

# Evaluation of seed soaking times on germination percentage, germination rate and growth characteristics of pistachio seedlings

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## Abstract

In traditional pistachio cultivation, prior to sowing seeds the latter are soaked in water for different times as farmers claim that this will contribute to germination. To our best knowledge, this is the first attempt to evaluate the effect of different water-soaking time periods on germination in pistachio. For this purpose, seeds of pistachio (*P. vera* L. 'Badami') were treated with water for 1, 6, 12 and 24 h. We monitored germination percentage, germination rate, mean time for germination rate, and seedling height at different stages, number of leaves, shoot and root dry weight, and trunk diameter. Our results indicated that germination percentage and rate significantly increased and seedling height increased in seeds, which were treated for 24 h. Although, plant root dry weight significantly decreased in seeds of this treatment, mean times for germination (MTG) values were not found to be statistically significant between treatments. We observed that the 6 h soaking treatment led to an increase in shoot and root dry weight, and trunk diameter. Also leaf numbers and root length values were highest in the 1 h soaked seeds, although differences were not significant. Positive relationship was observed between the plant shoot length and plant leaf number.

**Keywords:** mean germination time, *Pistacia vera*, length growth, plant dry weight

## INTRODUCTION

Pistachio species belong to the *Anacardiaceae* family. *Pistacia vera* L., i.e., cultivated pistachio, is the most important species in the genus that are all deciduous and dioecious. Pistachio is an important fruit tree grown in the sub-tropical areas in the world with highest production figures for Iran, Turkey, Syria and USA. Seed germination is the common procedure for large scale propagation of pistachio seedlings. Seedlings are grafted to desirable cultivars in nursery or field conditions to establish the pistachio productive orchards (Panahi et al., 2002).

Seed quality is affected by several factors and seed germination percentage, germination rate and seed vigor index have an important role in determination of seed quality (Black and Bewley, 2000; Rajjou et al., 2012). To begin the germination process in dried seeds, priming, pre-soaking, scarification, stratification and chemical treatments are necessary and the type of treatment used depends on species, cultivars and seed features. Pistachio species have different seed germination patterns; most of them have dormancy and need special treatment to break down their dormancy (Panahi et al., 2002). Acid treatment and gibberellic acid (GA<sub>3</sub>) are often used to break down especially thick seed coats (Rahemi and Baninasab, 2000) in wild pistachio rootstocks. Khan et al. (1999) reported wild pistachio seeds treated with KNO<sub>3</sub> at 1% solution significantly completed early germination in 135 days, increased the germination percentage and produced seedlings with higher length and diameter when soaked for 24 h. Soaking seeds in polyamines is a useful method to induce lateral root in pistachio seedlings (Sedaghat and Rahemi, 2011). Studies on germination of citrus seeds indicated that osmotic stress causes decline of seed water content, which results in inhibition of seed germination and seedling growth (Zekri, 1993).

There is no dormancy in *P. vera* seeds and additionally they have a high germination



percentage after being soaked in water (Panahi et al., 2002), the duration of soaking is however a very important parameter in achieving the maximum germination percentage. As, too lengthy periods of soaking cause bacterial decay of the embryo, prior to the actual germination, and inadequate duration causes inactivation of the enzyme facilitating the germination. Thus, both of these conditions cause a decrease in seed germination percentage and rate, and an overall decline in the subsequent seedling growth parameters.

The objective of this study was to evaluate the effects of seed soaking with varying durations in water to find, optimize and finally standardise a duration time for optimal seed germination features and subsequent growth parameters of seedlings of the most common pistachio cultivar, which is used as pistachio rootstock in Iran.

## **MATERIALS AND METHODS**

Two experiments were conducted in a greenhouse at the Bioscience Engineering Faculty, Ghent University (51°3'N, 3°42'E). Certified seeds of pistachio, *Pistacia vera* L. 'Badami' were obtained from the Iranian Pistachio Research Institute (30°39'N, 55°94'E), Rafsanjan, Iran. Seed size varied for 17.9 mm (length), 10.6 mm (width), 11.4 mm (thick) and 0.87 g (weight).

### **Experiment 1**

Seeds of 'Badami' pistachio cultivar were pre-treated with distilled water for 1, 6, 12 and 24 h as soaking time treatments. Then all pre-treated seeds were disinfected with 0.01% Captan, a broad-spectrum fungicide for 20 min (Panahi et al., 2002). Disinfected seeds were placed in petri dishes on Whatman filter paper and moistened with distilled water. Then seeds were kept in lab conditions for 21 days and the irrigation of them was performed by distilled water regularly. The germinated seeds were recorded every day and germination percentage calculated on the 21<sup>st</sup> day.

All germinated seeds were sown immediately in 4-L pots containing sand and organic material and kept in greenhouse conditions. Plant cultural requirements were done according to good agricultural practices during the experimental time. After 30, 40 and 50 days of sowing date, shoot height and root length were manually measured with a ruler; shoot diameter determined with a digital caliper and seedling (shoot and root) dry weight was determined after drying at 85°C for 48 h in an electric oven. Germination percentage and rate, mean of germination time and vigor index were calculated according to following equations (Ellis and Roberts, 1981):

- Germination percentage (GP):  $GP (\%) = S_{NG}/S_{NO} \times 100$ ; where  $S_{NG}$  is the number of germinated seeds and  $S_{NO}$  is the number of experimental seeds with viability, respectively.
- Germination rate (GR):  $GR (\%) = S_{NM}/S_{NG} \times 100$ ; where GR is the germination rate and  $S_{NM}$  is the number of germinated seeds on a given day.
- Mean of germination time (MGT): MGT is an index for germination rate.  $MGT = \sum (nd)/\sum n$  (day<sup>-1</sup>); where n is number of germinated seeds in day d,  $\sum n$  is total germinated seeds, D day of counting.
- Vigor index (VI):  $VI = GP \times \text{seedling dry weight (g)}$  (Abdul-Baki and Anderson, 1973).

### **Experiment 2**

The similar seeds of 'Badami' pistachio were put in petri dishes. Three petri dishes were considered for each treatment and control. Polyethylene glycol (PEG) solutions were prepared in 0.2, 0.8, and 1.5 MPa concentrations and 5 mL of each solution was added to petri dishes containing seeds. Also distilled water was applied to control petri dishes. All petri dishes were placed in lab conditions and every 24 h the number of germinated seeds was counted and in case of moisture deficiency distilled water was used.

### **Statistical analysis**

Treatments were arranged in a randomized complete design with five replicates per treatments in experiment 1 and three replicates per treatments in experiment 2. A two-way

analysis of variance was used to test for treatment differences and effects. Means were compared by a Duncan test. All analyses were performed in SPSS 20 (IBM Corporation, USA).

## RESULTS AND DISCUSSION

### Experiment 1

#### 1. Germination percentage (GP).

The germination percentage of seeds increased with increasing the soaking times for pistachio seeds (Figure 1). Seeds soaked for 24 h was the most effective treatment on GP and treatment for 1 h showed the lowest percentage. Statistical analysis showed that there were significant differences between these two treatments (Figure 1). These results are in agreement with the results of Sedaghat and Rahemi (2011) that reported application of polyamines for 12 h compared to 6 h increased percentage of germination capacity in 'Ghazvini' pistachio seeds. While, Khan et al. (1999) showed that normal water soaking had better germination percentage in 48 h duration compared to 12, 24 and 36 h duration in wild pistachio.

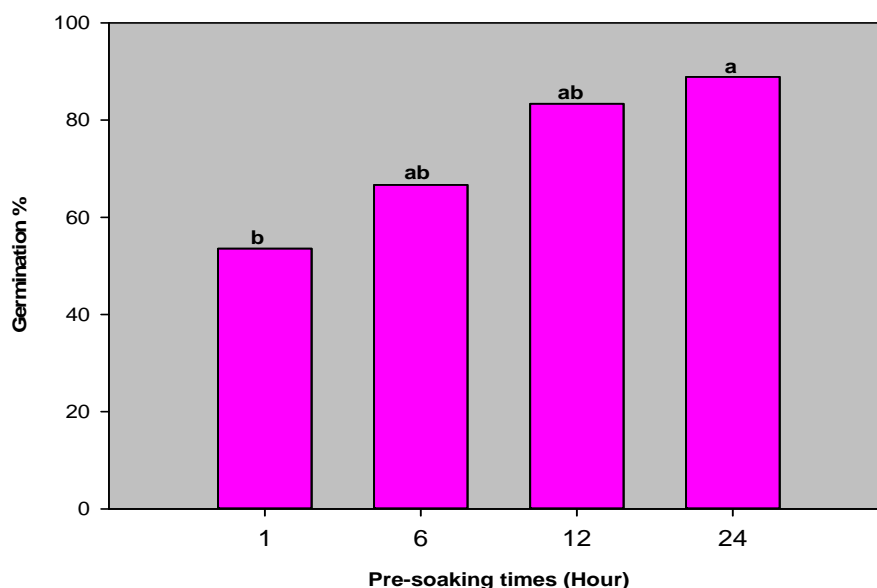


Figure 1. Changes in germination percentage in different treatments (h) of 'Badami' pistachio cultivar.

#### 2. Mean germination time (MGT).

Mean germination time significantly decreased by increasing soaking time duration of seeds to 12 h. Although soaking seeds for 24 h significantly increased compared to 12 and 6 h treatments (Figure 2). There was no significant statistical difference between 1 and 24 h treatments in 'Badami' pistachio cultivar (Figure 2). Sedaghat and Rahemi (2011) indicated that mean germination time of pistachio was enhanced by soaking time of seeds in polyamines.

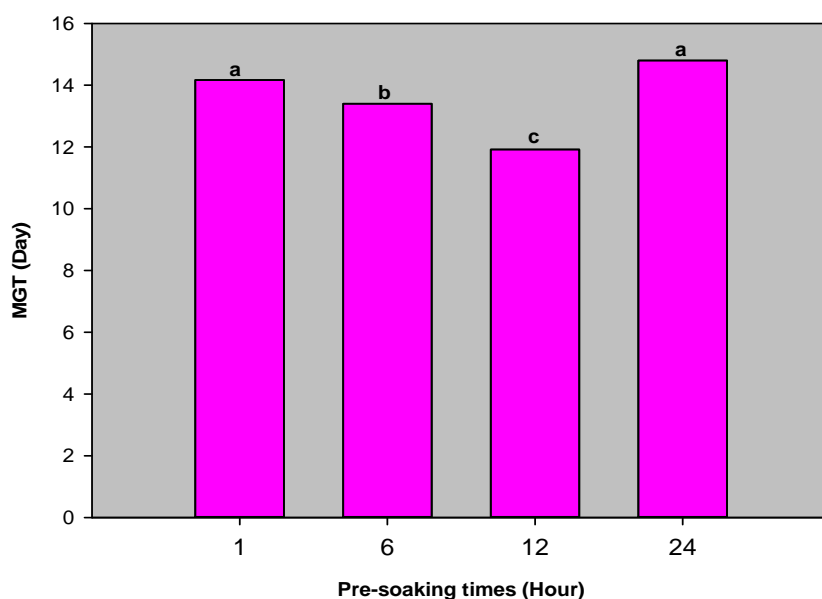


Figure 2. Changes in mean of germination time in different treatments (h) of 'Badami' pistachio cultivar.

### 3. Germination rate (GR).

Germination rate (%) was also affected by soaking time duration in this experiment. As GP significantly increased in seeds which were soaked in water for 24 h compared among other treatments, soaking of seeds for 6 h had the lowest percentage of GP in 'Badami' pistachio cultivar (Table 1). There were significant differences between soaking times on germination percentage in this cultivar.

Table 1. Effects of soaking of seeds on vigor index, germination rate, leaf number, root length, shoot dry weight, plant dry weight and plant diameter for 'Badami' pistachio cultivar.

Treatments (h)	Vigor index	Germination rate	Leaf number	Root length (cm)	Shoot dry weight (g)	Plant dry weight (g)	Diameter (mm)
1	85.4 a	25.3 b	14.5 a	23.2 a	0.763 a	0.998 a	4.7 a
6	76.6 a	20.4 c	14.0 a	11.7 a	0.886 a	1.130 a	5.4 a
12	54.7 a	25.4 b	13.5 a	14.1 a	0.758 a	0.975 a	4.7 a
24	59.3 a	33.2 a	18.0 a	11.8 a	0.607 a	0.743 a	4.0 a

### 4. Vigour index (VI).

The results of analysis showed that prolonging seed soaking times decreased the vigour index in pistachio seeds, although there were no significant differences among treatments (Table 1). These results are in agreement with the finding of Oskouie and Divsalar (2011) which showed that vigour was not affected by different level of nitrogen fertilizer on mother plants of rapeseed.

### 5. Seedling height.

Observation of experiment showed that there were no significant differences in plant height (cm) between different treatments in different periods after sowing the seeds (Figure 3), although, there were more height values in the 24-h treatment after 40 days in 'Badami' pistachio cultivar (Figure 3). These results are in agreement with the results of Sedaghat and Rahemi (2011) that reported application of polyamines for 12 h compared to 6 h increased the shoot length in 'Ghazvini' pistachio seeds. While, Khan et al. (1999) found

that normal water soaking for 48 h duration produced long seedlings compared to 12, 24 and 36 h duration in wild pistachio.

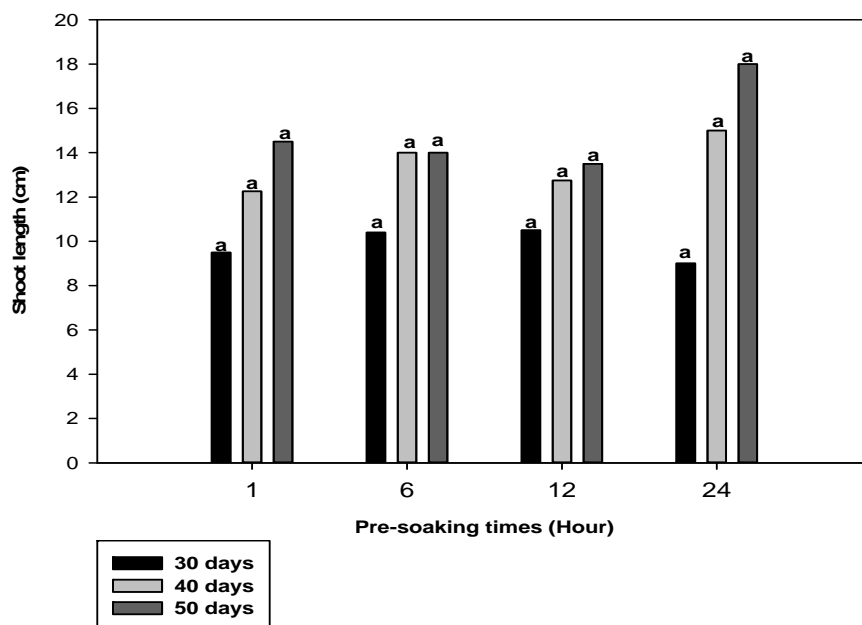


Figure 3. Changes in shoot length in different times after seed sowing in different soaking times in 'Badami' pistachio cultivar.

### 6. Root dry weight.

There were significant differences in root dry weight between 6 and 24 soaking treatments. Seeds which treated for 24 h had the lowest root dry weight among other treatments (Figure 4). Application of polyamines and distilled water (control) for 12 h compared to 6 h decreased the dry weight of roots in 'Ghazvini' pistachio seeds (Sedaghat and Rahemi, 2011).

There were no significant differences among different seed soaking times for leaf number, root length, shoot dry weight, plant dry weight, and trunk diameter (Khan et al., 1999) of pistachio seedlings (Table 1).

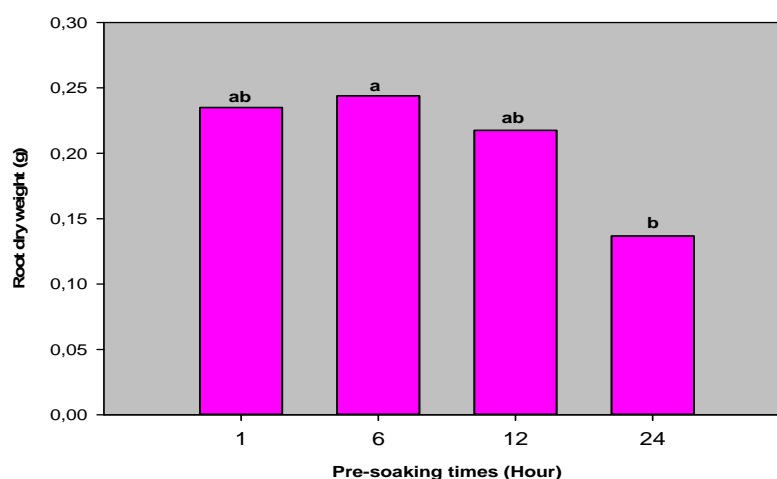


Figure 4. Changes in root dry weight in different soaking times in 'Badami' pistachio cultivar.

## Experiment 2

Evaluations of this experiment showed that there were no germinated seeds in any of the PEG solution treatments except the control. As we could not record any data on this part of our experiment, we were not able to compare the PEG treatments with control. In other words, osmopriming caused by all PEG treatments prevented the germination of pistachio seeds in this experiment. These results are in agreement with the finding of Zekri (1993) that indicated in germination of citrus seeds osmotic stress causes decline of seed water content, results in inhibition of seed germination and seedling growth. Although some references indicated that increasing PEG concentrations decreased germination percent in canola cultivars (Shahverdikandi et al., 2011) and in *Ziziphus lotus* (Maraghni et al., 2010).

## CONCLUSIONS

The result of this greenhouse experiment revealed that soaking seeds of pistachio for 24 h in distilled water improved germination capacity, germination rate and plant length growth compared to other treatments. Although, effects of this treatment on root dry weight and root length were decreased compared to 1 and 6 h treatments, a positive relationship was observed between the plant shoot length and plant leaf number. Thus, it is recommended to use distilled water for 24 h for *Pistacia vera* (different cultivars) seeds that lack dormancy to achieve the best germination parameters and plant length growth rates. The authors suggested evaluating the effects of PEG as priming or osmopriming treatments on other pistachio cultivars and species seeds.

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