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ORIGINAL ARTICLE

The Role of Gibberellic Acid, Sulfuric Acid and Cold Stratification on Seed Germination Parameters of Black Henbane (*Hyoscyamus niger* L.)

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ABSTRACT
One of the main problems that prevent sustainable use of medicinal plants, native to the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions or when cultivation is attempted. Black henbane seeds have low germination rate even under normal laboratory conditions. In this study, seeds of black henbane were soaked in GA_3 at four different concentrations (100, 150, 200 and 250 ppm) for 48 hours, and in H_2So_4 1% at 5 and 10 minute along with GA_3 at 250 ppm, and two duration of cold stratification (30 days and 60 days stored in a refrigertor at 2 °C) for seed breaking dormancy of three locations (Karaj, Kermanshah, Kandovan) with different latitude. Results showed that GA_3 stimulated the germination of all seeds. The highest germination percentage (95%) was recorded at 250 ppm GA_3 for Kermanshah and Karaj sites. In all seeds increasing the concentration of GA_3 up to 250 ppm and increasing the duration of soaking in H_2SO_4 from 5 min to 10 min, improved significantly both the germination percentage and rate. Seed treatment with cold stratification at 2 °C for 30 days was the best treatment only for germination percentage of Kandovan seeds, but this result was not observed in seeds those were collected from two other sites. The highest germination percentage (95%) and germination rate (0.17 seeds per day) and also the lowest mean germination time (5.68 day) was recorded in the concentration of 250 ppm GA_3 for seeds from Karaj site.
Keywords: *Hyoscyamus niger, Germination, Cold stratification, Gibberellic acid, Sulfuric acid.*

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INTRODUCTION

Seedling establishment is a critical stage in the life history of any plant species that relies on sexual reproduction for the persistence of its populations. Black henbane (*Hyoscyamus niger*) a species in Solanaceae family has long been used as a medicinal plant. This plant is a coarse, foul-smelling, and very hazardous herb and all parts are poisonous. Today, it has been cultivated as a medicinal plant and crop for drag companies' worldwide [1].

One of the main problems that prevent sustainable use of medicinal plants, native of the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions [2]. Undoubtedly medicinal plants are also affected by environmental factors. Germination characteristics in a single species can show local conditions. Besides, environmental factors play an important role in the derivation of species from each other and are responsible for genetic divergence.

Germination is a critical stage in the life cycle of the plants, and often controls population dynamics, with major practical implications [3]. Control of seed germination and growth is crucial to the survival of the next generation, and there are critical checkpoints at the transitions from dormancy to germination and from germination to growth. Generally germination rate of a species like black henbane is very low due to seed dormancy has been widely studied but the regulatory principles behind changes in several types of dormancy remain [4]. Nevertheless, plant growth regulators such as gibberellic acid (GA_3) and chemical substances such as sulfuric acid (H_2So_4) can be used to breaking seed dormancy [5].

Previous study on the germination of the seeds of *H.niger* was focused on the influence of growth regulators. The study revealed that gibberellic acid improved seed germination in this plant when compared to cold stratification [6]. Limited information is available on seed germination behavior of black henbane, other than descriptions of the alkaloids it contains. So, the purpose of this study is to

determine the effects of exogenous application of GA_3 in different concentrations, H_2So_4 treatment and two duration of cold stratification on breaking dormancy of black henbane seeds of three different locations in Iran (Karaj, Kermanshah, Kandovan).

MATERIALS AND METHODS

Seed Collection

The henbane seeds were collected from three different sites in Iran, Kermanshah, Kandovan and Karaj, which are located at 2200, 1900 and 1300 meter altitude as above sea level, respectively (Fig 1). After collection, immature seeds and those damaged were removed.

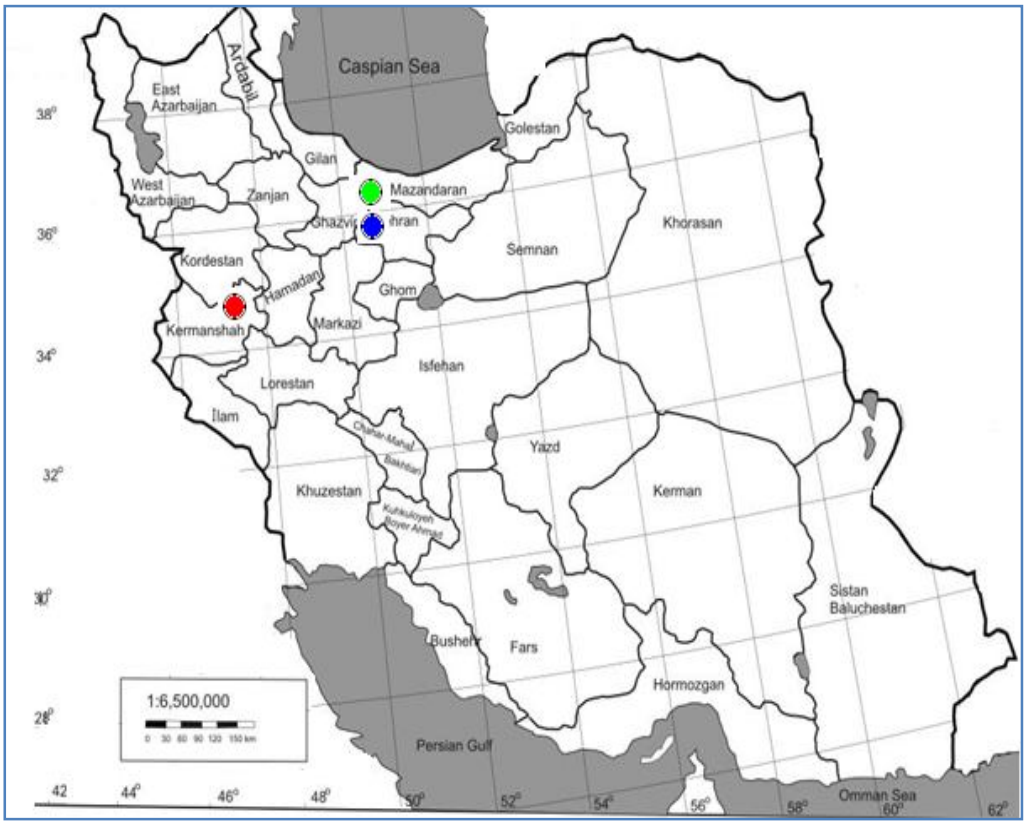

Fig 1. Map of three studied *hyoscyamus niger* seed collection sites in Iran (Kermanshah, Kandovan and Karaj, which is shown with green, yellow and pink colour, respectively).

Seed Germination

The seeds of *H. niger* were surface-sterilized in 70% ethanol for 2 min and then in 25% commercial bleach (containing 6% sodium hypochlorite) for 10 min and finally rinsed with sterile distilled water prior to germination tests and applying any treatment. All treatments consisted of three (n=3) replicates with 20 seeds in each. The seeds were placed on two layers of Whatman No.1 filter paper in disposable plastic Petri dishes (9 cm) moistened with 5ml of distilled water in sterilized Petri dishes.

Chemical scarification: Seeds of *H. niger* were soaked in GA_3 at four different concentrations (100, 150, 200 and 250 ppm) for 48 hours, and in H_2So_4 1% at 5 and 10 minute along with GA_3 at 250 ppm, and then washed thoroughly by distilled water, before transfer to the germination test process.

Cold stratification: Seeds after moisturized with distilled water were maintained at two duration of cold stratification (30 days and 60 days stored in a refrigertor at 2 °C).

Germination was recorded daily and was considered complete once the radicle protruded about 2 mm in length. The experiments were continued for 14 days. The germination percentage (G%), germination rate (GR) and Mean germination time (MGT) were calculated according to the following formulas [7,8]:

$$\text{Germination (\%)} = \frac{\text{Number of germination seed}}{\text{Number of viable seeds initiated}} \times 100$$

$$MGT = \frac{\sum TiNi}{S}$$

Where Ti is the number of days after beginning of experiment, Ni the number of seeds germinated on day i and S the total number of seeds germinated.

$$GR = \sum_{n=1}^{n} (\text{Number germinating since-1})/n$$

Where, n is the days.

Statistical Analysis

Data were processed by the analysis of variance (ANOVA) on the basis of completely randomized design (CRD) with 3 replications. The data were analyzed using computer SAS software (version 9.1; CoHort Software), and the means were compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

In our current experiment, application of GA_3 stimulated the germination of all seeds from different collection sites, when compared to the control treatment. This response was dependent on the concentration of applied GA_3. At the lower GA_3 concentrations (100 and 150 ppm) germination percentage of seeds was lower than that of higher concentrations (200 and 250 ppm). The highest germination percentage (95%) was recorded at 250 ppm GA_3 for Kermanshah and Karaj sites (Table 1, 2 and 3).

In three *H.niger* site seeds increasing the concentration of GA_3 up to 250 ppm and increasing the duration of soaking in H_2SO_4 from 5 min to 10 min, improved significantly both the germination percentage and germination rate.

Seed treatment with cold stratification at 2 °C for 30 days was the best treatment only for germination percentage of Kandovan seeds, but this result was not observed in seeds those were collected from two other sites. The highest germination percentage (95%) and germination rate (0.17 seeds per day) and also the lowest mean germination time (5.68 day) was recorded in the concentration of 250 ppm GA_3 for seeds from Karaj site (Table 3).

GA_3 is widely used to break dormancy of seeds of various plant species. It is reported that dormant seeds which require chilling, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy [2]. Washing and chilling are standard procedures which have been used to enhance the germination of dormant seeds [9]. Chilling at 2 °C for a period of 30 days resulted in the highest germination rate and percentage of Kandovan seeds (Table 1). In seeds from Kermanshah and Karaj sites, both germination rate and percentage of seeds were increased by increasing the number of exposure days at 2°C. However, when the number was increased from 30 – 60 days, a negative response was monitored for germination percentage of Kandovan seeds.

Results revealed that the seed germination behavior of Kandovan region (altitude; 1900 m) was slightly different compared to other two sites. The response to acid (H_2So_4) scarification was stronger when GA_3 was combined, which suggests a synergist response. The lowest germination percentage was determined in control untreated seeds.

GA_3 is one of the hormones proposed to control primary dormancy by inducing germination [10]. The effect of GA_3 as a germination promoter is hypothesized to increase with chilling treatment.

It is well known that acid scarification and cold stratification have been used successfully to overcome dormancy in mountain snowberry and related species. Gibberellic acid has been found to substitute for both warm stratification and cold stratification requirements in numerous species [11].

Endosperm weakening is also known to be required for radicle protrusion in Solanaceae species [12]. GA_3 might enhance the growth potential of the embryo [13] or induce degradation of food reserves in endosperm endosperm by stimulating hydrolytic enzyme activity [14] of *P. olympica* seeds. In nature, some of the ungerminated seeds may remain alive and serve as a soil seed bank, waiting for more favourable conditions. This strategy may be common among high-elevation wetland plants [15]. Li et al. [16] reported that GA_3, scarification and surface sowing promoted germination in the eight *Pedicularis* species they studied and suggested that allowing sufficient time for germination may be useful for germination under natural conditions.

Table 1. Seed germination (%), germination rate (seeds per day) and mean germination time (MGT, day) under employed treatments in *H. niger* for Kandovan site.

Dormancy breaking treatments	Germination (%)	Germination rate (seeds per day)	MGT (day)
GA_3 100ppm (48 h)	75^{d}	0.10^{b}	9.76^{d}
GA_3 150ppm (48 h)	75^{d}	0.07^{e}	13.06^{a}
GA_3 200ppm (48 h)	80^{c}	0.07^{e}	12.76^{b}
GA_3 250ppm (48 h)	85^{b}	0.11^{ab}	8.8^{e}
H2SO4 1%, 5min+GA3 250ppm, 48 h	70^{e}	0.08^{d}	10.35^{c}
H2SO4 1%, 10min+GA3 250ppm, 48 h	85^{b}	0.09^{c}	11.08^{c}
cold stratification (2^0C, 30 days)	95^{a}	0.10^{b}	9.23^{d}
cold stratification (2^0C, 60 days)	65	0.10^{b}	9.98^{d}
control	30^{f}	0.12^{a}	8.33^{e}

Different letters indicate significant differences in each treatment as determined by Duncan test at P = 0.05.

Table 2. Seed germination (%), germination rate (seeds per day) and mean time germination (MTG, day) under employed treatments in *H. niger* for Kermanshah site.

Dormancy breaking treatments	Germination (%)	Germination rate (seeds per day)	MGT (day)
GA_3 100ppm (48 h)	65^{f}	0.12^{b}	8.15^{d}
GA_3 150ppm (48 h)	75^{d}	0.11^{c}	8.5^{d}
GA_3 200ppm (48 h)	85^{c}	0.11^{c}	8.47^{d}
GA_3 250ppm (48 h)	95^{a}	0.14^{a}	7.01^{e}
H2SO4 1%, 5min+GA3 250ppm, 48 h	70^{e}	0.09^{d}	10.07^{c}
H2SO4 1%, 10min+GA3 250ppm, 48 h	90^{b}	0.14^{a}	7.11^{e}
cold stratification (2^0C, 30 days)	55^{g}	0.07^{e}	13.51^{a}
cold stratification (2^0C, 60 days)	60^{g}	0.08^{e}	12.01^{b}
control	30^{h}	0.12^{b}	8.33^{d}

Different letters indicate significant differences in each treatment as determined by Duncan test at P = 0.05.

Table 3. Seed germination (%), germination rate (seeds per day) and mean time germination (MTG, day) under employed treatments in *H. niger* for Karaj site.

Dormancy breaking treatments	Germination (%)	Germination rate (seeds per day)	MGT (day)
GA_3 100ppm (48 h)	75^{c}	0.11^{d}	8.4^{b}
GA_3 150ppm (48 h)	75^{c}	0.11^{d}	8.9^{b}
GA_3 200ppm (48 h)	85^{b}	0.17^{a}	5.82^{d}
GA_3 250ppm (48 h)	95^{a}	0.17^{a}	5.68^{d}
H2SO4 1%, 5min+GA3 250ppm, 48 h	70^{d}	0.13^{c}	7.64^{c}
H2SO4 1%, 10min+GA3 250ppm, 48 h	75^{c}	0.15^{b}	6.5^{c}
cold stratification (2^0C, 30 days)	65^{e}	0.08^{e}	11.51^{a}
cold stratification (2^0C, 60 days)	75^{c}	0.09^{e}	11.02^{a}
control	30^{f}	0.12^{cd}	8.33^{b}

Different letters indicate significant differences in each treatment as determined by Duncan test at P = 0.05.

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