Effects of Temperature, Water and Salt Stress on Seed Germination of *Pinus Pinea L* in the Saida Region, Algeria

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Abstract
Pinus pinea (L) is one of the characteristic species of the Mediterranean flora, formerly introduced and naturalized in Algeria to stabilize and fix the western coastal dunes. The natural regeneration of Pinus pinea, like some other Mediterranean species, is facing serious problems. However, little or no research has been carried out on Pinus pinea in Algeria in terms of natural regeneration and the factors that determine it. Drought and salinity are abiotic constraints that can have a detrimental effect. In order to provide basic information for its multiplication, the effect of incubation temperature (15°C, 20°C,) and the impact of salt stress and water stress on seed germination were studied. Salinity treatments used were 2.92g/l, 5.84g/l, 11.68g/l, and under different osmotic potentials of Polyethylene glycol (PEG 6000) at 3g/l, 13g/l, 35g/l. Germination tests were carried out in Petri dishes. A maximum germination percentage (83%) was obtained under a temperature of 20°C. A negative correlation was obtained between NaCl concentration and seed germination, whereas in the concentration of 11.68g/l. germination was nil (0%), This suggests that Pinus pinea is moderately salt-tolerant at the germination stage. Pinus pinea seeds are moderately tolerant of water stress, with a germination capacity of 37% when exposed to a PEG 3 g/l solution, making this species a candidate for reforestation programs and consequently for extending its range.
Key words: *Pinus pinea*, germination, salt stress, water stress, PEG6000.

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1. Introduction

Pinus pinaster (L) is one of the characteristic species of the Mediterranean flora. Its total area worldwide is estimated at 600,000 ha, of which over 400,000 ha are in Spain. It can be found in

a pure state or mixed with maritime pine (Pinus pinaster Ait) or certain shrub species such as juniper (Juniperus) and oak (Quercus) (Barbeito, 2009).

Pinus pinaster is a heliophilous and thermophilous species. It is found in the Mediterranean bioclimatic zone, which varies from humid to semi-arid. It is sensitive to low temperatures, especially when the atmosphere is humid. It requires sunshine to ensure good fruiting (Seigue, 1985). This species has been used since ancient times for its economic importance, principally the production of wood and pine nuts (Moussouris and Rigato, 1999; Calama et al. 2003). In Algeria, Pinus pinaster was first introduced in 1935 to stabilize coastal dunes in the west, and subsequently in 1974 to fix coastal dunes. However, little or no research has been carried out on Pinus pinaster in Algeria in terms of natural regeneration and the factors that determine it. Drought and salinity are abiotic constraints that can have a detrimental effect on plants by limiting their growth, development and even their chances of survival (Sharma, 2019).

In this work we studied the effect of temperature, salt stress and water stress on Pinus pinea seed germination with the aim of providing basic information for its propagation.

2. Materials and methods

The aim of this study was to determine the impact of salt and water stress on the germination behaviour of Pinus pinea seeds, which can be considered as a good indicator of the degree of plant tolerance to these two stresses.

2.1 Plant material

For this study, we used mature Pinus pinea seeds collected in September 2021 in the Constantine region (Algeria).

Figure 1: Pinion pine seeds.

2.2 Experimental set-up

Seeds were sterilized with a 5% sodium hypochlorite solution for 5 min, then rinsed five times with distilled water. The seeds were then rinsed five times with distilled water to prevent any fungal attack.

Several batches of seeds were germinated at two different temperatures: 15 and 25°C on absorbent paper in Petri dishes. Each dish contained 10 seeds, and each NaCl and PEG-6000 treatment was repeated three times. A few drops of each solution were added to each Petri dish, every 48 h. The number of germinated seeds was counted every day during our experiment.

2.3 Application of stress

Experiment 1: Effect of seed incubation temperature on germination

To determine the optimal germination temperature and test seed reliability and dormancy, seed germination was studied under different incubation temperatures15°C and 20°C.

Experiment 2: Effect of water and salt stress on germination.

Seed germination was studied under different concentrations of NaCl 2.92g/l, 5.84g/l, 11.68g/l. and under different osmotic potentials of Polyethylene glycol (PEG 6000) at 3g/l, 13g/l, 35g/l.

Figure 2: Sodium chloride and PEG 6000.

Figure 3: Preparing seeds for germination under the three different stresses studied.

3. Results and discussion

3.1 Effect of seed incubation temperatures on germination rates

According to our results, the optimum temperature for germination of Pinus pinaster seeds is 20°C, with a germination percentage equal to 83%. This behavior may explain the strong adaptation of this species to ecological conditions in arid environments characterized by relatively high temperatures.

Thanos et al (2000) and Baskin and Baskin (1998) reveal that this behavior reflects a possible survival strategy typical of Mediterranean plants with optimum temperatures ranging from 15°C to 30°C .

Temperature remains a determining factor for germination, which can largely influence the capacity and percentage of germination by eliminating the different types of dormancy. Adili (2012), confirms that Pinus
pinaster is among the seed species without dormancy. The germinative capacity of the seeds varies between 70 and 90%, and their longevity is 4 to 6 months on average, and can extend to over a year when kept in dry cold. Favourable temperature ranges for germination depend largely on the time of year when the plants complete their life cycle, the geographical origin of the species and the quality of the seeds.

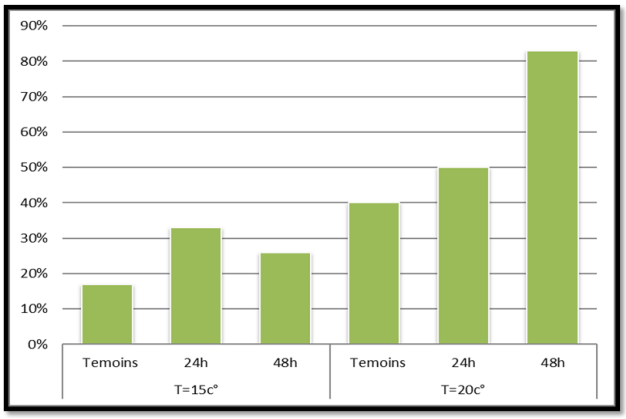

Figure 4: Germination rate of Pinus pinaster seeds at different temperatures (15C°; 20C°).

Figure 5: Pinion pine seedlings germinated at 20C°.

3.2 Salt stress effect

The results show significant differences in seed germination rates. Indeed, increasing NaCl concentration levels resulted in a reduction in germination percentage, which can be attributed to a decrease in water uptake created by salinity, consequently inhibiting germination and radicle elongation (agrimi, 1993). The concentration of 11.8 g/l marked a germination rate of 0% (Fig 6), this salt content presents a critical threshold for pinion pine seeds.

Touaba&Alatou (2018), attest that the species is indifferent to the chemical nature of the soil; however, active limestone and soil salinity, without prohibiting the tree's development, can limit its growth.

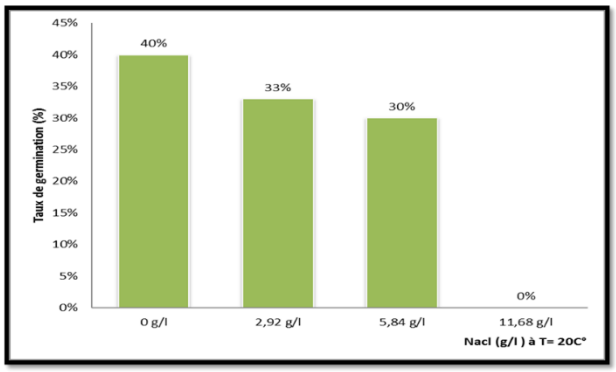

Figure 6: Germination rate of Pinus pinaster seeds under different NaCl concentrations.

3.3 The effect of water stress

A high negative correlation was obtained between germination percentage and PEG 6000 concentrations, showing that any decrease in osmotic potential led to a reduction in pinion seed germination and vice versa. The results of this study indicate that pinion seeds are moderately tolerant to water stress (Fig 7). In a Mediterranean climate, germination is often limited to short wet periods, either in spring or autumn and not during the summer (García-Fayos et al. 2000, Quilichini and Debussche, 2000).

Moisture has always been considered an important factor in seed germination and emergence during the first growing season (Vaartaja, 1950; Tirén, 1952; Winsa, 1995; de Chantal, 2003). Optimum water content (soil moisture close to the point of permanent wilting) is required to trigger seed germination, while increasing water content is required for seedling growth (Wangchuk, 2007).

Most plants in the Mediterranean basin are exposed to water stress during the summer. Thus, high soil moisture during summer drought is often crucial for seedling establishment (Pardos et al. 2007).

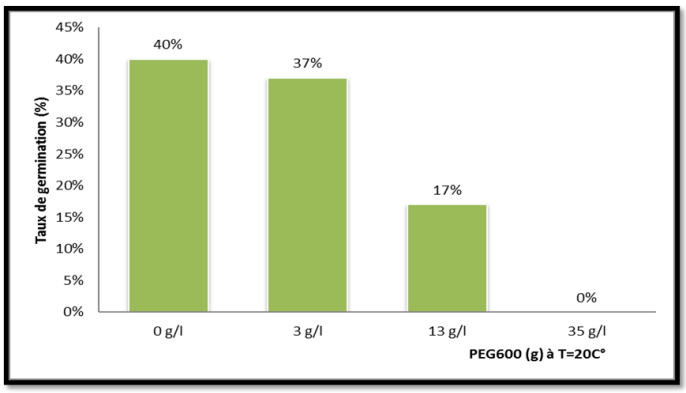

Figure 7: Germination rate of pinion pine seeds under different osmotic potentials based on PEG 6000 (polyethylene glycol).

4.Conclusions

Seed germination is under the effect of various external environmental factors, although the most essential are the combination of temperature, humidity and light (Adili, 2012). This can be particularly critical for species whose seeds do not undergo dormancy and therefore cannot form a persistent seed bank and will therefore be vulnerable to climatic hazards. The impact of salt and

water stress on germination behavior can be considered a good indicator of the degree of plant tolerance to these two constraints. Although it does not fully reproduce the behavior of plants under stress conditions, germination rate provides more or less accurate information on their ability to tolerate stress.

Based on the data from the present study, we concluded that Pinus pinaster seeds were not dormant, and that germination rate increased in response to increasing temperature, until an optimum of around 20°C was reached. High concentrations of NaCl inhibit germination. It should also be noted that Pinus pinaster seeds are fairly tolerant of water stress, which means that this species can be considered for reforestation programs and, consequently, for extending its range. By virtue of its moisture resistance, Pinus pinaster wood is used for the construction of boats, pilings, mine timbers, lumber, poles and rough joinery. It is also used in paper mills for the production of cellulose and mechanical pulp (Sghaier et all, 2006).

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