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NOTE

SEED DORMANCY IN *PISTACIA MUTICA* F. & M.

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ABSTRACT

Seed dormancy of 'beneh' (*Pistacia mutica* F. & M.) rootstock was the subject of this investigation. Scarification with 98% cold sulfuric acid (90 min), stratification (10, 20, 30, 40 d at 5±1°C) and soaking in gibberellic acid (GA_3) (100, 250, 500, 750, and 1000 mg l^{-1}) were used to increase seed germination of 'beneh'. Scarification plus 20 d stratification at 5±1°C increased seed germination from 27.7% to 62.1%. The highest seed germination occurred when seeds were soaked in GA_3 at 500-1000 mg l^{-1} Inhibitors extracted from 'beneh' seeds had properties similar to abscisic acid (ABA). Concentration of these inhibitors decreased as stratification time increased. The lowest concentration of inhibitors was found 20 d after stratification. A hard endocarp and internal dormancy were the main causes of seed dormancy.

Key words: Abscisic acid, 'Beneh', Gibberellic acid, Scarification, Seed dormancy, Stratification.

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خفتگی بذر در بنه (*PISTACIA MUTICA* F. & M.)

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به ترتیب دانشجوی سابق کارشناسی ارشد (اکنون مربی گروه باغبانی دانشکده کشاورزی دانشگاه صنعتی اصفهان، اصفهان، جمهوری اسلامی ایران) و دانشیار بخش باغبانی دانشکده کشاورزی دانشگاه شیراز، شیراز، جمهوری اسلامی ایران.

چکیده

در این تحقیق ماهیت خفتگی در بذربنه (*Pistacia mutica* F. & M.) مورد مطالعه قرارگرفت. به منظورافزایش تنزگی در بذرهای بنه تیمارهای مختلفی مانند خراش دهی به وسیله سولفوریک اسید غلیظ (۹۸ درصد) و سرد، سرمادهی مرطوب در دمای ۱±۵ درجه سانتی گراد به مدت ۱۰، ۲۰، ۳۰ و ۴۰ روز و خیساندن بذرها در جیبرلیک اسید در غلظت های ۱۰۰، ۲۵۰، ۵۰۰، ۷۵۰ و ۱۰۰۰ میلی گرم در لیترانجام شد. خراش دهی به همراه ۲۰ روز سرمادهی مرطوب باعث افزایش تنزگی بذرها از ۲۷/۷ درصد به ۶۲/۱ درصد شد. بیشترین تنزگی در تیمارهای خیساندن بذر درغلظت های ۵۰۰ و ۱۰۰۰ میلی گرم در لیتر مشاهده شد. ترکیبات بازدارنده موجود در بذرهای بنه استخراج و معلوم شد که خصوصیاتی شبیه ابسایزیک اسید داشتند. میزان این ترکیبات بازدارنده با افزایش دوره سرمادهی مرطوب کاهش یافت و غلظت بازدارنده ها ۲۰ روز پس از سرمادهی مرطوب به کمترین میزان رسید. نتایج نشان داد که دلیل اصلی خفتگی دربذر بنه وجود درون بر سخت و خفتگی درونی است.

INTRODUCTION

'Beneh' (*Pistacia mutica*) is a wild pistachio species native to the dry, semi-dry and semi-wet regions of Iran (13). It was selected as a rootstock because it was resistant to root-knot nematodes (15). *P. mutica* is graft compatible with the commercial pistachio cultivars (6). In Iran, *P. mutica* is propagated from seed. Germination controlling mechanisms are important in nature because they contribute to natural survival and to the dissemination of species (12). In horticulture, various treatments such as scarification with

sulfuric acid, low temperature or growth regulators are used to break seed dormancy (5, 6, 7, 14).

Seeds of *Pistacia* species vary in their capacity to germinate, depending on the hardness of the endocarp (17). The effect of seed scarification on the germination of *P. terebinthus* and *P. atlantica* has been studied by Crane and Ford (2). They observed that seeds of *P. terebinthus* had 53% germination following scarification with H_2SO_4 for 1.5 hr. None of the seeds germinated in the control group after two weeks from sowing. Treated seeds of *P. atlantica* had a germination time of 46 and 80% after 2 and 4 wk, respectively, as compared with 26% in the untreated seeds. Pair and Khatamian (9) reported that germination of *P. chinensis* seeds ranged from 63% to 92% after 60 d stratification at 4° C compared to 0-24% when sown directly without chilling treatment. Casini and Conticini (1) reported 50 to 79% increase in germination of unshelled *P. terebinthus* seeds immersed in 50 mg l^{-1} GA_3 for 7 d.

Abscisic acid has been associated with dormancy. Changes in ABA content have been studied in several species to determine if any relationships exist between ABA and changes in dormancy during chilling (3, 4, 8). Rudnicki (10) found that much of the ABA in apple seeds had disappeared after 3 weeks of stratification. The purpose of this study was to determine the effects of scarification, stratification, gibberellic acid treatments and abscisic acid-like content in dormancy of *P. mutica* seeds.

MATERIALS AND METHODS

Seed Material

Fruits of *P. mutica* were obtained from the wild population of 'beneh' trees in southwest of Sirjan in the Kerman province. Fruits were dehulled and blanks separated by floating in water. Seed were air-dried and kept in cold storage (4° C) for the subsequent experiments.

Scarification

Seeds were divided into two portions: one portion was submerged in concentrated sulfuric acid (98%) for 90 min. Treated and untreated seeds were then washed for 24 hr in running water.

Stratification

The scarified and non-scarified seeds were treated with a 10% chlorox solution for 10 min, rinsed with distilled water and left to dry at room temperature before use. Seeds were mixed with moist peat-moss and kept at 5±1° C for 10, 20, 30, and 40 d.

Gibberellin Treatment

The scarified and non-scarified seeds were soaked in gibberellic acid (0, 100, 250, 500, 750 or 1000 mg l^{-1}) for 24 hr. At the end of these experiments, seed germination percentages and germination time (d) were recorded.

Extraction of Seed

To determine the effects of internal inhibitors on seed dormancy, 10 g of seeds previously stratified for varying lengths of time were homogenized in 20 ml ice-cold methanol (80%), and kept at 5° C for 24 hr. The filtrate was centrifuged, the pellet re-extracted with 80% methanol and the washing added to the supernatant. The extract was evaporated at 36° C to about 10 ml, adjusted to pH 8.8 with 10% NaOH, and extracted three times with an equal volume of ethylacetate. The aqueous fraction was adjusted to pH 2.4 with 1 M HCl and again partitioned into ethylacetate. The acidic ethylacetate fraction was reduced to dryness at 36° C and taken up in to 2ml absolute ethanol. A 0.2 ml of extract was applied to pre-washed Macherey-Nagel Duren silica gel thin layer chromatography plate and was developed in ethylacetate:chloroform: acetic acid (15:5:1 v/v).

Growth inhibitors were assayed by the wheat coleoptile test (16). A standard curve of synthetic ABA was used to determine the relative inhibitor concentration.

Experimental Design

The experimental design was a completely randomized design with four replications using 100 seeds per replication, and means were compared using Duncan's multiple range test (DMRT). Data recorded as percentage were analyzed after $\arcsin\sqrt{x}$ transformation. Germinated seeds were counted between 2 to 40 d.

The second experiment (ABA-like substances determination) was a completely randomized design with four replications, each plot consisting of one petri dish with 13 wheat embryos, followed by DMRT.

RESULTS

Germination Percentages

Various durations of stratification affected seed germination. Scarification with concentrated sulfuric acid followed by stratification at 5±1° C for 20 d increased seed germination of 'beneh' from 27.7% to 62.1%, but was not significantly different from 30 or 40 d stratification at 5±1° C (Table 1). Scarified seeds followed by 500 mg l^{-1} GA_3 treatment increased seed germination of 'beneh' to 59.9% (Table 1).

Table 1. Effect of scarification, stratification and GA_3 treatments on germination percentages of *P. mutica* seeds.

Treatment	Scarified seeds	Non-scarified seeds	Means
Control	27.7h†	7.3n	17.5G
10 d STR	54.6d	10.0m	32.3D
20 d STR	62.1a	12.0l	37.1BC
30 d STR	60.9ab	11.8l	36.3C
40 d STR	61.6a	12.8kl	37.2BC
GA_3 (100 mg l^{-1})	43.7g	13.6k	28.7F
GA_3 (250 mg l^{-1})	45.8f	15.7j	30.7E
GA_3 (500 mg l^{-1})	59.9bc	15.9j	37.9B
GA_3 (750 mg l^{-1})	59.4c	20.7i	40.0A
GA_3 (1000 mg l^{-1})	52.6e	21.2i	36.9C
Means	52.8A	14.1B	

† Means in each columns and rows followed by the same small (capital for means) letters are not significantly different at P≤0.05 by DMRT.

Seed Germination Time

Scarification alone had no significant effect on the time of seed germination, but the addition of GA_3 at 500 mg l^{-1} significantly reduced the time of seed germination in 'beneh' (10.2 d). Among the treatments, stratification for 20 d resulted in the shortest time to germination (11.7 d), (Table 2).

Effect of Stratification on ABA-like Substances of Seeds

The concentration of ABA-like substances in seeds decreased as stratification time increased (Fig. 1). The extract of seeds stratified at 5±1°C

for 20 d resulted in the least reduction of wheat coleoptile growth. The extract of seeds without stratification had the highest concentration of ABA-like substances and significantly reduced wheat coleoptile length (Fig. 1).

Table 2. Effect of scarification, stratification (STR) and GA_3 treatments on germination times (d) of *P. mutica* seeds.

Treatment	Scarified seeds	Non-scarified seeds	Means
Control	16.9g†	29.8a	23.3A
10 d STR	12.5h	27.5b	20. 0B
20 d STR	11.7j	26.7ef	19.2F
30 d STR	12.2i	27.4bc	19.7CD
40 d STR	12.3hi	27.2bcd	19.7CD
GA_3 (100 mg l^{-1})	12.3hi	26.7ef	19.5DE
GA_3 (250 mg l^{-1})	12.2hi	27.5b	19.8BC
GA_3 (500 mg l^{-1})	10.2k	27.0cdef	18.6G
GA_3 (750 mg l^{-1})	11.6j	27.1cde	19.3EF
GA_3 (1000 mg l^{-1})	12.4hi	27.0cdef	19.7CD
Means	12.4B	27.4A	

† Means in each columns and rows followed by the same small (capital for means) letters are not significanty different at P≤0.05by DMRT.

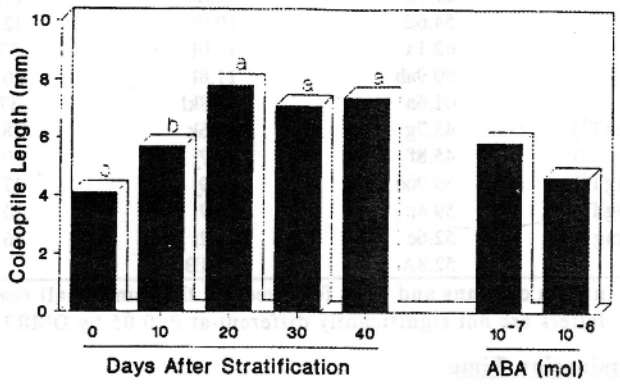

Fig. 1. ABA content of methanol extracts of *P. mutica*. Different letters on bars denote significant differences at P≤0.05 according to DMRT.

DISCUSSION

Untreated seeds generally failed to germinate but acid treated seeds had a germination time of 27.7% after 16.9 d. If the hard endocarp was the only cause of dormancy, germination time should have increased with

scarification, but stratification was necessary to overcome internal dormancy. The data showed that 'beneh' seeds had a double dormancy which is consistent with earlier reports by Shekafandeh and Shaybany (12). Stratification treatments in combination with scarification increased seed germination. Acid treated seeds needed a 20-d stratification period to reach 62.1% germination (Table 1). These data agree with the results of pair and Khatamian (9) and Shao (11) in germination studies on *P. chinensis*. Acid treatment did not sufficiently improve germination time and treatment with GA_3 was necessary. Our results showed scarification by acid without stratification did not improve the germination.

These data support the contention that germination is hindered by a hard endocarp not the inability of the embryo to absorb water or oxygen. ABA has been reported as a control agent in both types of dormancy in *Rosa* species as well as in the subsequent germination processes (18).

Stratification decreased ABA-like content (Fig.1). These data agree with the results of Martin *et al.* (7), in walnut studies. It seems evident from Fig.1 that 20 d chilling at 5±1°C which broke seed dormancy also resulted in decrease of seed inhibitor content.

These results demonstrate stratification can be replaced by GA_3 which is in agreement with previous reports on *P. atlantica* (6) and *P. terebinthus* (1).

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