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Original Contribution

A COMBINATION OF CHEMICAL SCARIFICATION AND 6-BENZYLAMINOPURINE (BAP) TREATMENT PROMOTE SEED GERMINATION IN *DRACOCEPHALUM KOTSCHYI* SEEDS

K. Moradi, M. Otroshy*

Agricultural Biotechnology Research Institute, Center of Iran (ABRII), Isfahan, Iran

ABSTRACT
Dracocephalum kotschyi Boiss. is a herbaceous wild medicinal plant native to Iran. Due to high degree of seed dormancy, seeds always show a low germination rate. In this study, different treatments were compared to overcome seed dormancy. Different chemical scarifications, including dilution in concentrated sulfuric acid for 15 minutes, culturing seeds in gibberelic acid, 6-benzylaminopurine (BAP) and Kinetin, chilling at 4°C for 1, 2, 3 and 4 weeks and 25°C for 4 weeks were studied. Results showed that chemical scarification treatment using sulfuric acid for 15 minutes and applications of BAP hormone were efficient in promoting seed germination.

Key words: *Dracocephalum kotschyi*; Dormancy; Chilling; Seed germination; Chemical scarification

INTRODUCTION

The *Labiatae* family (*Lamiaceae*) is one of the largest and most distinctive families among flowering plants, with about 220 genera and almost 4000 species worldwide. This family is best known for their essential oils and many biologically active essential oils have already been isolated from its various members (1, 2). Moreover, this family is also well known for production of diterpenoids. Seed dormancy is a mechanism that insures the survival of the species.

Many seeds fail to germinate after processing and placement in favorable growing conditions. Such seeds are said to be dormant. In some dormant seeds morphological changes must take place before germination can start, (3). Seed dormancy is a nuisance in propagation programmers and removing or breaking dormancy has always been a challenge for plant physiologists, (4). There are different methods to overcome seed dormancy, for instance; acid treatments are often used to break down especially thick impermeable seed coats. Since seeds placed in concentrated sulfuric acid (H2SO4) will become charcoal in time, the temperature of the acid and duration of which the seeds are soaked are very important, (5).

Among different hypotheses which have so far been proposed to describe the mechanism of seed dormancy, the hormone-balance theory and the metabolic theory, (6) have received more attention. According to this theory, dormancy is controlled by the combine action of different inhibitors (ABA), and activators (gibberellins, cytokinins and ethylene). Study has shown that application of exogenous gibberellins has proved to be effective in breaking seed dormancy and in substituting the requirement of cold stratification in many seeds, (7). Moreover, gibberellins stimulate seed germination and reverse the effects of ABA (8, 9).

The *Dracocephalum kotschyi* seeds have a long dormancy. In natural condition, the seeds generally after approximate 5-6 weeks germination in natural. This present study was undertaken to investigate the role of GA3 in breaking *Dracocephalum kotschyi* dormancy.

***Correspondence to**: *Mahmoud Otroshy, Agricultural Biotechnology Research Institute, Center of Iran (ABRII), P.O. Box 85135-487, Isfahan, Najaf abad, Iran, tel. 00983312234694, 00989133687622, E-mail: otroshy@yahoo.com*

MATERIALS AND METHODS

The experiment was conducted at the Tissue Culture Lab of the Agricultural Biotechnology Research Institute of Iran in 2008. Seeds were obtained from the Collection of the shahid Fozveh Agricultural Research Center.

The seeds were washed with tap water for 5-10 minutes to remove surface contamination to further minimize the risk of infestation by fungi and bacteria, then sterilized by immersing in 70% ethanol for 1 minute with shaking followed by 20 minutes in 20% sodium hypochlorite containing one drop of Tween 20. The seeds were then rinsed three times with sterile distilled water in a laminar flow cabinet to remove minor amounts of disinfection liquid.

The germinated seeds were transferred to the Petri dish containing a standard MS medium, (10) containing 17 different hormone treatments, concentrated sulfuric acid and one chilling treatment on 28 days were applied, Hormone treatments included GA3(0-5 and 15 mg/l) plus combinations or BAP(0- 0/2 and 0/5 mg/l), KIN (0- 0/2 and 0/5 mg/l), 3% (m/v) sucrose and 0.7% (m/v) agar.

Cultures were incubated in a growth chamber at 24°C, 16 h photoperiod provided and with a light intensity of 2000 lux using, white fluorescent lamps.

To apply chilling treatments, petri dishes containing seeds were placed in a dark refrigerator at 5° C for 1, 2, 3 and 4 weeks. Then petri dishes were transferred to a growth chamber at 24° C. Germination rates were recorded 2, 3 and 4 weeks after transferring them to a 24° C growth chamber.

The experiment was conducted as a completely randomized factorial design with 5 replications. Data were statistically analyzed using SAS software statistical computer program and Duncan's Multiple Range Teats was used to compare the Mean values.

RESULTS

Effects of Chemical Scarification on Seed Germination:

The effect of acid scarification on increasing seed germination was found significant ($P<0.05$). In principle, treatment with sulphuric acid was effective in breaking the seed dormancy. Seeds soaked in concentrated acid for 15 min resulted in the highest germination of 89%. However, prolonged treatment decreased the rate of seed germination and the layer around the seeds was destroyed. The fact that concentrated sulphuric acid gave the highest percentage of germination within 15 min indicates that the more rapidly the seed coat is ruptured the faster the rate of germination. However prolonged emersion may harm the seeds as the acid can rapture vital parts of the embryo.

Effects of Chilling on Seed Germination:

Chilling treatment (5°C) data suggested no significant ($P<0, 05$) influence on germination rate. In this experimenet that showed Chilling treatment (5°C) had no effect on germination rate.

Effects of Gibberellic Acid and Hormones Seed Germination:

Different GA3 concentrations had a significant ($P<0, 05$) effect on breaking dormancy and the rate of seed germination. The different days and concentration after planting it was observed that the percentages of sprouting, was higher of GA3 15 mg/l **(Table 1)**. The higher sprouting rate was showed in MS medium plus BAP 0.2 mg/l **(Table 1 and 2)**.

GA3 and BAP were showed best treatment than the others plant regulation when they were in 24°c after 28 days **(Table 3)**.

DISCUSSION

This experiment showed, chemical scarification the percentage of germination was significantly different at all treatments of breaking dormancy. The maximum number of germinated seed was found with concentrated sulphuric acid within 15min after that BAP and gibberellic acid treatment was showed the maximum number of germinated seed. Two types of seed dormancy have been recognized, coat imposed dormancy and embryo dormancy, (11). Coat-imposed dormancy is dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extra floral organs. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues are either removed or damaged. From the investigations carried out, such treatment as soaking in application of sulphuric acid were found to induce germination of seeds of *Rubia tinctorum*. Auxins and ethylene, which could increase nucleic acid metabolism and

protein synthesis (13, 14). Softening the seed coat by means of imbibing in concentrated sulphuric acid greatly enhance the germination percentage of the seed (12, 11).

Table 1. *The effect of different hormones and acid scarification application on germination and seedling characters in Dracocephalum kotschyi*

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
GA_3 5 mg/l	83.22 a	1	2.2
GA_3 15 mg/l	86.22 a	1.2	2.8
Kin 0.2 mg/l	85.22 a	1	2.5
BAP 0.2 mg/l	87.88 a	1.8	3
GA_3 5 mg/l + Kin 0 mg/l	75.80 a	1	1.8
GA_3 5 mg/l + Kin 0.2 mg/l	73.02 a	1	1.8
GA_3 5 mg/l + BAP 0.2 mg/l	81.40 a	1.1	2
GA_3 15 mg/l + BAP 0 mg/l	72.13 a	0.5	1.5
Kin 0.5 mg/l + BAP 0 mg/l	75.87 a	0.8	1.5
Kin 0 mg/l + BAP0.2 mg/l	73.93 a	0.6	1.5
Cold stress for 1, 2, 3 and 4 week	0	0	0
Acid scarification for 15min	90 a	3	5
Control	20 b	1	1

Mean values followed by the same letter(s) are not significantly different ($p \leq 0.05$)

Table 2. *Mean comparison of BAP concentrations*

Concentration of BAP	Mean of germinated seed (%)
0.00 mg/l	33.78 f
0.20 mg/l	87.89 a
0.50 mg/l	81.89 b

Mean values followed by the same letter(s) are not significantly different ($p \leq 0.05$)

Table 3. *The effect of hormones on percentage of additional germination at different intervals during germination without chilling*

	germination		
Treatments	2 nd week	3 rd week	4 th week
GA_3 5 mg/l	83.22 a	76.32 b	38.00 abcde
GA_3 15 mg/l	86.22 a	82.33 b	44.89 abcd
Kin 0.2 mg/l	85.22 a	79.67 ab	66.22 abcde
BAP 0.2 mg/l	87.88 a	83.11 bcd	55.44 f
GA_3 5 mg/l + Kin 0 mg/l	75.80 a	70.00 ef	62.67 fg
GA_3 5 mg/l + Kin 0.2 mg/l	73.02 a	64.33 f	49.33 fg
GA_3 5 mg/l + BAP 0.2 mg/l	81.40 a	76.67 b	46.00 cd
GA_3 15 mg/l + BAP 0 mg/l	72.13 a	64.33 ab	46.67 cd
Kin 0.5 mg/l + BAP 0 mg/l	75.87 a	57.00 cd	48.00 cde
Kin 0 mg/l + BAP0.2 mg/l	73.93 a	70.00 ab	50.00 cde

Mean values followed by the same letter(s) are not significantly different ($p \leq 0.05$)

Clued sulphuric acid treatment was the best treatment in seed dormancy of *Dracocephalum kotschyi*, the optimum period for acid scarified seed germination was 15 min (89%),the others thus found sulphuric acid for 15 min to set forth best treatment to decreases germination, (12) but, Veasey and Teixeira de (2002) that showed Concentrated sulphuric acid for 40 min provided the best results for breaking seed dormancy in all three species Sesbania, that this examination germination decreases when

seeds were stayed in acid for more than 15 min, (12).

Chilling treatment in this experimentation was showed unimpressive for seed break dormancy, but others investigation told chilling indicated that *Rubia tinctorum* L. seed germination significantly increased with pre-germination chilling for 6 week (47%) and 8 week (76%), (12).

In this manner GA3 treatment with Hormones were effective treatments in break dormancy seeds. In this test BAP and GA3were best treatment, the other certain also were reporter; (15, 16) reported that maximum sprouting of garlic was obtained after GA3 treatment.

The results obtained from this investigation exhibited influence of GA3 on the sprouting and other early growth behaviors of garlic and gave an indication of breaking dormancy in garlic by the application of GA3.

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Abbreviation: GA3: Gibberellic Acid, BAP: 6-benzylaminopurine, KIN: kinetin
Sprouting