Capparis spinosa*) [26], and *Alstroemeria ligtu* hybrid seeds [27].

Research results have shown that *D. stramonium* and *D. ferox* showed high sensitivity to the light; also vegetation and some other techniques can be affected by seed dormancy [28]. Although the penetration of light into the soil at a depth of 4 cm decreasing about 0.01% but still this less amount of light would be irritating Datura seed germination [29]. Light is the main ecological factor that regulates germination [30]. The condition of darkness can be one of the major reasons for seed dormancy in Datura (in non-scarification seeds) and even the seeds are kept in dry condition in the darkness for 24 months; the seed will be still in dormancy mode [17]. The poor environmental condition for mother plants promotes some changes in dormancy seeds. Another study revealed that light reduction and water stress during ripening seeds in *D. ferox* caused a decrease in the degree of dormancy due to lower level of hormones (or basic fraction inhibitors) related to inhibiting germination [18].

Based on these results, lack of Datura seed germination probably was not related to the hard seed coat, but also some physiological factors such as internal inhibitors using GA_3 and KNO_3 help to remove dormancy or induced them. It should be noted that some of the seeds after applying treatments on them did not germinate and most desirable condition for germination percentage was only 63%. Previous studies exhibited that compounds contain nitrate and gibberellins could effect on the cell membrane and affected grain by physiological processes. Seeds contain sufficient resources of gibberellins and/or soluble nitrogen compounds can easily induce germination [23]. However, other nitrogen compounds are effective to stimulate seed germination [31]. After applying scarification, the effect of prechilling around 3 and 6 months on germination including using gibberellin hormone showed germination on *T. platyphyyllos* seeds [10], and the plants from treated seed by gibberellin exhibited high germination rate than other treated seeds. However, GA_3 concentrations would help in increasing breakage of seed dormancy and germination rate [32]. In the present study, the combined use of these two chemical substances on seed scarification had significant results which demonstrate the synergistic effect to stimulate germination of *D. stramonium.* Previous studies have shown that gibberellin and potassium nitrate had significant effects together on *Avena fatua* [33], *Papaver dubium* and *P. Rhoeas* L. [34].

The rapid increase in plumule and radicle length cause accelerate the germination and better establishment of seedlings which is very important for researches related to medicinal plants and weed plants [35]. In this study, using different concentrations of GA_3 and KNO_3 significantly affect the uniformity of radicle and plumule, but no significant trend found.

CONCLUSIONS

According to this study, to stimulate germination of Datura seeds, using the mechanical method like scarification for seed coats; it can be a prerequisite for the effectiveness of gibberellins and potassium nitrate as chemical treatments. The simultaneous use of gibberellic acid and potassium nitrate concentrations between 100 to 500 ppm on treated seeds of *D. stramonium* given the best results in germination and elongation of plumule and radicle seedlings and it will cause to accelerate the germination.

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