

## Effect of Scarification, Gibberellic acid and Stratification on Seed Germination of Three *Pistacia* Species

تأثير التخديش، حامض الجبرليك والتنضيد على إنبات بذور ثلاثة أنواع من الفستق

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### Abstract

Germinability of *P. atlantica* Desf., *P. palaestina* (Bioss) Post. and *P. lentiscus* L. seeds as influenced by various treatments was investigated. This included four treatments: Acid scarification for 15 minutes, soaking of acid scarified seeds in 1000 ppm GA<sub>3</sub> for 24 hours, stratification of seeds at 5C° for 30 days and control (untreated seeds). Significantly, the highest germination (60%) was obtained for *P. palaestina* acid scarified plus cold stratified seeds over the control of the three *Pistacia* species (15, 10, 0%), this percentage (60) was on the same level of significance with the other *Pistacia* species. Scarified seeds of *P. lentiscus* resulted in 13.3% germination, scarified plus GA<sub>3</sub> soak of *P. lentiscus* and *P. atlantica* Desf. (34, 39.9%), and scarified plus cold stratified *P. lentiscus* (32%) seeds. Early seed germination was obtained with seeds of *P. lentiscus* after one week of incubation with scarified plus GA<sub>3</sub> (6%) and scarified seeds of *P. palaestina* (5%), other treated seeds of the three species started germination after two weeks. *P. palaestina* seeds continued with the highest germination percentage thereafter. Significantly, the longest mean time to complete germination (MTG) was obtained from the control of the three *Pistacia* species (27.94, 24.50, 30.04), Scarified *P. atlantica* (25.84), and scarified plus GA<sub>3</sub> soak of *P. atlantica* (26.25) as compared with the other treatments for the three *Pistacia* species which were all in the same level of significance.

**Key words:** *Pistacia palaestina*, *Pistacia atlantica*, *Pistacia lentiscus* germination, seed dormancy.

## ملخص

تمت دراسة تأثير معاملة بذور ثلاثة أنواع من الفستق جنس *Pistacia* (*P. atlantica*<sup>1</sup> Desf, *P. palaestina*<sup>2</sup> (Bioss) Post., *P. lentiscus*<sup>3</sup> L.) باستخدام تجريح كيميائي، حامض الجبرليك، والتنضيد البارد على درجة حراره ٥ م' لمدة ٣٠ يومياً على إنباتها. تمت عملية تخديش البذور بواسطة نقعها بحامض الكبريتيك المركز ومن ثم معاملتها بحامض الجبرليك ١٠٠٠ جزء بالمليون لمدة ٢٤ ساعة او التنضيد او بدون معاملة (تخديش فقط) كما تركت مجموعة كشاهد بدون تخديش او معاملات أخرى. أدت معاملة البذور الى تحسين نسبة إنباتها بدرجة معنويه احصائيا وظهرت فروق واضحة بنسبة الإنبات بين الأنواع الثلاثة، حصلت أعلى نسبة انبات ٦٠ % بعد ٣٠ يوم من التنضيد البارد في بذور الضرو الفلسطيني (البطم) المخدشه على الشاهد في الأصناف الأخرى (١٥،١٠،٠) على التوالي، حصلت معاملة التخديش للصنف السريس على (٣، ٣١) % وتخديش مع حامض الجبرليك للصنفين سريس وبطم على (٩، ٣٤، ٣٩) % على التوالي. كذلك معاملة التخديش والتنضيد البارد للصنف السريس (٣٢) %. حصل أسرع أنبات بعد أسبوع في الصنف (سريس) للبذور المخدشه (٦) % وبذور الضرو الفلسطيني المخدشه (٥) %، بخصوص بذور الأصناف الأخرى فقد بدأ أنباتها بعد اسبوعين من الحضانه . من ناحيه أخرى وبخصوص معدل الزمن لاكمال الانبات والذي يعبر عن سرعة الانبات، فقد كان اقل في البذور المعاملة عن الشاهد وحصل اقل رقم (١٩ يوماً) في بذور الضرو الفلسطيني. يتضح من نتائج التجربة التأثير المعنوي للتخديش الكيماوي على تحسين قدرة بذور البطم على الانبات.

**الكلمات المفتاحية:** البطم. فستق بري فلسطيني (ضرو فلسطيني). سريس.

## Introduction

Pistachio is an economically important crop in the central and west Asia and north Africa (Kaska, 2001). Approximately 66 million wild *Pistacia* trees are existed in Turkey, belonging mainly to *P. terebinthus*, *P. khinjuk* and *P. atlantica*. (Kafkas, & et.al. 2001). Fifty five million Pistachio trees are also distributed as wild forests in Iran. (Behboodi, 2002). Several species of *Pistacia* have been identified in Palestine (Ali-Shtayeh, & et.al. 2006), (Ellis, & et.al. 1985), (Post, 1932), (Zohary, 1966, among them are *P. atlantica*, *P. palaestina* (Bioss) Post. and *P. lentiscus* L. *P. atlantica* trees are mainly growing in the southern area of Palestine, mainly Jerusalem and Hebron (Post, 1932). Another species has been recorded in the 1930's as a hybrid between *P. palaestina* and *P. lentiscus*. (MOA, 2002). These species have shown a great adaptation to the harsh environmental conditions and therefore, could be used as good

genetic resources (Chaabouni, Gouta, 2002). However, these resources have been under threat by both genetic erosion and human activities. No trials have been conducted to conserve these resources. Propagation of these plants are difficult. Seeds of these species are surrounded by a hard sclerotic endocarp, which make the germination of these seeds difficult and with low percentage (Isfendiyaroglu, & zeker, 2002). Different seed pretreatments were used to enhance *Pistacia* seed germination. Scarification and cold stratification were found to improve the seed germination. (Ayfer, Serr, 1961), (Crane, & Forde, 1974). It was found that the mean time to complete germination of *P. atlantica*, *P. terebinthus* and a hybrid was higher with seeds treated with 1000 ppm GA<sub>3</sub> and stratified at 4 C° for 15-45 days. (Isfendiyaroglu, & zeker, 2002). On the other hand, there was no significant effect of GA<sub>3</sub> treatments on the germination of *P. atlantica* seeds, however, acid scarification of the seeds for two hours enhances germination rate and percentage (Chaabouni, Gouta, 2002).

The germination rate of seven types of *P.khinjuk* seeds ranged from 40% to 96% after the seeds were exposed to different treatments, the highest germination rates were obtained with stratified seeds (Kafkas, 1998). In another study, it was found that the highest germination percentage (73.3%) was obtained when the seeds were soaked for 48 hours in 125 ppm GA<sub>3</sub> solution (Ak, & et.al. 1995). More than 95% seed germination was obtained in three *Pistacia* species (*P. vera*, *P. khinjuk* and *P. atlantica*) after the seeds were soaked for 7 days in 100 ppm GA<sub>3</sub> solution (Kuru, Aksu, 1995). According to the above discussed literature no work was done regarding the germination of the local *Pistacia* species except for *P. atlantica*, therefore the aim of this work was to investigate the effect of different seed treatments on the seed germination of three *Pistacia* species.

### **Materials and Methods**

Seeds of three *Pistacia* species (*P. atlantica* Desf., *P. palaestina* (Bioss) Post. and *P. lentiscus* L.) were collected from naturally growing trees from Hebron and Jenin areas. The fruits containing seeds were

soaked for 24 hours in water to remove the pulp. They were then rubbed in a screen to separate the pulp from the seeds and then washed. Shell containing seeds were floated in water to separate viable seeds from those floating ones. Viable seeds were then used in the germination experiment. Seeds from the three species were scarified with concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 15 minutes, and were immediately put under running tap water for 24 hours to remove acid residue. A group of seeds was kept without scarification as a control treatment. Four treatments were compared for each species in a factorial treatment design. The treatments were; control (unscarified and unstratified), acid scarification, soaking of scarified seeds in 1000 GA<sub>3</sub> for 24 hours and cold stratification of the scarified seeds. The seeds were stratified at 5± 2 C° for 30 days in moist vermiculite. The treatments were arranged in a completely randomized design with 5 replicates with 20 seeds used in each replicate. Therefore, a total of 100 seeds were used for each treatment and 400 seeds for each species.

The seeds were sown in sterile Petri dishes on double layer filter paper, the dishes were incubated at 22C° under dark condition in an incubator (Sanyo MLR-350), and they were watered with sterile water according to need. Germinated seeds were counted daily, seeds with protruding radicle and plumules were scored as germinated. The germination percentage and the mean time to complete germination (MTG) was calculated for each species. MTG was calculated according to the formula described by (Isfendiyaroglu, & zeker, 2002) as follows:

MTG = (t x n)/n; where t is the time in days starting from day zero, and n is the number of seeds completing germination on day (t). Two way analysis of variance was conducted followed by mean separation using the Least Significant Difference Test (LSD) at 5% probability level. The data were analyzed using the SAS software package (SAS Institute, 1990).

## Results

The statistical analysis indicated a significant interaction between the seed treatments and the *Pistacia* species at all reading periods. The

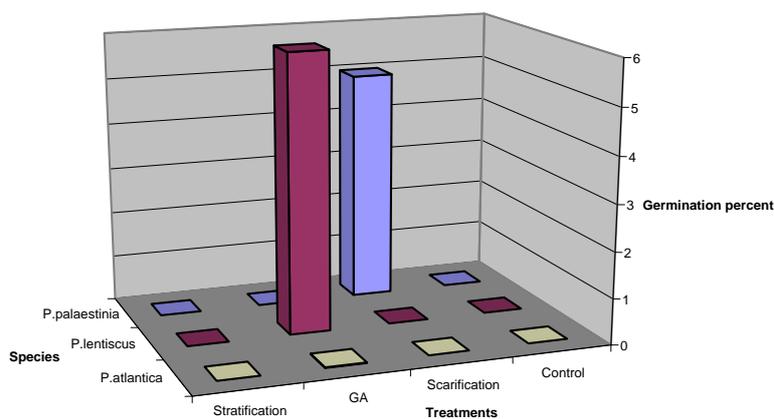
treated seeds exhibited higher germination percentages than the control in all species (Table 1). Seed germination started after one week of planting with the exception of scarified *P. lentiscus* seeds. Scarified plus GA<sub>3</sub> treated seeds of *P. lentiscus* resulted in 6% seed germination and scarified seeds of *P. palaestina* in 5%. (Fig. 1a). After two weeks of planting germination was obtained from all treated seeds except the control and the higher germination percentage was obtained with *P. palaestina* treated seeds (Fig. 1 b). A similar trend of seed germination was observed at week 3 and week 4 with the higher germination percentage observed with *P. palaestina*. (Fig. 1c, 1d). At the last reading period (week 5), the highest germination percentage was obtained with *P. palaestina* (60%) after cold stratification, however, this percentage was at the same significant level of that obtained from scarified seeds treated with GA<sub>3</sub> or acid scarification of the same species (56 and 57.5%), respectively (Table 1). No germination was obtained with the control treatment of *P. atlantica* species, however 15% and 10% germination percentages were obtained in the control treatment of both *P. palaestina* and *P. lentiscus*, respectively. For *P. lentiscus*, the highest germination was obtained from seeds treated with GA<sub>3</sub> and cold scarification (34 and 32%), respectively. On the other hand, scarified and stratified *P. atlantica* seeds exhibited the highest germination percentages (47.5 and 50%) respectively (Table 1). However, these percentages were not significantly different from those obtained with seeds treated with GA<sub>3</sub> of the same species (39.9%).

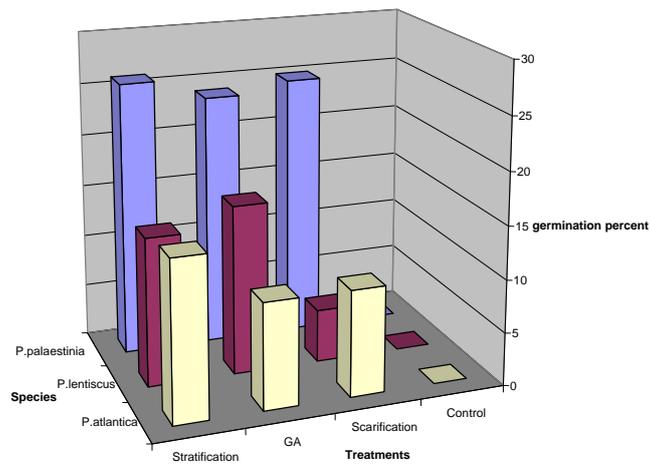
Regarding the germination rate, the MTG of all treatments was less than the control treatment of all species (Table 1). Stratified seeds of the three species and scarified seeds of both *P. palaestina* and *P. lentiscus* exhibited significantly a low MTG which ranged from 19.43 to 23.92 days with the lowest obtained in *P. palaestina* scarified seeds (19.43).

**Table (1):** Effect of seed treatments on the germination percent and rate of three pistachio species after 5 weeks of incubation.

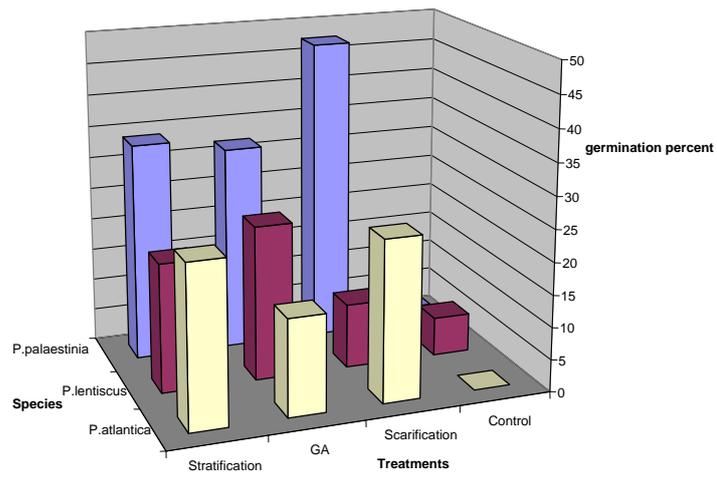
Treatment	Species	Germination %	MTG* (days)
Control	<i>P. palaestina</i>	15.0 d	27.94 cd
	<i>P. lentiscus</i>	10.0 d	24.50 bc
	<i>P. atlantica</i>	0.0 d	30.64 d
Acid scarification	<i>P. palaestina</i>	57.5 a	19.43 a
	<i>P. lentiscus</i>	13.3 d	19.83 a
	<i>P. atlantica</i>	47.5 abc	25.84 bc
Scarification + 1000 ppm GA <sub>3</sub>	<i>P. palaestina</i>	56.0 ab	23.02 abc
	<i>P. lentiscus</i>	34.0 c	20.42 a
	<i>P. atlantica</i>	39.9 bc	26.25 bcd
Scarification + Cold stratification	<i>P. palaestina</i>	60.0 a	21.60 ab
	<i>P. lentiscus</i>	32.0 c	21.14 ab
	<i>P. atlantica</i>	50.0 ab	23.92 abc

MTG: Mean time to complete germination.

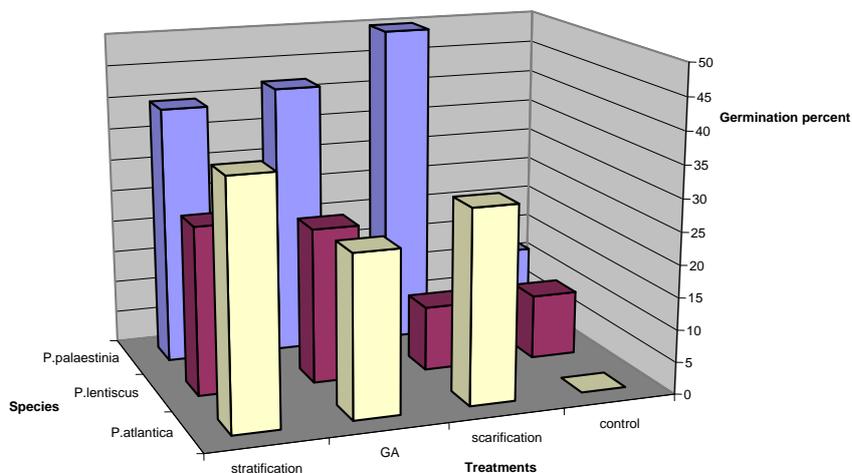
**Fig (1 a):** Effect of different treatments on the seed germination of three *Pistacia* species (Week 1).



**Fig (1b):** Effect of different treatments on the seed germination of three *Pistacia* species (week 2).



**Fig (1c):** Effect of different treatments on the seed germination of three *Pistacia* species (week 3).



**Fig (1d.):** Effect of different treatments on the seed germination of three *Pistacia* species (week 4).

## Discussion

Seed dormancy could be derived from either tissues enclosing the embryo or from the embryo itself, (Bewley, Black, 1994). Therefore, the germination barrier in *Pistacia* species could be due to the seed covering. The result also indicated the variation in seed germination ability among the three species. These findings are in agreement with (Ayfer, Serr, 1961), (Chaabouni, Gouta, 2002), (Isfendiyaroglu, & zeker, 2002). However, the results are in contrast with the finding of (Ak, & et.al. 1995), (Kafkas, 1998), (Kuru, Aksu, 1995). Similar result was reported by (Pitto, 1995) who found no significant difference in the seed germination of scarified and (scarified and stratified) seeds of *P. lentiscus*. The result of the current study indicated that scarification was the primary factor enhanced the seed germination. However, the germination percent obtained after scarification was relatively low, this indicated that other factors contributed to the low germination percent.

Polyphenols and flavonoids produced in the fruit or seed could inhibit germination. (Baskin, C.C. & Baskin, J. M. 1998). The inhibitory effect of phenolic compound on seed germination is closely related to the regulation of the endogenous auxin, oxygen supply and seed coat permeability (Bewley, Black, 1994). However, it was found that the concentration of phenolic compounds varied with GA<sub>3</sub> and scarification seed treatments of the three *Pistacia* species (Isfendiyaroglu, & zeker, 2002). The highest phenolic compounds were found in *P. atlantica*. This finding could be a possible cause of the lowest seed germination obtained in our experiment.

Regarding the germination rate, the MTG of all treatments was less than the control treatment of all species. The three species exhibited different MTG, this is in agreement with the results of (Isfendiyaroglu, & zeker, 2002) with three *Pistacia* species, and the seeds of *P. chinensis* were more rapid and completed germination in 15 days after they were exposed to cold stratification for 45 days.

Our results indicated the significant effect of acid scarification and the improvement effect of other treatments (GA<sub>3</sub> and stratification of the scarified seeds). The physical effect seems to be more efficient in inducing seed dormancy. However, further investigation is necessary to support this finding and more are needed to improve *Pistacia* seed germination, studies could handle the issue of phenol compound, stratification period and other chemical enhancing agents.

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