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EFFECT OF COLD AND SCARIFICATION ON SEEDS GERMINATION OF *PISTACIA ATLANTICA* L. FOR RAPID MULTIPLICATION

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Abstract

This study was carried out to determine the effects of mechanical scarification and cold treatment of seeds at + 4°C before sowing seed of *Pistacia atlantica*. In these cases, the durations at cold temperature, which have been taken into account, are successively 60, 50, 40, and 30 days. After these periods, the rate of germination and the speed of germination are calculated every 4 days. The rates of germinations concerning the not scarified seeds having undergone a cold treatment lasting 50 days are the most important that is a value of 39% from the 09-05-2010 until the 29-05-2010. The speed germination is maximum that is 89 germinated seeds /4 days. In addition this same rate reaches a maximum of 70%. Concerning the pistachio tree seeds of the atlas treated mechanically with duration of cold treatment at + 4°C during 30 days. For the same category of seed the speed of germination is maximum that is 214 seeds/4 days. Thus the scarification and the 30 days stay are the fastest means which allow to obtain the seedlings of pistachio tree in a rather short time.

Introduction

The pistachio tree of the Atlas constitutes an important forest inheritance, it is a characteristic species of the Algerian Atlas as its name indicates it (Yousfi *et al*., 2003). It is a tree that exists from the margins of the Sahara to the medium sub-humid mountains. (Benabid & Fennane 1994) Belhadj (2007) notes that *P. atlantica* is very widespread in the Algerian south (arid and semi arid area).

The bark produces a resin-mastic, which naturally exudes in an abundant way in hot weather, the gum is used as disinfectant to cure the wounds (Jafri & El Gadi, 1978). This essence enters within the framework of the fight against desertification, it is used for the fixing of the dunes due to its well-developed root system (Herrera, 1997), and as a windbreak (Belhadj, 2002 and Maamri, 2008). The tree is very useful to receive the graft of *Pistacia vera*; the grafted trees are of a great strength, very rustic and of a remarkable longevity (Monastra *et al*., 2000). Today, this species undergoes a strong degradation, and many authors (Quezel & Santa, 1963, Monjauze, 1968, 1980, Quezel & Medail, 2003) qualified it as a "highly residual one and in phase of decline".

Wajid & Shoukat (1993) mention that the stage of germination results in the appearance of the root. By definition germination incorporates those events that commence with the up-take of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley & Black, 1994). The seed germination is a crucial and most sensitive stage in the life cycle of all plants (Al-Yemeni & Basahy, 1999; Afsheen *et al*., 2013). In addition Benmehioul *et al*., (2010) add that the emergence of the root is the indicator of germination. In fact Javaid *et al*., (2010) report that parthenium seeds show a variable dormancy during different months of the year. In nature, dormancy is used to protect seeds from conditions temporarily favourable to the germination, but which does not last and becomes quickly harmful for the survival of the young plants (Willan, 1992). According Sadeghi *et al*., (2009) the seed dormancy is mechanism that insures the survival of the species. The embryonic dormancy occurs when the embryo is not completely developed or caused by the presence of inhibitor of growth. The embryonic dormancy is mainly due to the abscisic action of the acid in the embryo, which blocks the action of the gibberellic acid responsible for the starting of germination. Embryonic dormancy would be related to the environmental conditions and can be due to the conditions in which the harvest and the maturation of the seeds are made (Vallée *et al*., 1999).These authors reported that , the tegumentary dormancy may be lifted by the scarification which is a mechanical treatment, which breaks or weakens the tegument (hulling). In fact Rabotovao, (2003) noted that germination is the perforation of the envelopes by the root due to a lengthening of the root cells representing the final stage of germination, it recovers the sequence of the events starting from the seed at rest until obtaining an autotrophic seedling (viable). However Cheema *et al*., (2010) add that the various factors, which affect the germination of seed during harvest, are the temperature and moisture. The dormancy of the seeds expresses a state in which a viable seed does not germinate even if it is under conditions regarded as favourable to germination. According to above discussed literature no work was done on effect of the duration of the cold and scarification of seeds of *P. atlantica,* in this context that the present study was realized.

Material and Methods

The vegetable material concerning this work is the pistachio tree of the Atlas (*Pistacia atlantica*) the *Pistacia atlantica* seeds have been collected in September 2009. The seeds of *P. atlantica* coming from semi arid area Djelfa to 20 km in the South-East of Messaâd (longitude: 03°40'22"E, Latitude:

34°02'11"N and altitude: 629 m) (Fig. 1). This study relates to the effect of mechanical scarification and the duration of the various thermal treatments of the *P. atlantica* seeds and its effect on germination. The seeds of the *P. atlantica* are divided into two categories of seeds of which the first contains 160 scarified seeds (Fig. 2a) and the second contains the same number of seeds which are not scarified (Fig. 2b). The seeds are put in the refrigerator at a + 4°C temperature during successively a month, 40, 50 and 60 days for each batch. Then they are treated by a solution of fungicide (10 ml of this fungicide + 90 ml of distilled water during 15 mn), the fungicide called Tachigazole, a fungicide against the *phythium*, *Aphanamyce* caticum and *Fusariums* fungus. The test of germination is led in 6 repetitions of 35 seeds in pots of 1.5 liter each. Each pot contains a mixture of ground made up of topsoil at a rate of 4 measures, a measure of sand and a measure of composts. The seeds were put in the ground at a depth equal to twice and half their diameters and sprinkled each day with tap water; the dates of sowings are shown on Table 1.

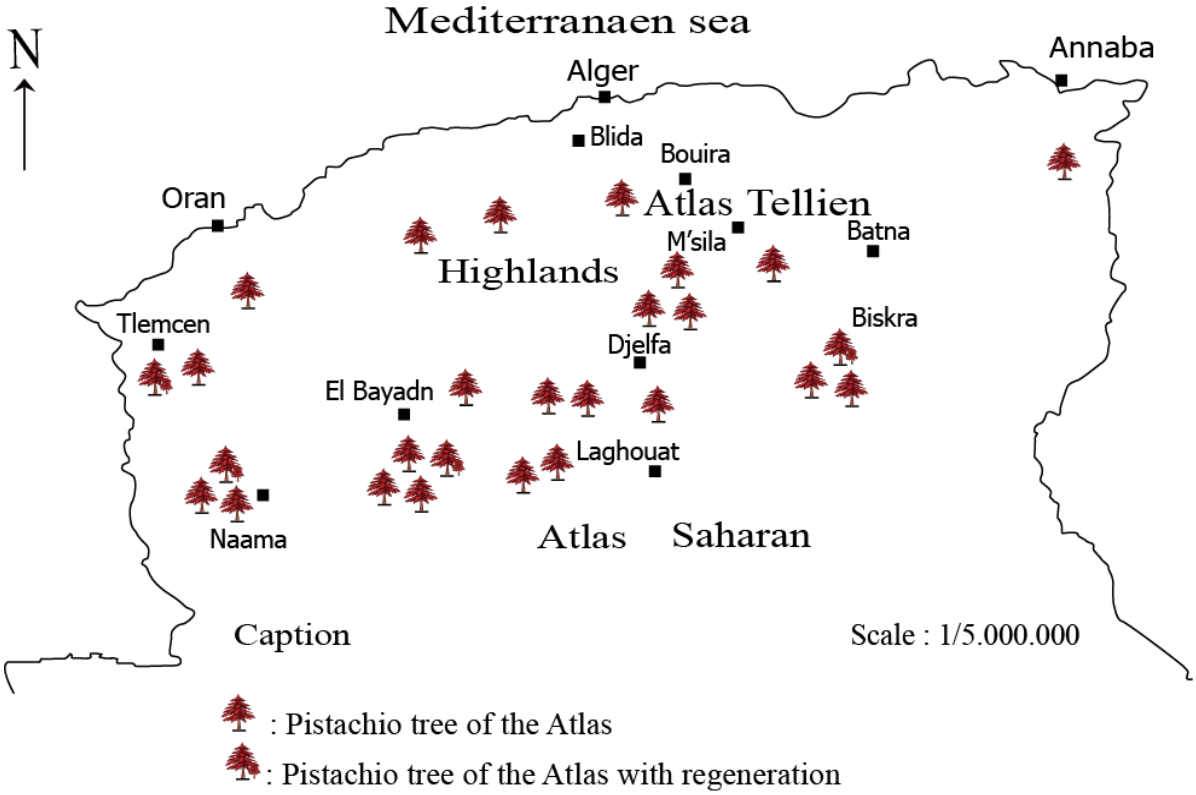
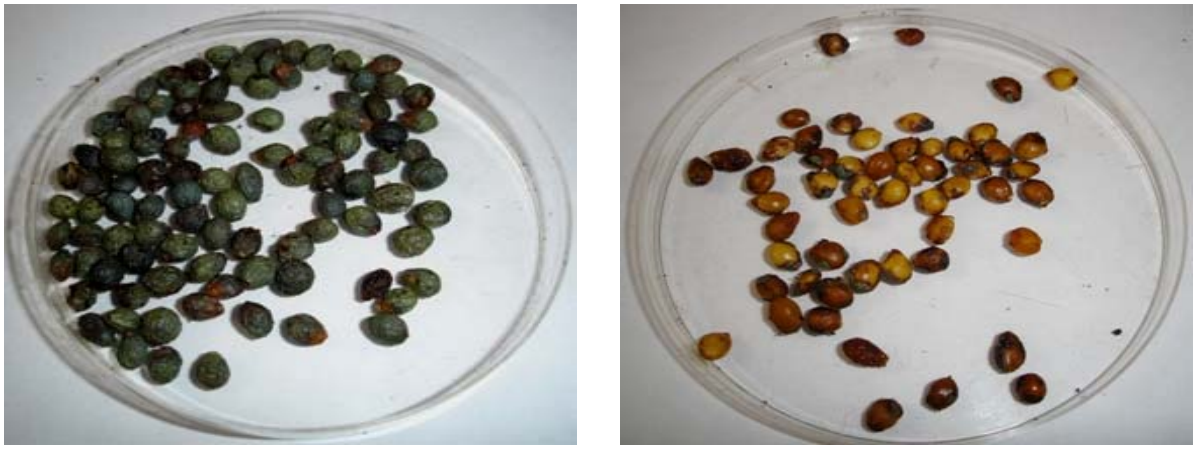


Fig. 1. The distribution of *P. atlantica* in Algeria.



a: Not scarified seeds b: Scarified seeds

Fig. 2. Seeds of *P. atlantica*.

Table 1. The sowing dates for the various heat treatments.

The dates of sowings	4th March 2010	8th March 2010	9th March 2010	10th March 2010
Duration of heat treatment	60 days	50 days	40 days	30 days

The counting consisted germinated scarified seeds and the non-scarified ones in each pot compared with the total number of seeds put in germination every 4 days after the sowing date. The test of germination was carried out at the room temperature of the laboratory. The following was noted.

The rate of germination: Evaluation of seed germination was evaluated each four days during two months, seed are considered germinated when the radicle emerged from the testa.

The rate of germination is calculated according to the following percentage:-

$$CG\ \% = (NGG / NTG) \times 100$$

CG (%) = Capacity of germination (percentage of seeds able to germinate under the conditions of the experimentation)

NGG = Number of germinated seeds.
NTG = Total number of seeds tested.

The speed of germination: The germination speed of seeds put under cultivation was equal to (Scott *et al.,* 1984):

$$V = NGG / T$$

V = Speed of germination

NGG = Number of germinated seeds.

T = Times of germination (days)

Statistical analysis: Data were processed using statistical 6.0 variance analysis (ANOVA) was applied in order to find a significant difference (at $\alpha = 5\%$ and confidence interval of 95%) between the different category of seeds and effect of duration on seeds efficiency of seed germination.

Results and Discussion

The pistachio tree seeds of the atlas underwent a cold stratification with an aim of lifting dormancy. It is in this context that Yaaqobi *et al*., (2009) note out that the duration of cold stratification at + 4°C improves the germinate potential of the pistachio tree seeds of the Atlas. This enabled us to obtain good results as long as the duration of putting in cold is prolonged.

For this purpose Rehman *et al*., (2011) who worked on seed germination of black raspberry added that the germination rate was observed that more 50% seeds germinated even during stratification after 45 days in case of seeds stratified in distilled water or Gibberellic acid. The preliminary treatment of seeds by the cold exerts a positive effect on activation of the germination process, under the effect of cold; the embryo that is in seed has completed its dormancy. The scarification of seeds started the process of germination after 20 days of putting under cultivation (Table 2). The result of scarification of seeds agrees with that of Ahoton *et al*., (2009) who has showed that the scarification of seeds favours significantly the process of germination, therefore it involved the fast inhibition of the tegument of seeds and the entry of water in the reserves that allows the fast exit of the root and the starting of the metabolic reactions of the embryo and the cotyledons.

Table 2. First starting date of seeds germination having undergone various periods of cold treatment.

Dates of sowing and heat treatment duration	4th March 60 days		8th March 50 days		9th March 40 days		10th March 30 days	
Categories of seeds	G N S	G S	G N S	G S	G N S	G S	G N S	G S
Number of germinated seeds	06	07	06	04	01	03	00	03
Number of days taken to germination	26 days 30 March 2010		22 days 30 March 2010		21 days 30 March 2010		20 days 30 March 2010	

G N S = Not scarified seeds, G S = Scarified seeds

Concerning the heat treatment

Abu-Qaoud (2007) has also found the same results, indeed he has applied various pre-treatments to the seeds of three pistachio trees species including *P. atlantica*, he has concluded that scarification was the fundamental factor that had raised the germination of seed. But the percentage of germination is finely larger when he has applied cold. In the present study the rates of germinations concerning the non-scarified seeds having undergone a cold treatment lasting 50 days are the most important, that is, a value of 39% from the 09-05-2010 until the 29-05-2010, this stability of the rate during 20 days explains the stopping of germination with the exhaustion of the tegumentary reserves (Fig. 3). Also the similar result was reported by Kafkas & Kaska (1998).

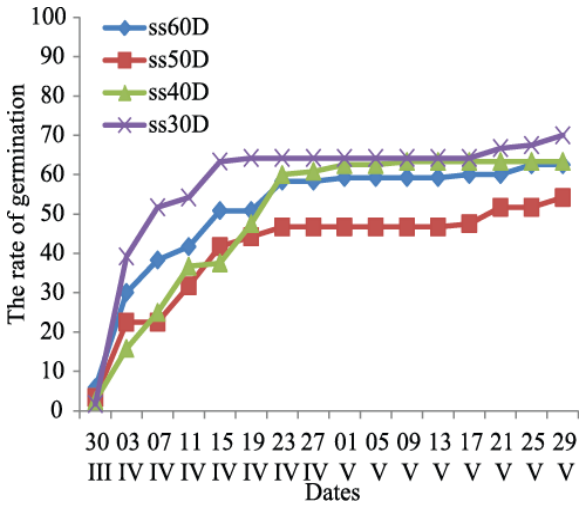


Fig. 3. The rate of germination scarified seeds of *P. atlantica*.

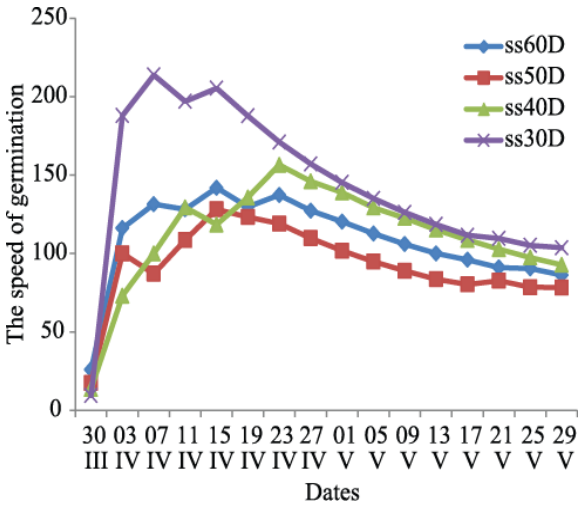


Fig. 5. The speed of germination scarified seeds of *P. atlantica*.

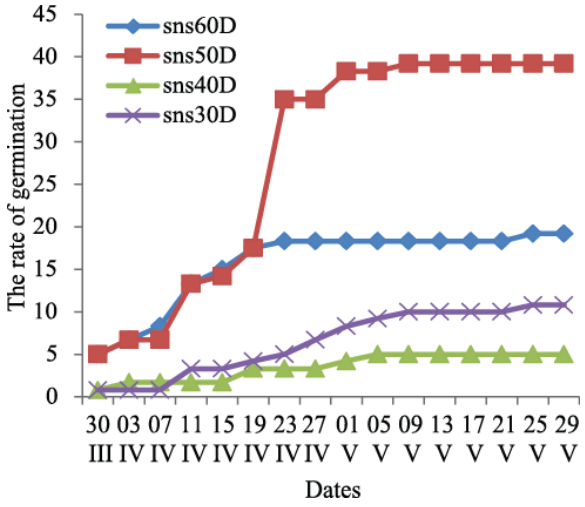


Fig. 4. The rate of germination non-scarified seeds of *P. atlantica*.

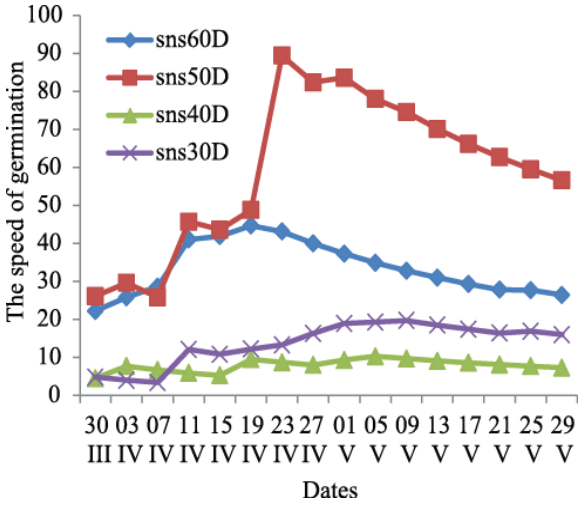


Fig. 6. The speed of germination not scarified seeds of *P. atlantica*.

On the other hand, the speed of germination is maximum for 89 germinated seeds/4 days on the 23-04-2010 (Fig. 5) In addition, this same rate reaches a maximum of 70% on the 29-05-2010, concerning the pistachio tree seeds of the atlas treated mechanically with a duration of cold treatment + 4°C during 30 days (Fig. 4). For the same categories of seeds the speed of germination is maximum on the 07-04-2010, that is, 214 seeds/4 days (Fig. 6). It is in this context that Werker (1981), Hanna, (1984) and Cavanagh, (1987) add that the seeds have impermeable teguments to water and to oxygen and require a scarification allowing the inhibition and germination. The latter is a dormancy of tegumentary origin or tegumentary inhibition since the embryo, removed from the tegument of seed, is ready to germinate, (Côme, 1970). In addition, Hadj Brahim, (1993) adds that the germination of the pistachio tree seed of the atlas put under the normal conditions in cultivation without any physical treatment may last 45 to 65 days.

The variance analysis of scarified and not scarified seeds with various cold stays shows that there is a significant difference knowing that the probability is of 0.0001 that is lower than 0.005 ($p<0.05$) for a confidence interval of 95%. The same results are also observed by Abu-Qaoud, (2007), he applied various pre-treatments to the seeds of three species of pistachio trees and among them *P. atlantica*, he concluded that scarification was the fundamental factor that has raised the germination of the seed. This same author adds that the variance analysis indicates that the treatment by stratification at + 4°C during 15, 30 and 45 days of selected seeds is very highly significant ($p<0.001$). But the percentage of germination is finely larger as he applied cold. Thus the results obtained in this present work indicate that the treatments given to the seeds of *P. atlantica,* be it: mechanical (scarification), or the cold have a positive effect on the rate of the germination of the seed (Table 3).

Table 3. The variance analysis of scarified and not scarified seeds with various cold stays.

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F of fisher	Probability> fisher's test
Model	22	64039.551	2910.889	48.941	0.0001
Residues	105	6245.188	59.478		
Total	**127**	**70284.739**			

Conclusion

The seeds *Pistacia atlantica* when subjected to a preliminary pre-treatment in cold of show that the rate and the speed of germination of scarified seeds are more important than those of not scarified seeds. In addition the scarified seeds require a 30 days stay only at one temperature of + 4°C. On the other hand this present study also demonstrates the utility of scarification and the duration of the heat treatment in order to accelerate the process of germination of this species, which take part in the intensive installation of the seedbeds of pistachio tree.

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