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The Effects of Scarification, Cold Stratification and Gibberellic Acid Treatment on Germination of Kholkhong Seeds

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Abstract: Kolkhong (*Pistacia khinjuk* Stocks) is a native tree of Iran. Economically, its importance is known in the production of edible pistachio nuts when grafted with *Pistacia vera*. Its role in soil stabilization has been appreciably recognized in the recent past. This study concerns the determination of the effect of scarification by sulfuric acid (98%), cold stratification at 5+1°C (10, 20, 30 and 40 days) and gibberellic acid (100, 250, 500, 750 and 1000 mg L^{-1}) on the germination of Kolkhong seeds which have a dormancy period with low germination percentage. The results showed that Kolkhong had physical and internal dormancy. Scarification of seeds using cold acid for 20 min, followed by stratification for the period of 30-40 days or soaked in concentrations of 500-1000 mg L^{-1} gibberellic acid gave the highest percentage of seed germination.

Key words: Kolkhong, seed germination, scarification, stratification, gibberellic acid

INTRODUCTION

Kolkhong (*Pistacia khinjuk* Stocks) is one of the native deciduous trees of Iran. It occurs as a common associate of Beneh (*P. mutica*) in the mountainous area (Tabatabaee, 1966). Although it is slow growing, this species is resistant to drought and thrives well on poor soil (Raeder-Roitzsch, 1969). The species has a good range of distribution, being found in Turkey, Iran, Afghanistan, Pakistan, Iraq, Syria and Palestine (Khatamsaz, 1987). It has a number of local uses. It yields good quality fuel and charcoal, the trees are commonly pollarded by villager for using their foliage as fodder for the cattle and as tanning and dyeing material (Fatahi, 1996). The seeds are also edible. In spring and early summer the tress are sometimes tapped for a gum which flows from the blazes into small earthen pots hung to the tree poles below the blazes. The product is collected as a kind of chewing gum or as mastic.

P. khinjuk can be successful grafted with *P. vera* which produces the edible pistachio nuts (Thakur and Rathore, 1991). The seeds are the only source for propagating the species but these have been found to possess a substantial degree of dormancy as well as germinating irregularly. In horticulture, various treatments such as scarification with sulfuric acid, low temperature and growth regulators were used in breaking various types of seed dormancy (Khosh-Khui and Bassiri, 1976; Kuru and Aksu, 1995; Sladan, 1973). Seeds of *P. khinjuk* and other species are enclosed in a hard shell (Ak *et al*., 1995). Scarification of shells with sulfuric acid increases germination rates (Crane and Forde, 1974). Pair and Khatamian (1983) showed that the germination of *P. chinensis* seeds ranged from 63-92% after 60 days stratification at 4°C in comparison with 0-24% when sown directly without chilling treatment. Gibberellic acid (GA_3) plays an important role in seed germination (Khan, 1971).

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Martin (1968) reported that treating the seeds of *Duboisia leichhardtii* with GA_3 gave almost 100% germination. Also Casini and Conticini (1979) reported that unshelled seeds of *P. terebinthus* when immersed in 50 ppm GA_3 for seven days, the germination increased from 50-79%. Changes in abscisic acid (ABA) content were studied in seeds of several species to determine if a relationship exists between ABA and changes in dormancy during chilling (Diaz and Martin, 1972; Hawison and Sanders, 1975). Martin *et al.* (1969) determined that there was ABA in *Juglans regia* before stratification; however, ABA decreased during the stratification period.

The present study was under taken to investigate the effects of scarification, stratification and growth regulator (GA_3) on dormancy of *P. khinjuk* seeds.

MATERIALS AND METHODS

Seed Materials

Fruits of *P. khinjuk* were obtained from the wild population of Kolkhong trees in north of Neiriz in the Fars province, Iran. Fruits were dehulled and blanks separated by floating in water. Seeds were air-dried at 25±3°C and kept in cold storage at 5±1°C for the following experiments.

Scarification

Seeds were divided in two portions, one portion of seeds were placed in a container and covered with concentrated sulfuric acid (98%) for 20 min at room temperature. Treated and untreated seeds were washed for 24 h in running water.

Stratification

The scarified and non-scarified seeds were treated with 10% chlorox for 10 min and then rinsed with distilled water before use. Seeds were mixed moister peat-moss and kept at 5±1°C for 10, 20, 30 and 40 days.

Gibberellic Acid Treatment

The scarified and non-scarified seeds were soaked in GA_3 (100, 250, 750 and 1000 mg L^{-1}) for 24 h.

At the end of these experiments, seed germination percentages and germination rate (days) were recorded. The formula used in determining germination rate values is as follows (Pieper, 1952):

$$GR = \frac{(n_1 \times t_1) + (n_2 \times t_2) + (n_3 \times t_3) + (n_i \times t_i)}{T}$$

Where,

GR : Germination rate.
n : No. of days for each counting of germinated seeds.
t : No. of germinated seeds in each counting day.
T : Total No. of germinated seeds.

Experimental Designed Statistics

The statistical analysis of germination experiment was carried out according to the completely randomized design with four replications and 100 seed per each replication then followed by Duncan's New Multiple Range Test (DNMRT). Germinated seeds were removed once every two days a total period of 40 days.

RESULTS AND DISCUSSION

The result of the experiment shown in Table 1 indicated that clearly all the pre-treatments gave a highly significant increase in the average percentage of germination compared with nontreated seeds except 10 days stratification in which no significant effect on germination was observed. Considering the treatments, scarifications to be an effective method for breaking the seed dormancy of *P. khinjuk,* where 73.26 mean % germination was obtained as compared with 22.75% in the unscarified seeds. These findings are in agreement with the results of Crane and Forde (1974) and Sehgal and Singh (1990) in germination studies on *P. atlantica* and *P. integerrima* respectively. Scarification by concentrated sulfuric acid and stratification at 5±1°C for 40 days increased seed germination in Kolkhong up to 90.72%, but had no significant difference with 30 days stratification (Table 1). This data was in agreement with the results of Pair and Khatamian (1983) and Shao (1989) in germination studies on *P. chinensis.*

Scarified seeds plus GA_3 at 500 mg L^{-1} increased seed germination in *P. khinjuk* upto 98% (Table 1), but had no significant difference with GA_3 at 750 and 1000 mg L^{-1}, 30 and 40 days stratification (90.16, 89.16, 90.11 and 90.72%, respectively). This is in accordance with the results obtained by several other investigators (Ak *et al*., 1995; Casini and conticini, 1979; Kuru and Aksu, 1995) who worked on various *Pistacia* species. The results of this study showed that scarification alone had low effect on the rate of seed germination. Scarification plus GA_3 at 500 mg L^{-1} significantly reduced rate of seed germination in Kolkhong (12.30 days), but had no significant difference with 1000 mg L^{-1} (12.70 days). Among the chilling treatment, stratification for 40 days had the lowest rate of seed germination (13.21 days) (Table 2). Present results showed that untreated seeds almost failed

Table 1: Effects of scarification, stratification (STR) and gibberellic acid (GA_3) treatments on germination percentages of *P. khinjuk* seeds

Treatments	Scarified	Non-scarified	Means
Control	22.13gh*	3.73k	12.93G
10 days STR.	46.01d	9.27jk	27.64F
20 days STR.	71.65bc	12.32ij	41.98E
30 days STR.	90.11a	19.21ghi	54.16C
40 days STR.	90.72a	22.03gh	56.38BC
GA_3 (100 mg L^{-1})	67.12c	17.36hi	42.24E
GA_3 (250 mg L^{-1})	74.22b	24.65fg	49.44D
GA_3 (500 mg L^{-1})	91.38a	29.19f	60.29AB
GA_3 (750 mg L^{-1})	90.16a	38.52e	64.34A
GA_3 (1000 mg L^{-1})	89.16a	32.24e	62.70A
Means	73.26A	22.75B	

*: In each row or column, means with the similar letter(s) are not significantly different at 1% level of probability using DNMRT

Table 2: Effects of scarification, stratification (STR) and gibberellic acid ($_{GA3}$) treatments on germination rates (days) of *P. khinjuk* seeds

Treatments	Scarified	Non-scarified	Means
Control	19.54g*	28.02a	23.78A
10 days STR.	17.84h	25.95b	21.90B
20 days STR.	17.28i	25.15c	21.22C
30 days STR.	13.25lm	24.56d	18.90E
40 days STR.	13.21lm	24.65cd	18.93E
GA_3 (100 mg L^{-1})	16.70j	22.16e	19.43D
GA_3 (250 mg L^{-1})	15.56k	22.27e	18.92E
GA_3 (500 mg L^{-1})	12.30n	22.23e	17.27F
GA_3 (750 mg L^{-1})	13.46l	20.96f	17.21F
GA_3 (1000 mg L^{-1})	12.70mn	21.30f	17.00F
Means	15.18B	23.72A	

*: In each row or column, means with the similar letter(s) are not significantly different at 1% level of probability using DNMRT

germinate (3.73%), but acid treated seeds germinated up to 22.13%. If the hard endocarp was the only cause of dormancy, germination should have increased with scarification, but in the case of internal dormancy, stratification was necessary to overcome it. The data showed that Kolkhong have double dormancy with agrees with earlier reports by Shekafandeh and Shaybany (1989). Present results showed that scarification by acid without stratification did not improve the germination capacity. It supports the idea that germination hindrance caused by hard endocarp is not due to an inability of the embryo to absorb either water or oxygen. ABA has been reported as a control agent on both of these types of dormancy as well as in the subsequent germination processes (Tillberg, 1983). A hypothetical scheme to describe the internal control of Kolkhong seed dormancy could include the following: prior to stratification, ABA concentration is sufficient to induce dormancy and GA concentration is low (Mathur *et al*., 1971). As stratification proceeds, ABA decreases and GA and cytokinins are synthesized or released from bound forms (Diaz and Martin, 1972). The results of the present study showed that stratification can be replaced by GA_3 and this finding was in agreement with previous reports on *P. atlantica* (Kuru and Aksu, 1995) and *P. terebinthus* (Casini and Conticini, 1979). Gibberellins appear to play a role in two different stages of germination. One occurs at the initial enzyme induction in their transcription from the chromosomes. The second is in the activation of reserve food mobilizing system (Hartman *et al*., 1990).

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