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Research Article

Storing cork oak (*Quercus suber*) seeds under different temperatures and the effect of storage treatments on the germination and growth of stored seeds

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Abstract
The cork oak "Quercus suber L." is one of the most important forest trees in the world, especially in the Mediterranean Basin, due to its high environmental, socioeconomic, and landscape value throughout the year. In general, these trees face particular problems that affect their germination, growth, survival, and the other biotic and abiotic causes that affect natural regrowth.Thus, the most important of these problems is the annual irregularity in tree seed production. For this reason, The storage problems of this species are of concern to managers, who are using new alternative techniques to deal with the problem of the irregular annual production of oak seeds and to preserve them for as long as possible. The present work aimed to evaluate the effect of storing cork oak *Quercus suber* L. seeds at different temperatures and for different periods of up to 300 days on their vitality and ability to germinate. For this, the seeds were subjected to four storage parameters at four temperature levels: -0.5°C, -1°C, +4°C with thermotherapy, and +4°C without thermotherapy as a control from January 2019 to September 2019. Analysis of the obtained results showed that the treatments studied significantly affected the measured parameters. After 30 and 60 days, the results of the 4 treatments concluded that the fourth storage treatment (storage of seeds directly at +4°C) presented a good performance in terms of the length of the seedlings, which was high (52.45 cm), the production of seedlings with an average diameter of 5.28 mm, and the important germination percentage (88.57%), unlike the other treatments. After a storage period of 270 days, the fourth storage treatment, the control, stored at 4°C without thermotherapy, still showed the best growth and development. This treatment also achieved a higher germination percentage (17.14%), plant height (11.33cm), and average diameter (1.85 mm) than the other treatments.

Keywords: Cork oak seeds, Germination, Growth, Storage parameters, Vitality

INTRODUCTION

According to E.P.F.-CT97-3480 (2001), the world's forests contain many of the most prosperous ecosystems on Earth, where the forests are a source of many products such as volatile oils, herbal medicines, fuels, food, furniture, and clothing. Furthermore, forests prevent soil erosion, help regulate the climate, and provide clean water, essential to people's lives and well-being worldwide. In fact, the forests are exposed to enormous pressures and challenges such as deforestation, increased carbon emissions, and shortages of water and nutrients. Despite the knowledge of the threats to the survival of endemic species in forests, many people are not aware of the serious condition in which some trees exist. Also, the studies indicate that many types of trees are threatened with extinction, as many types of trees of economic importance are at risk of extinction due to the unsustainable use of forests (UNEP, 1987)

The cork oak trees face problems in their natural regrowth to meet the challenges faced by forests in general, and oak trees face special problems that affect them in terms of germination, growth, and survival. In this regard, the most important of these problems is the annual irregularity of trees in the production of seeds. Perez-Ramos *et al*. (2014) confirmed this in their study, where they proved a discrepancy between cork oak individuals in seed production during the same year.

In another study conducted by Vieitez *et al*. (2012) for the germination of *Quercus suber* L. seeds and addressing the problem of annual irregularity in its production and its impact on the natural renewal of oak forests, new mechanisms and techniques of seed storage and cryopreservation were used to maintain the viability of embryos in cork oak seeds. Also, previous studies showed a clear fluctuation in the annual production of cork oak seeds. Martin *et al*. (1998) studied the effect of environmental factors on cork oak seed production. The results showed a fluctuation in the number of seeds produced. Seed productivity fluctuated from year to year in all the regions under study. The present study aimed to study the role of the different storage processes of cork oak seeds in forest nurseries to determine the most appropriate heat treatment for storing oak seeds. Additionally, the goal was to achieve optimal germination and growth rates for seeds, preserve their vitality for as long as possible during storage, and solve this problem.

MATERIALS AND METHODS

Experimentation

The process of collecting the seeds needed to store the acorns of *Quercus suber* was carried out in the Maamora Suberaie Forest on December 11, 2018. These seeds were processed at the level of the laboratory of the forest research center; after sorting, according to Pintos *et al*. (2008) and Monteleone *et al*. (2002), two methods were used:

Method I

The acorns were first treated with thermotherapy for two hours and thirty minutes at 41 °C. Secondly, the acorns were taken out for a five-minute break. Thirdly, a fungicide was applied to the acorns. After this, the acorns were kept at 4°C for 24 hours. Following the cold treatment, the seedlings received a second treatment with Previcur, a fungicide, at a ratio of 10 milliliters per seven liters of water.

Subsequently, three groups of treated seeds were divided, with twelve bags each. There were fifty seeds in each bag. Lastly, a plastic coating was applied to the bags holding the seeds. The seed packets were subjected to three temperatures: +4°C, -0.5°C, and -1°C.

Method II

The acorns were put in just after harvesting, sorted, and then treated against fungi with a fungicide (Previcur) at a rate of 10 ml per 7 liters of water. They were put in containers filled with wood and closed hermetically. After that, they were placed in a refrigerator at a temperature of 4 °C.

Seed storage modalities

Seeds of cork oak were stored at five treatments as follows:

Control: No antifungal treatment (Prévicur) or storage procedure was applied to the seeds.

Treatment I: The seeds were treated in a 41 °C water bath for two and a half hours, then cooled and stored at -0.5 °C.

In Storage Treatment No. I, 12 plastic bags were stored in the cooler at a temperature of -0.5 °C, so each bag represented the monthly planting of seeds. The seeds were treated in a water bath at 41 °C for two and a half hours, then exposed to air for cooling and treated with a fungicide (Previcor) before storage.

Treatment II: Seeds were treated in a 41°C water bath for two and a half hours, then cooled and stored at -1°C.

In Storage Treatment No. II, 12 plastic bags were stored in the cooler below -1 °C, and a water bath treated the seeds at a temperature of 41 °C for two and a half hours, then exposed to air for cooling and treated with a fungicide (Previcor) before storage.

Treatment III: Seeds were treated in a 41 °C water bath for two and a half hours, then cooled and stored at +4 °C.

In Storage Treatment No. III, the seeds were treated in a water bath at a temperature of 41 °C for two and a half hours, then exposed to air for cooling and treated with a fungicide (Previcor), and the seeds were packed in 12 plastic bags so that each bag contained 50 seeds. The bags were stored in coolant at +4 °C.

Treatment IV: The seeds were treated directly at +4 °C in addition to the standard treatment (no storage).

Treatment IV: The storage treatment in which the seeds were stored directly without a water bath, as in Treatments I, II, and III, and storage is at +4 °C in 12 plastic bags, the first planting process of seeds for all storage transactions was 30 days after the start of storage, and the cultivation process was repeated every 30 days, where the second cultivation was after 60 days of storage, the third cultivation after 90 days of storage, and so on until the last cultivation of all different types of storage was after 300 days from the beginning of storage (Fig. 1).

Experimental design

The planting medium that was prepared was the soil (natural or artificial), which was a means of material and nutritional support for the plant where the root system of the plant developed. The local soil that was used was compost from Maamora Forest. It consisted of materials rich in humus and soil rich in organic materials mixed with commercial compost by mixing the soil with peat moss as a growth medium.

The medium for germination and growth was mixed with organic fertilizer, and the experimental units were filled and moved to compact them. Once the units were entirely filled, they were moved for the last time, and the excess soil was removed. The experimental units were placed on platforms with a height of 20 cm from the ground's surface. The plastic experimental units used in agriculture were solid plastic units in the form of a truncated pyramid. These ponds were perforated in the base and fixed from the inside with grooves, or "root guides.

The units for planting had dimensions (cm 37 * cm 49), and the units were divided from the inside into 35 sections. The size of the experimental unit was 500 cc. One seed was placed in each section, and one unit represented one experimental unit with 35 seeds. All agricultural operations were carried out, starting with planting seeds, irrigation, removing harmful weeds, and using some treatments against fungal or insect diseases when necessary (Fig. 1).

Parameters measured

The results were taken at the end of October 2019 by taking measurements of the traits studied, such as the percentage of germination (%), the diameter of the stem (mm) in plants, and the height of the plants (cm). Thus, the average was calculated for each of them by taking the arithmetic mean of all the studied traits and thus, the average percentage of germination per treatment, the average diameter of plants per treatment and the average height of plants per treatment were obtained.

Statistical analysis

The results were compared with the results of the control cultivation (cultivating seeds without storage) and the percentages of all studied traits were compared with the results of the control cultivation and converted

Fig. 1. *Production and storage of cork oak acorns after 300 days in the nursery, A : Sorting of acorns , B : Thermotherapy of acorns at 41 °C for 2 hours and 30 minutes, C : drying of acorns after thermotherapy, D : coating of the glands for their preservation in different temperatures: +04°C, -0.5°C and -01°C, E : acorn storage in wood sweat, F : filling of cultivation racks with substrate for cultivation of preserved acorns, G : cultivation of preserved acorns, H : acorn germination, I : development and growth of cork oak seedlings after conservation*

into graphs to clarify the differences between germination and growth rates of *Q. suber* seeds. The results were subjected to statistical analysis using SAS version. 9.1 (2002) to find out the least significant difference (LSD) between the averages of the transactions under study and the significance of their impact on the studied factors with a value of 0.05 to find an answer to the study's questions.

RESULTS AND DISCUSSION

Experiment studied the effect of four oak seed storage treatments on seed germination, seedling length, and diameter for ten months (300 days) from January 2019 to October 2019. The results on oak seed storage treatments (Table 1) show that the studied treatments had a significant effect, with a probability (P<0.01), on both germination rate (2749.085**) and seedling length (772.746**). In contrast, the differences between treatments did not reach a significant degree in their impact on seedling diameter (35.175). However, the information in the figures shows the averages of the four storage treatments under study during the ten months, in addition to the standard treatment. So, the results indicate (Fig. 2) the superiority of the fourth storage treatment (treatment IV) (storing seeds directly at +4°C) for thirty days in seedling length (52.45 cm), followed by the first treatment (seeds treated in a water bath, then cooled and stored at -0.5°C) for 120 days. Stem length (49.33 cm) Then, in the second and third treatments (seeds treated in a water bath, then cooled and stored at -1°C; seeds treated in a water bath, then cooled and stored at +4°C), the seedling length reached 42.16 and 41.79 cm, respectively.

On the other hand, in Fig. 3, no significant differences were recorded between the tested treatments during the ten months in the diameter of the resulting seedling, except that the fourth treatment (storing seeds directly at +4°C) for 30 days produced the most seedlings in diameter with an average of 5.28 mm, but it did not differ significantly from the rest of the transactions. Also, the effect of treatments on acorn seed germination rate was significant (P<0.01). The maximum germination percentage (88.57%) was recorded for the standard treatment and the fourth storage treatment (treatment IV) (storage directly at +4°C) for 30 and 60 days, respectively. It is clear from the results that the fourth treatment after 30 days was significantly superior to seedling length and seedling diameter in a non-significant degree and to the percentage of germination, whether after 30 days or 60 days, which did not differ from the standard treatment. This may be due to the cork oak seeds retaining their moisture content, which was not affected by storage periods of 30 or 60 days. This was confirmed by Pintos *et al*. (2008), who could preserve oak seeds at a temperature of +4 °C for two months without a significant decrease in their vital abilities.

In addition, the results (Fig. 4) showed that storage periods could maintain the vitality of oak seeds if they retained their moisture content. The 180-day storage treatment in treatments No. III, II, and IV succeeded in

Table 1. Effect of seed storage periods and germination coefficients on germination percentage, length and diameter of oak seedlings

S.O.V.	d.f.	Length	Diameter	Germination%
Rep	2	2.861	29.542	0.313
Treatments	40	772.746**	35.175 ns	2749.085**
Error	80	0.385	28.030	0.015

**: highly significant differences with a probability (<0.01); Ns: non-significant differences

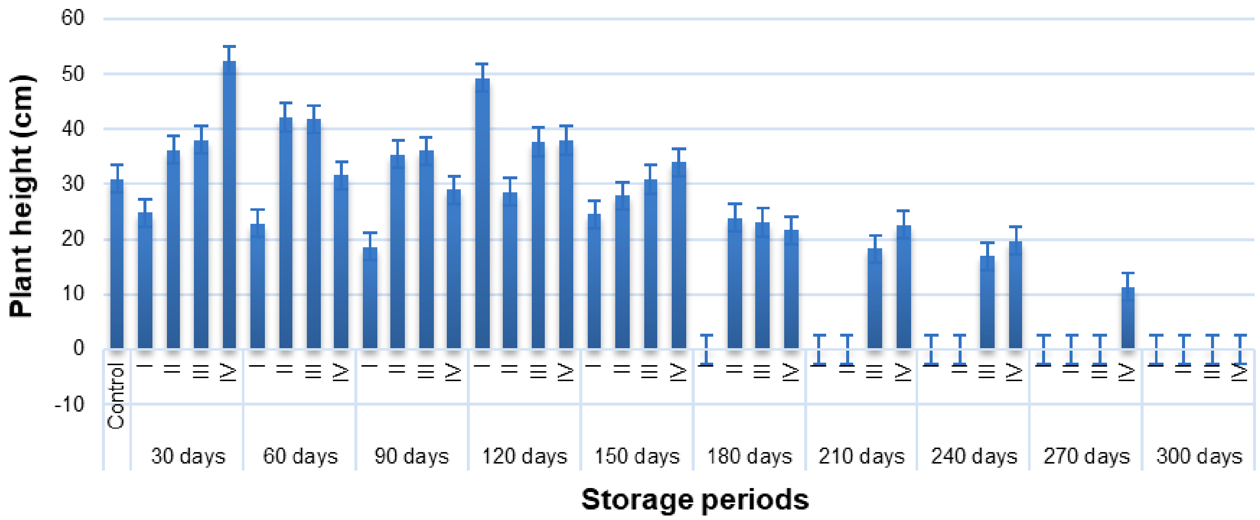

Fig. 2. *Effect of storage periods on plant height (cm)*

retaining their vitality and achieved a good germination rate and stem length. It is reported by Monteleone *et al*. (2002) that the seeds stored for 9 months, in which the moisture content did not decrease to 42%, had the same germination rate as the non-stored seeds, while in present study, the treatment, the stored seeds in treatment I failed to achieve a germination percentage and did not germinate completely after a 180-day storage period, as did treatment II after a storage period of 210 days and treatment III after 270 days. This is due to several reasons, including the low moisture content of stored seeds, as Zulueta and Montoto (1992) reported. The germination percentage decreases significantly when it loses 40% of its moisture in long-term storage and becomes a suitable medium for the growth of fungi. It increases the rate of infection of the seeds with fungi, which causes a decrease in the percentage of germination and growth, as confirmed by Merouani *et al*. (2005).

Therefore, Fig. 4 shows the continuation of germination in treatment IV (storage at +4 °C), where this treatment achieved a germination rate of 17.14% and a plant height of 11.33 cm after a storage period of 270 days (9 months), while the rest of the seed storage treatments failed to achieve a germination rate at all. This may be because the seeds were able to maintain a moisture content of no less than 40% at a low temperature of +4°C, which enabled them to produce a percentage of germination as reported by Santos and Bernardino (1995), which requires finding a medium that ensures that there is no increase in moisture in the storage medium and the growth of fungi during storage is not allowed, with the seeds maintaining their moisture during storage periods.

The studies have shown the effect of some climate factors, such as moisture stress in the summer and temperatures in the spring, on the annual production of oak seeds (Pons and Pausas, 2012). In addition, Cao *et al*. (2013) indicated that the resulting seed yield was affected by the mite infestation rate and pointed to several other reasons that affect the annual variation in seed production, as they estimated the number of seeds produced from two types of oak trees (*Q. suber*, *Q. canariensis*).The results of this study showed that trees living in fertile soil and a humid environment were more productive of seeds than those planted in specific fertility areas, and that seed production depends mainly on soil fertility. In addition to the surrounding environmental

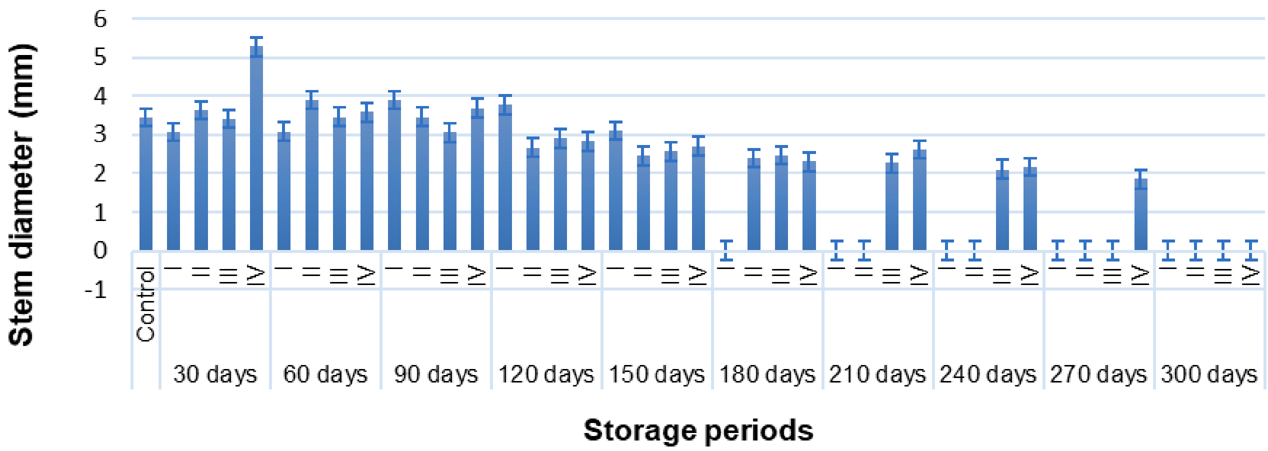

Fig. 3. *Effect of storage periods on stem diameter (mm)*

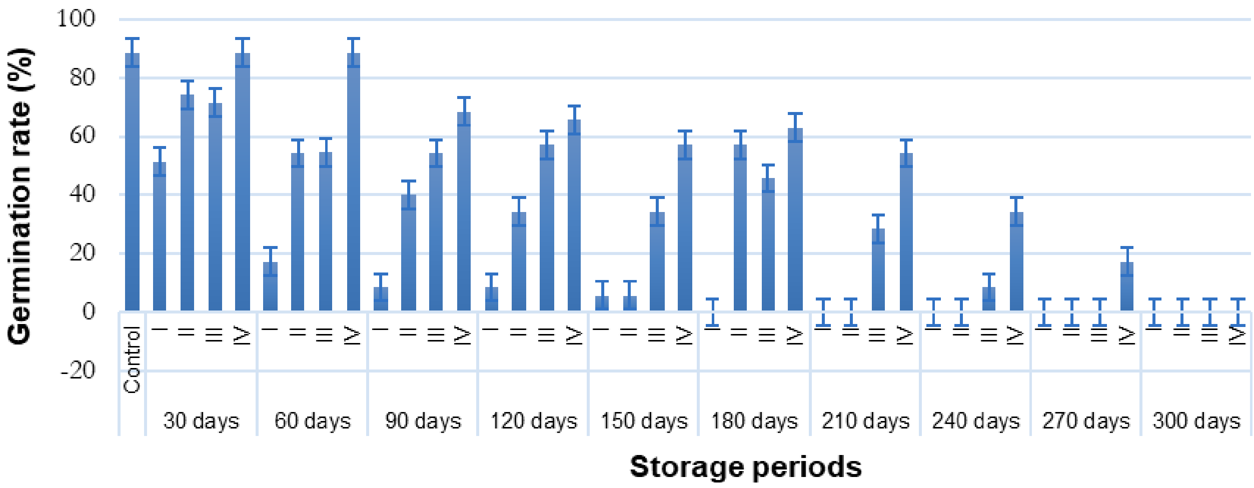

Fig. 4. *Effect of storage periods on germination rate*

factors and its genetic makeup, the studies have also indicated that the annual production of seeds is affected by the quality of trees, whether they are deciduous or perennial (Perez-Ramos *et al*., 2015).

Here, the researchers found that deciduous oaks had fewer seeds and were more volatile from year to year than evergreen oaks (*Q. suber*). This may explain why seed production is affected by defoliation and drought conditions, which lead to a decrease in seed production. For these reasons, it was necessary to think about finding alternative techniques to address the problem of irregular oak trees in the production of seeds annually and to keep them for the longest possible period, considering that they retain their vitality and ability to germinate again after storage. This means intending to use and plant them, achieving the necessary re-germination of forests throughout the year, and compensating for the fluctuation in the production of trees for seeds annually.

Many studies have dealt with many ways to store cork oak seeds at different temperatures in an attempt to provide seeds with germination, vitality, and good morphological characteristics for the longest possible period to compensate for the fluctuation and difference in the annual seed production of oak trees, but the seeds faced many difficulties when storing them, such as low humidity, moisture content, and the percentage of moisture inside the seeds when stored (Zulueta and Montoto, 1992). This last results indicated that the percentage of germination decreased significantly when *Q. suber* seeds lost 40% of their moisture, in addition to the difficulty of storing oak seeds for a long time due to their high moisture content. (Santos and Bernardino, 1995) showed that the seeds with a moisture content of more than 50% and less than 40% have a low life, and this meaning was confirmed by Belletti *et al*. (2001) that the moisture content of the seeds should be more than 40%. Here, the percentage of germination and germination of seeds with a moisture content of 42%, stored at zero centigrade, were similar to fresh seeds. This was also confirmed in a study conducted by Merouani *et al*. (2001), where he dried the cork oak seeds and stored the seeds for six months at a temperature of zero degrees Celsius. The seeds were identical in morphological and physiological maturity; their moisture content was 44%-47%, and the germination rate exceeded 92%.

It was observed that there is a relationship between the vitality of the seeds and their moisture content, and the germination rate decreased sharply when the moisture content of the seeds was less than 30%. Monteleone *et al*. (2002) studied the effect of seed storage on temperatures (-5, -2, 0, and 3°C). The seeds were stored at different temperatures and humidity levels for 3–12 months. The results indicated that after 9 months, the percentage of germination of seeds with a moisture content of 42% and stored at zero percent was similar to that of non-stored seeds. Branco *et al*. (2002) studied the effect of immersing the seeds in a water bath (45°C) on the vitality of insects and seeds. Therefore, the temperature did not affect the quality and vitality of the embryo, seed germination, or the growth of oak seedlings. In addition, the fungal infections during the storage period affected the vitality of the seeds and their germination rate due to the high humidity during storage. This was confirmed by Merouani *et al*. (2005). The studies showed the sensitivity of *Q. suber* seeds during storage at low temperatures (much less than zero Celsius), and this was confirmed by Xin *et al*. (2010).

Conclusion

The present study showed that the best storage treatment for cork oak seeds was treatment IV (storage at +4°C). The maximum germination percentage (88.57%) was recorded for each standard treatment (control) and the IV treatment of storage (storage directly at +4°C) for 30 or 60 days.The fourth treatment after 30 days was significantly superior to seedling length and seedling diameter by a non-significant degree and to the germination percentage, whether after 30 days or 60 days, which did not differ from the standard treatment. This treatment also achieved a germination rate of 17.14% and a plant height of 11.33 cm after a storage period of 270 days (9 months). This may be because the seeds retained some moisture content while being stored at a low temperature of +4°C, while the rest of the seed storage treatments failed to achieve a germination rate. This was due to several reasons, such as the low moisture content of stored seeds and the germination percentage decreased significantly when it lost 40% of its moisture. The decrease or absence of germination of *Q. suber* seeds is also due to the increase in humidity of the storage medium over long periods, as it turns into a medium suitable for the growth of fungi and the increase in the infection rate of the seeds with fungi. This leads to a decrease in the percentage of germination and growth, which requires finding solutions to the problem of high humidity during storage and ensuring that moisture does not increase during storage and does not allow the development of fungi and infection of seeds during storage while maintaining the seeds of humidity and not losing moisture during storage periods.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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