Trakia Journal of Sciences, No 1, pp 82-87, 2013
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Available online at:
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ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

SEED GERMINATION AND DORMANCY BREAKING TECHNIQUES FOR *DUCROSIA. ANETHIFOLIA* (DC.)

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ABSTRACT
Ducrosia. Anethifolia DC. (Apiaceae), endemic in Iran, Afghanistan and Pakistan is an important medicinal plant. Public usage of medicinal plant is restricted in order that they are wild and must be domesticated but, there are limitations for germination under laboratory conditions. Seeds of Apiaceae family have long period of dormancy and seeds of this species show poor germination. The aim of this project was to study the effects of different chemical and physical factors as running water, temperature and GA_3 (1000, 2000 and 3000 ppm) at 23±2˚C and 4˚C, scratching (seed pre-treatment with H_2SO_4 (50%) at 23±2˚C alone and in combination with GA_3 (1000-2000 ppm) on germination and seed dormancy breaking on filter paper and sand for 2 months. These seeds presented low germination that was due to the high degree of seed dormancy. Different methods to overcome seed dormancy were compared. The efficacy of chilling, acid scarification, and GA_3 treatments in germination improvement was tested. The most effective treatments in seed germinations of *D. Anethifolia* were 14 days running water. And chemical scarifications (H_2SO_4) in combination with GA_3 were efficient in promoting germination. Also, GA_3 with chilling was more effective than GA_3 alone. Socking in H_2SO_4 (50%) 3 min, running water 24h and control did not show any beneficial effect upon germination of *D. Anethifolia*.

Key words: *Ducrosia. Anethifolia*, seed germination, dormancy breaking, medicinal plant.

INTRODUCTION

Dormancy is a natural reaction for encountering with bad condition in nature that has caused various species conserves in especial environments. *Ducrosia. Anethifolia* (DC.) of family Apiaceae is biennial or perennial forbs that have a restricted distribution, mainly in Afghanistan, Iran, Iraq and Pakistan. In traditional medicine, it is used to treat catarrh, headache and backache. The aerial parts of *D. Anethifolia* demonstrated activity against a panel of fast growing mycobacterium (1). Seeds of *D. Anethifolia* (DC.) have long period of dormancy.

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Generally the germination capacity of Apiaceae family is very low due to seed dormancy (2, 3, 4, 5).

Unfortunately, there is limited information concerning the potential seed dormancy problems of for *D. Anethifolia*. We evaluated the effect of temperature, addition of gibberellic acid (GA_3), H_2SO_4 (50%) and running water on the germination of seeds of *D. Anethifolia* in order to provide information about germination requirements which could be use for propagation.

A period of chilling (stratification) relieves primary dormancy of many northern hemisphere species (6). Dormancy is efficient mechanism to insure the existence of the species.

Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination (7). The effect of GA_3 as a germination promoter is hypothesized to increase with chilling treatment (2). Chilling also plays an essential role in providing the motive required to conquer dormancy. Chilling has been reported to induce an increase in GA_3 concentration (8). The goal of this project was to define treatments which can motivate and increase germination of an important medicinal plant in Iran.

MATERIAL AND METHODS

1. Source of Seeds

The mature seeds of *D. anethifolia* were collected in July 2007 from a biological reserve in the nearby county of Karaj of Iran. Altitude ranges 1360 m above sea level and the climate is typically Mediterranean (annual mean temperature of 14.1°C and an annual total rainfall of 251 mm).

The seeds were dried under sunlight. Next, immature and incomplete seeds were separated. They were gathered in bag. After that, the seeds were surface sterilized by soaking in 70 % alcohol from 30s to 1 min, and immediately soaked in 2.5 % sodium hypochlorite (NaOCl) for 3 min. They were rinsed thoroughly with double sterilized water before applying any treatment. Germination experiments were tested using three replications of 25 seeds per each treatment.

2. Experiments' Procedure

Before germination experiments, the seeds of *D.anethifolia* were exposed to various physico-chemicals and hormonal treatments as follows:

Rinsing with running water: Seeds were soaked in tap water for periods of (24 and 72) h, (7and 14) days thereafter, transferred to the germination test process.

Acid scarification: Seeds of *D. anethifolia* were treated with H_2SO_4 (50% v/v) for varying durations. Thus, 1, 3 min treatments were given; and after that, washed thoroughly by distilled water, before transfer to the germination test phases.

Usage of GA_3 treatment: seeds were soaked in 1000, 2000 and 3000 ppm for 48 h and 72 h.

Composite treatment

Chemical scarification and GA_3 treatment: Seeds were put in H_2SO_4 (50% v/v) for 1 min and then washed wholly by distilled water pursued by soaking in 1000 and 2000 ppm GA_3, for periods of 48 h. and 72 h.

Cold stratification and GA_3 treatment: seeds were held at moist sand at temperature of 5ºC, for 60 days next, soaked in GA_3 (1000, 2000, and 3000 ppm) for 48 h and 72 h.

After any treatment, seeds were located on 15 cm sterilized Petri dishes containing double layered Wathman No.1 filter papers wetted with 10 ml of double sterilized water. Afterward, they were incubated at 22–24 °C with a 16/8 h (light/dark) photoperiod in a growth room fitted with a light intensity of 8075 m mol m^{-2} s^{-1} provided by cool white fluorescent lamps.

Treated seeds were put away to germinators with proximate darkness, steady temperature of 25°C and relative humidity between 70% and 75%. Germinated seeds were counted and transferred every week for 60 days after each treatment. A seed was scored as germinated when the tip of the radical had grown free of the seed coat. There was a treatment as control that there was no treats on it.

3. Data analysis

The germination rate was calculated as follows

$$\text{Germination rate: } = \sum_{n=1}^{60} \left(\frac{Gt}{Wt}\right)$$

Gt is the number of germinated seeds after per week. (Wt)

Percentage of seed germination was computed according to the following equation (Maliro and Kwapata 2000)

$$\text{Percentage of germination} = \left(\frac{G}{N}\right) \times 100$$

G= the number of germination seed

N= number of total seeds

Data were analyzed statistically using MSTATC software. The data were calculated using a randomized complete design with 3 replications and the Duncan test with probability of $P<0.05$.

RESULT

The attached result of different treatments in percentages germinations and rates in *D. Anethifolia* is demonstrated in the Table 1. There was a significant difference in germination percentages and rates of *D. Anethifolia* among various treatments. No germination was recorded by soaking in H_2SO_4 (50%) for 3 min and running water for 24 hours where germination was failed. In H_2SO_4 (50%) for 1 min, germination percentage was 25.33% but, treatment soaking in the H_2SO_4 (50%) for 3 min failed to germination that may be due to embryos destruction. Seed germination of *D. Anethifolia* was significantly affected by running water treatments. The most effective treatments in breakage of seed dormancy were running water for 2 weeks. Increasing running water time increased both germination percentages and rates.

In this experience application of GA_3 enhanced the germination of *D. Anethifolia*. Among three concentrations of the GA_3, concentration 3000 ppm had the higher germination percentage than 1000 ppm and 2000 ppm. There weren't any significant difference ($p<0.05$) between concentrations 1000 ppm and 2000 ppm of GA_3. **(Table 1)**

Table 1. *Appropriate treatments for seed germination and dormancy breaking in Ducrosia. Anethifolia*

Dormancy breaking treatments	Germination (%)	Germination rate (Seeds per day)
GA_3 1000 ppm (48 h)+cold Stratification (60 d)	20^{c-g}	0.19^{d-g}
GA_3 1000 ppm (72 h)+cold Stratification (60 d)	20^{c-g}	0.23^{d-g}
GA_3 2000 ppm (48 h)+cold Stratification (60 d)	8.33^{gh}	0.07^{g}
GA_3 2000 ppm (72 h)+cold Stratification (60 d)	45^{ab}	0.52^{b}
GA_3 3000 ppm (48 h)+cold Stratification (60 d)	28^{b-e}	0.33^{b-f}
GA_3 3000 ppm (72 h)+cold Stratification (60 d)	13^{e-h}	0.29^{b-f}
GA_3 1000 ppm (48 h)	4.76^{h}	0.08^{g}
GA_3 1000 ppm (72 h)	8.57^{d-h}	0.16^{e-g}
GA_3 2000 ppm (48 h)	7.61^{f-h}	0.15^{e-g}
GA_3 2000 ppm (72 h)	6.66^{i}	0.22^{c-g}
GA_3 3000 ppm (48 h)	15.24^{h}	0.31^{b-f}
GA_3 3000 ppm (72 h)	10.47^{b-f}	0.21^{c-g}
Running Water (24 h)	0^{i}	0^{h}
Running Water (72 h)	5^{i}	0.12^{fg}
Running Water (7 d)	25.33^{b-f}	0.32^{b-f}
Running Water (14 d)	60^{a}	0.88^{a}
H_2SO_4 (50%) 3min	0^{i}	0^{h}
H_2SO_4 (50%) 1min	25.33^{b-f}	0.34^{b-e}
H_2SO_4 (50%) 1min + GA_3 1000 ppm (48 h)	34.66^{a-c}	0.47^{cb}
H_2SO_4 (50%) 1min + GA_3 1000 ppm (72 h)	32^{b-d}	0.42^{b-d}
H_2SO_4 (50%) 1min + GA_3 2000 ppm (48 h)	37.33^{a-c}	0.52^{b}
H_2SO_4 (50%) 1min + GA_3 2000 ppm (72 h)	40^{ab}	0.59^{ab}
Control	0^{i}	0^{h}

Means within a column followed by the same letter are not significantly different by Duncans' multiple range tests ($P > 0.05$)

Chemical scarifications (H_2SO_4) in combination with GA_3 were efficient in promoting germination. Among this combined treatments concentration 2000 ppm of GA_3 for 72 h in combination with H_2SO_4 (50%) for 1 min had the highest germination percentage and rates (40%, 0.52). GA_3 with chilling was more effective than application of GA_3 alone. The affect of Concentration 2000 ppm of GA_3 for 72 h with chilling for 60 days in germination percentage and rates (45%, 0.59) was more beneficial than these combined treatments.

DISCUSSION

Owing to break of seed dormancy, Different methods were compared. In this study, seeds were located under the tab water for 1, 3, 7 and 14 day. By leaching inhibitors, tab water treatment improved germination percentage and rates (Table 1). Chemicals that accumulate in the seed-coat during development and remain in the seed after harvest can act as germination inhibitors. Dormancy is often caused by inhibitors such as phenols, coumarin and abscisic acid which can be leached out by soaking in water (9, 10). If it was used water bath, it must be changed every 24 hours for accelerating this process. In nature-overcome by heavy rains which can leach the inhibitors in tropics, deserts; some soil absorbs toxins such as ammoniac given off (11). These outcomes represent that chemical may be main inhibitor of germination of *D. Anethifolia*. Leaching is said to be useful for *Prunusand Magnolia*. (12).

In H_2SO_4 (50%) for 1 min, germination percentage was 25.33% but soaking in the H_2SO_4 (50%) for 3 min failed to germinate. The failure of seeds germination may be due to embryo death, then before penetration any acid into the seed coats they must be transferred from acid. Al-Yemeni and. Basahi (1999) reported Scarification of seeds of *D. anethifolia* (DC.), in concentrated sulphuric acid is most effective than boiling water or ethanol treatment to break seed dormancy (13). Increased germination has been recorded in scarified seeds of *Chordospartium stevensonii* (14) and stratified seeds of *Hoheria glabrata* (15).

GAs are known to obviate the requirement of seeds for various environmental cues, promote germination, and counteract the inhibitory effects of ABA, frequently in combination with cytokinins (16). In this experience application of GA_3 improved the germination of *D. Anethifolia*. Among three concentrations of the GA_3, concentration 3000 ppm had the higher germination percentage than 1000 ppm and 2000 ppm. Chemical scarifications (H_2SO_4) in combination with GA_3 were efficient in promoting germination. The application of acid scarification followed by imbibitions with 10^{-4}M GA_3 is a technique widely used to germinate seeds with a hard seed coat and embryo dormancy. Such treatment did not improve the germination of seeds of three species of *Pseudopanax* (17). Acid scarification significantly increased imbibitions percentages and rates over control irrespective of the GA_3 concentrations used (18).

Cold winter conditions are simulated with Cold stratification or pre-chilling for seeds with internal dormancy. The effects of moist chilling in establishing hormonal levels that favor germination have been suggested to result from cold-stimulation of appropriate enzyme activity (19). Moist chilling had significant influence on *Ferula* seed germination. Moist prechilling for 7-9 weeks was the best treatment for dormancy breaking of *Ferula* seed (5, 20). Stratification at 4˚C was very successful in breaking dormancy of seeds and increasing the duration of stratification percentage in *Bunium Persicum* (21). GA_3 with chilling was more effective than application of GA_3 alone. The benefits accruing from affect of Concentration 2000 ppm of GA_3 for 72 h with chilling for 60 days in germination percentage and rates was more than these combined treatments.

These results show that application of GA_3 in scarified seeds with H_2SO_4 significantly increases germination in comparison with exogenously applied GA_3. Exogenous application of GA_3 significantly enhanced germination of scarified seeds *Cyclocarya paliurus* (22).

Considerable research on gibberellins as seed germination promoters shows that application of gibberellic acid to dormant seeds can eliminate their natural chilling requirement (23). It was found that a significant number of *C. paliurus* seeds germinated that had been treated with exogenously applied GA_3 (22). The addition of GA_3 at two concentrations did not enhance seed germination of *T. terscheckii* neither under white light nor under darkness conditions, which

coincides with the results obtained in other cacti species (24, 25). Seed coats have been shown to impose dormancy because they may restrict the supply of oxygen to the seed or may contain growth inhibitors or may prevent the leaching of inhibitors from the embryo (6). The role of plant growth regulators, particularly gibberellins in the breaking of seed dormancy has been studied extensively (16).

CONCLUSION

In conclusion, the present work has established an effective approach for breaking seed dormancy and enhancing seed germination of *Ducrosia. Anethifolia* (DC.) through Rinsing with running water, Acid scarification and Usage of GA_3 treatment. Other possibilities are Composite treatments GA_3 with Chemical scarification and Cold stratification in various concentrations and durations. Running water for 2 weeks is essential treatments in order to achieving to appropriate germination in this specie. It seems that dormancy is caused by an inhibiting chemical in the exterior surface or interior neighbor layers of seeds. Further studies are required to elucidate the cultivation requirements of this endemic plant. Combination treatments of GA_3 and cold stratifications were suitable for seed germination. The study suggests cold stratification treatments are economic and easily applicable by nursery workers and poor farmers in developing mass planting stock, over costly plant growth regulators and associated technicalities. This laboratory germination results can be applied to propagation projects that would support conservation programs within the study site. But totally germination of *D. Anethifolia* via seed is very difficult and approximately low.

ACKNOWLEDGEMENT: We thank Dr. Davazdah Emami for collecting seeds. We are grateful to Dr. Etminan who helped us with the statistic analyses and especially thanks to M.Sc. Irvani for her assistance in preparing this manuscript. This study supported by the Institute of Medicinal Plant of Iran.

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