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Effects of substrate on the germination and seedling growth of *Quercus suber* L.

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Abstract. The seedling quality is one of the most important factors for the success of reforestation programs. In this sense, this work aimed to evaluate the effects of substrate on the germination of cork oak acorns from El Aouana forest, located in the Jijel region of north-eastern Algeria, and on the performance of seedlings, particularly their growth. The experiment was performed in the nursery of the Tlemcen Forest Conservation. For this purpose, five substrates were used: S1 (sand), S2 (topsoil), S3 (potting soil), S4 (1/2 sand + 1/2 topsoil) and S5 (1/3 sand + 1/3 topsoil + 1/3 potting soil). Germination and survival rates, and seedling morphological traits: average height of seedlings, average root collar diameter, stem height/root collar diameter ratio (H/D), average number of leaves per plant, leaf length, leaf width and leaf area, were evaluated. Results obtained after 16 months of monitoring in the nursery showed high germination rates of 91.4%, with an average survival rate of 89.5%, and significant differences were recorded between the substrates tested. In terms of growth, the best results were obtained with the potting soil substrate (S3) for all parameters. The lowest yields were recorded in seedlings grown on sand alone (S1).

Key words: cork oak, substrates, growth parameters, nursery, El Aouana-Jijel

1. Introduction

The cork oak *Quercus suber* L. of the Fagaceae family, is an evergreen sclerophyllous oak restricted to the western part of the Mediterranean Basin, whose origin dates back to the Tertiary (Natividade 1956; Tutin *et al.* 1964). It is part of the upper Pliocene flora (Lamey 1893; Emberger 1930; Vilar 1934; Boudy 1950; Quezel 2000; Manos & Stanford 2001; Merouani *et al.* 2001). It is part of the upper Pliocene flora (Lamey 1893; Emberger 1930; Vilar 1934; Boudy 1950; Quezel 2000; Manos & Stanford 2001; Merouani *et al.* 2001). The range of this species covers approximately 2,7 million hectares (Mendes & Graca 2009). The largest areas are in the Iberian Peninsula, particularly in Portugal and Spain, corresponding to more than 50% of the global distribution area (Silva & Catry 2006). Cork oak is also present in other southern European countries, including France and Italy, and in North Africa, Algeria, Morocco and Tunisia.

In Algeria, the cork forests initially covered an area varying between 410,000 and 480,000 ha (Saccardy 1937; Boudy 1952; Natividade 1956; Seigue 1985; Richard 1987; Iprocor 1999; Quezel & Medail 2003), and extended over the territory of 23 departments, from the Mediterranean coast in the North to the Tellian mountain range in the South, with 4/5 of them in the east of the country (Bouhraoua *et al.* 2014). Currently, 220,000 ha are productive. The low cork production in recent years is mainly due to decline of the cork production areas (Dehane *et al.* 2013). This regression is the result of many factors, including low natural regeneration, repeated fires on the same plots, pest attacks, competition from other woody species, tree aging and overgrazing.

The problem of regeneration of the cork oak was posed to foresters as early as 1930 (Marion 1955). Natural regeneration by seeds does not occur every year. It depends on years of good fruiting and good density and spatial distribution of a forest stand (El Antry & Piazzetta 2014; Varela & Piazzetta 2014). Indeed, the stock of acorns, the main source of generative stand regeneration, suffers great losses on the ground and on the tree, due to multiple predators: wild boars, deer, rodents, birds, insects, as well as man and his animals, which are very active and feed both on acorns and seedlings.

To this must be added the low and irregular acorn production (Nsibi 2005). To overcome these difficulties, artificial regeneration is an interesting alternative.

The production of forest seedlings with the required biological and physiological characteristics to ensure their survival and growth after transplantation is an essential step for successful reforestation programs (Ammari *et al.* 2006).

Many researchers have been interested in producing growing substrates that meet the requirements of a forest plant and improve its ability to resist transplanting stress (Landis 1990; Miller & Jones 1995; Benmahioul *et al.* 2010; M'sadak et *al.* 2012). Substrate preparation is still an issue in most of our forest nurseries, as we continue to use traditional soil and sand mixtures of poor physical and chemical quality.

In this context, the aims of this study were (*i*) to assess whether the substrates used to grow cork oak seedlings in the nursery affect the germination of acorns and (*ii*) to examine whether the substrate tested has an effect on the performance of the seedlings, particularly their growth. We hypothesized that (*i*) the potting soil substrate is more favourable to germination than the other substrates studied and (*ii*) the growth of seedlings in the nursery is conditioned by the nature of the substrate used.

2. Material and Methods

Acorns were harvested at maturity in November 2018 from ten trees selected from a natural cork oak stand of the state forest of El-Aouana-Jijel, located in eastern Algeria (36°45'29.11"N and 05°39'57.60"E) with an altitude of 30 m; 860 mm of annual average precipitation and 17.35ºC of annual average temperature. After sorting and cleaning, the acorns were placed in plastic bags and stored in a refrigerator at +4°C until use.

The experiment was conducted in the nursery of the Tlemcen Forests Conservation in a greenhouse backed by steel and glass walls with an east-west orientation, ventilated by windows placed laterally on both sides and heated in winter by heating devices to better manage seedlings growth.

2.1. Determination of moisture content

In the cork oak, as in other species of the genus *Quercus*, the germination rate decreases with the decrease of acorns' moisture content (Schroeder *et al.* 1987). The moisture content level (TE) of fresh acorns was determined on 5 replicates of 10 acorns, weighed separately to determine fresh weight (Pf) using an electronic scale (OHAUS). Their dry weight (Ps) was measured after drying at 103°C for 17h (ISTA 1999). The moisture content, expressed as a percentage of the acorn fresh weight (Willan 1992; ISTA 2009) was calculated by the formula: TE = 100 x (Pf - Ps)/Pf

2.2. Acorn germination

Fresh acorns (3 to 5 days after harvest) were sowed, only one acorn per polyethylene WM container (height: 17 cm, length: 8 cm, width: 5 cm, weight: 22 g, volume: 400 cm^3), without any pre-treatment. Five substrates were used to examine their effects on seedling germination and development: sand (S1), topsoil (S2), potting soil (S3), 1/2 sand + 1/2 topsoil (S4), and 1/3 sand + 1/3 topsoil + 1/3 potting soil (S5). These substrates were chosen because they are widely used for seedling production of many fruit and forest species (Piva *et al.* 2013).

Two replicates of 30 acorns – 60 acorns/substrate, i.e., in total, 300 acorns were used in this experiment. Watering was done every two days using a gardener's watering can and repeated as needed. No fertilisation was applied to the young seedlings. The number of emerged seedlings was counted weekly and their growth was monitored for 68 weeks at a temperature of 18°C/25 °C ± 2°C (night/day). An acorn is considered to be germinated when the radicle pierces the pericarp and manifests its positive geotropism.

2.3. Measured variables and data analysis

The variables measured per substrate were: average acorn germination rate (%), seedling survival rate (%), average seedling height H (cm), root collar diameter D (mm), stem height/root collar diameter ratio (H/D), average number of leaves per seedling, leaf length and width (cm) and leaf area (cm^2). For each treatment, the monthly average of the quantitative variables for each plant was calculated at the end of each month, during the 16 months of follow-up in the nursery, and then, the final average for all months was calculated at the end of the experiment.

The mean values of studied parameters were calculated and then entered into a database in Excel 2007 format. The effect of the growing substrates on different parameters was assessed using one-way ANOVA, with software R 2.2.0. The Shapiro-Wilk and Levene tests were used to verify the normality and homogeneity of variances of the data. A comparison of means was made using Tukey test (Couty *et al.* 2014). Results were considered significant at $p < 0.05$.

3. Results

3.1. Effect of substrates on acorn germination

The acorns used in this experiment had 45.35% moisture content. The average germination rate (%) was determined starting from the 35th day after sowing

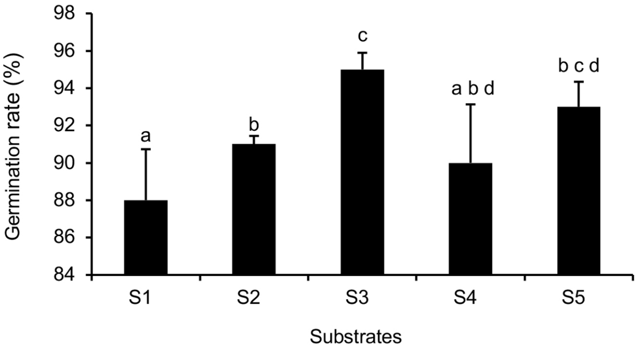

Fig. 1. Germination rates of cork oak seedlings grown on different substrates (S1: sand, S2: topsoil, S3: potting soil, S4: 1/2 sand + 1/2 topsoil, and S5: 1/3 sand + 1/3 topsoil+ 1/3 potting soil). Bars above the histograms are the standard deviations; significant differences at P < 0.05 are shown by different letters

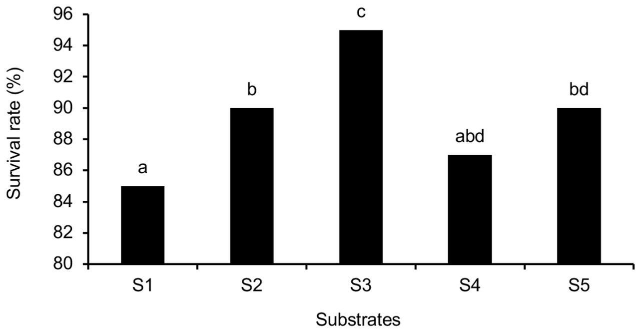

Fig. 3. Survival rates of cork oak seedlings grown on different substrates (S1: sand, S2: topsoil, S3: potting soil, S4: 1/2 sand + 1/2 topsoil, and S5: 1/3 sand + 1/3 topsoil + 1/3 potting soil). Significant differences at P < 0.05 are shown by different letters

in sand and from the 28th day for other substrates. The germination rates of cork oak acorns were influenced by the type of growing substrate used. The highest average rates were recorded for potting soil (substrate 3) and the sand/topsoil/potting soil mixture (substrate 5) with values of 95% and 93%, respectively. Sand alone (substrate 1), sand/topsoil (substrate 4) and topsoil alone (substrate 2), gave average rates of 88%, 90% and 91%, respectively (Fig. 1).

The comparison of the means revealed a significant differences between the substrates tested (p=0.0006). It should be noted that after the fourth week, the germination kinetics followed the same pattern for all substrates (Fig. 2).

To determine the survival rate, both dead and living plants were counted over a period of 16 months. It was defined as the ratio of the number of dead seedlings to the number of emerged seedlings per 100. Analysis of Fig. 3 shows that the survival of seedlings was significantly affected by the substrate used. Indeed, the survival rates varied between 85 and 95% recorded respectively for plants raised on sand (S1) and potting soil (S3).

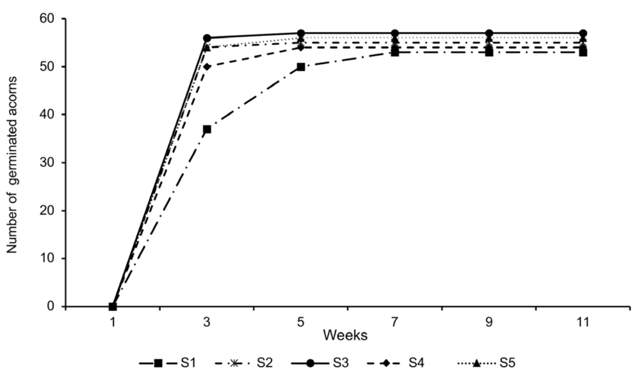

Fig. 2. Acorn germination kinetics per tested substrate

3.2. Effect of substrates on growth parameters

3.2.1. Seedling height

Seedling height was influenced by the type of growing substrate in a highly significant way (p < 0.000). Indeed, the obtained average heights formed three different groups: the first group contained plants with the highest growth raised on the potting soil substrate (S3=39.05 cm), the second group consisted of seedlings of average size grown in two mixtures – sand/topsoil (S4=26.51 cm) and sand/topsoil/ potting soil (S5=27.82 cm). Finally, the third group contained seedlings with a below-average size obtained on the sand (S1=17 cm) and topsoil (S2=20.43 cm) substrates with the respective growth differences of 18.62 and 22.05 cm compared with that recorded for the potting soil substrate (Fig. 4).

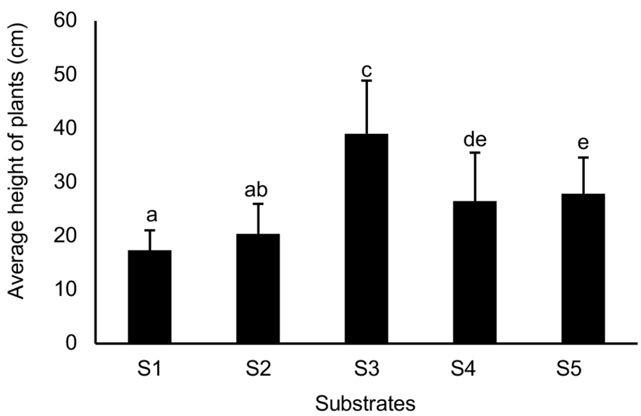

Fig. 4. Average heights of cork oak seedlings grown on different substrates (S1: sand, S2: topsoil, S3: potting soil, S4: 1/2 sand + 1/2 topsoil, and S5: 1/3 sand + 1/3 topsoil + 1/3 potting soil). Bars above the histograms are the standard deviations; significant differences at P < 0.05 are shown by different letters. (nS1=53; nS2=55; nS3=57; nS4=54; nS5=56)

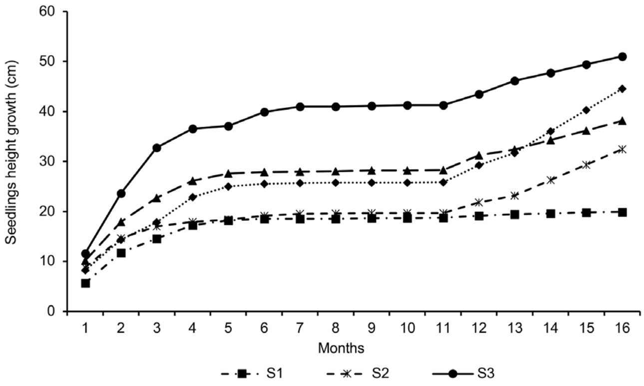

Fig. 5. Changes in the growth of cork oak seedlings on different substrates

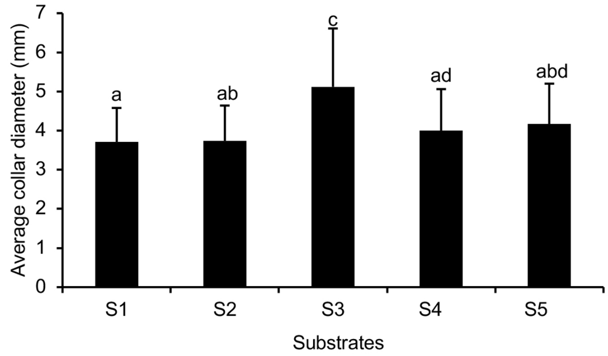

Fig. 6. Average root collar diameters of cork oak seedlings grown on different substrates (S1: sand, S2: topsoil, S3: potting soil, S4: 1/2 sand + 1/2 topsoil, and S5: 1/3 sand + 1/3 topsoil + 1/3 potting soil). Bars above the histograms are the standard deviations; significant differences at P < 0.05 are shown by different letters

The analysis of the average heights of plants grown on various substrates in the nursery for 16 months shows that the growth of the stem axis is rhythmic and characterized by successive three phases (Fig. 5). The first phase corresponds to the emergence and then the rapid growth of the seedlings, with a duration of about 16 to 20 weeks. During this period, an increase in seedling height varied depending on the type of substrate tested. The highest value (40 cm) was recorded in the plants grown on the potting soil substrate (S3), compared to only 18.5 cm, 19 cm, 25.5 cm and 28 cm measured for the sand (S1), topsoil (S2), sand/topsoil (S4) and sand/topsoil/potting soil (S5) substrates, respectively. A second phase of about 24 weeks was characterized by weak or slowed growth in the majority of seedlings grown on different substrates. The third phase began from the 11th month and was characterized by restarting growth, more marked for the plants grown on the potting soil substrate (S3). Indeed, a difference was observed between the cumulative growth recorded for this substrate (S3=51cm) and other substrates: S4, S5, S2 and S1, where the average heights were 44.5cm, 38cm, 32.5cm and 20cm, respectively.

3.2.2. Root collar and stem diameter

The development of root collar diameter was slightly influenced by the substrate type (Fig. 6). Potting soil (S3) supported the highest radial growth (5 mm) after 68 weeks. Seedlings grown in sand (S1), topsoil (S2), 1/2 sand + 1/2 topsoil (S4) and 1/3 sand + 1/3 topsoil + 1/3 potting soil (S5) had average collar diameters of 3.7mm, 3.8mm, 4mm and 4.2 mm, respectively.

The stem height/root collar diameter ratio (H/D, cm/mm) varied with the sowing substrates used (Table 1). The highest ratio (7.6) was recorded for potting soil (S3). The other substrates: sand (S1), topsoil (S2), 1/2 sand + 1/2 topsoil (S4) and 1/3 sand + 1/3 topsoil + 1/3 potting soil (S5), showed ratios of 4.46; 5.46; 6.62 and 6.67, respectively.

Table 1. Stem height/root collar diameter (H/D, cm/mm) ratio of cork oak seedlings grown on different substrates S1: sand, S2: topsoil, S3: potting soil, S4: 1/2 sand + 1/2 topsoil, and S5: 1/3 sand + 1/3 topsoil + 1/3 potting soil. Significant differences at P < 0.05 are shown by different letters

Substrate	H/D ratio
S1	4.66 a
S2	5.46 b
S3	7.62 c
S4	6.62 d
S5	6.67 d
Average	6.20

3.2.3. Leaf development

The trend of foliar organogenesis, expressed as an average number of leaves per stem and leaf area was similar to that observed for height increase. The variability of leaf number per plant revealed highly significant differences between the substrates tested, with the exception of the two mixtures: 1/2 sand + 1/2 topsoil (S4) and 1/3 sand + 1/3 topsoil + 1/3 potting soil (S5), where the comparison of the means showed that they were not significantly different at $p < 0.05$ with respective averages of 46.5 and 47.5 leaves/plant (Table 2). Topsoil (S2) and sand (S1) had the lowest yields with 32 and 22 leaves/seedling, respectively.

The same tendencies were observed for leaf area, which varied significantly with substrate. The best values (3.8 and 3.1 cm^2) were recorded for the plants grown in potting soil (S3) and sand/topsoil/potting soil mixture (S5), respectively. The lowest leaf area was observed on sand (S1), with a value of 1.7 cm^2. A

Table 2. Average measurement values for different leaf parameters of cork oak seedlings grown on different substrates. Values in the same column denoted by the same letter do not differ significantly at P < 0.05

Substrates	Number of leaves/plant	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
S1	22.00 a	3.76 a	2.29 a	1.74 a
S2	32.00 b	4.08 a	2.40 a	2.25 b
S3	59.40 c	4.82 c	2.74 c	3.83 c
S4	46.50 d	4.13 a	2.36 a	2.31 bd
S5	47.50 de	4.38 ae	2.61 ce	3.12 e
Averages	41.48	4.23	2.48	2.65

non-significant difference was recorded between the averages for topsoil (S2) and 1/2 sand + 1/2 topsoil (S4) (Table 2).

The longitudinal and transverse growth of leaves follows precisely an identical pattern in the different substrates. Potting soil (S3) seems to be the most favourable for the development of cork oak leaves. Leaf length varied between 3.8 and 4.8 cm, while width from 2.3 to 2.7cm (Table 2). The comparison of averages showed that the differences between them were significant only for the potting soil S3 and the mixture 1/3 sand + 1/3 topsoil + 1/3 potting soil (S5), while for the other substrates, they were not significant at $p < 0.05$.

4. Discussion

4.1. Germination of acorns

The obtained results of germination rates and analysis of variance showed that the effect of substrate was significant. The highest percentage of germination was recorded with acorns sown on potting soil. These results are comparable to those obtained by Sarir & Benmahioul (2017) in three oak species (Holm oak, Cork oak and Zen oak) grown in nurseries, and by Cemagref (1983) and Vinagre *et al.* (2005), who considered that cork oak acorns have very high germination rates, more than 80%, if they are handled properly. It is possible that the high seedling germination rate obtained with potting soil substrate (S3) is due to its characteristics, i.e., the excellent storage capacity of water and oxygen (Alvino & Rayol 2007). These factors provide a very good environment for the activation of enzymes responsible for the hydrolysis of reserve substances in seeds, to start germination process and seedlings emergence (Taiz & Zeiger 2009; Piva *et al.* 2013).

Furthermore, after germination and emergence, a loss of 5 to 15% of plants was observed in all substrates. This could be due to multiple causes. Thus, Koumiche and Benmahioul (2016) observed that Holm oak seedlings growing on a substrate composed of topsoil had a high mortality rate. According to the same authors, this is due to pathogens, in particular, fungi responsible for damping-off disease. These parasites have resting forms that allow them to persist in the soil and subsequently cause new infections. Abourouh *et al.* (1995) and Benmahioul *et al.* (2010) also reported a relationship between root mortality of plants due to attacks by phytopathogenic fungi, such as *Phytophthora*, and the lack of aeration of certain substrates. On the other hand, Assi *et al.* (2018), asserted that the poor quality of root systems of seedlings grown in containers and left in them for too long is one of the major causes of young plant dieback and mortality.

4.2. Seedling growth parameters

Biometric measurements on young seedlings showed significant differences between substrates tested for the growth parameters. At the end of the 16 months of observations, the best results were obtained for the potting soil. The growth levels recorded for this substrate can be justified by its high organic and mineral matter content compared to other types of substrates used, particularly sand. M'Sadak *et al.* (2013) reported that in the nursery, the physical properties of the growing substrates are among the determining factors of the morphological quality of seedlings. Weawer (1958) has long shown that root morphology can be modified by environmental factors, such as: the seed size and type and structure of the substrate. Moreover, Hillel (1982), Landis (1990), Lamhamedi *et al.* (2000), and Lamhamedi *et al*. (2006) have shown the influence of the physical characteristics of the substrate on all root functions, including the absorption of water and mineral elements necessary for plant growth and development.

The comparison of the mean values of root collar diameters also shows the dominance of plants from potting soil substrate (S3). The plants grown on sand substrate (S1), apart from poor growth in height, showed also the lowest radial growth. Overall, the diameters obtained are acceptable and these results agree with those of Lamhamedi *et al.* (2000), Bouchaour-Djabeur *et al.* (2011) and El Boukhari *et al.*

According to our research, the use of organic substrate had a positive effect on stem height/root collar diameter ratio obtained after 16 months of growing. This ratio was significantly higher than the standard ratio for potting soil compared to other substrates. This may be due to the composition of this substrate. According to Lamhamedi et *al.* (1997, 2000), this ratio should be lower than 8 when the plant reaches a target of 28 to 40 cm and the root collar diameter varies between 4 and 5mm. Our findings confirm those of Bouchaour-Djabeur *et al.* (2011) and Zine El Abidine *et al.* (2016) for cork oak seedlings.

The analysis of the effects of substrates on leaf organogenesis trends showed that the tested substrates significantly affected the number of leaves per plant, as well as the leaf area. The observed lower values of these two parameters measured for seedlings grown on sand (S1) and topsoil (S2) are mainly related to these substrate textures. Indeed, Guehl *et al.* (1989) reported a significant effect of substrate type on the photosynthetic characteristics and leaf area of *Cedrus atlantica* Manetti. The use of a substrate composed of soil organic matter (S3) significantly increased the number of leaves per plant and the leaf area. These observations confirm those reported by Benseighir-Boukhari & Argillier (2006) for *Quercus suber* L. and those of Hamidi *et al.* (2017) for *Pistacia vera* L. According to the studies of Weigel (1994) and Schippers (2007), conducted in a nursery, a good substrate must be composed of easily degradable organic matter to give good results. In terms of leaf growth and development, our findings agree with those found by Piazetta (2005) for the same species.

5. Conclusion

This study presents the results of experiment on the germination and seedlings growth of *Quercus suber* L. on different substrates.

It was found that the use of substrate rich in organic matter gave very satisfactory germination rates compared to other substrates. As for biometric parameters expressed statistically, we have shown that the best results for different growth parameters considered were obtained with the potting soil (S3) substrate. These results were comparatively superior to other substrates, i.e., to classic substrates used in forest nurseries in Algeria (sand S1 and topsoil S2). For cultivated plants, the S3 substrate can be recommended for the production of seedlings from seeds, as it guarantees vigorous and early seedlings with a shorter stay in the nursery, and therefore a reduction in production costs.However, other complementary and diversified studies, both in a nursery and on reforestation sites, are needed to validate the most appropriate substrates for the production of cork oak seedlings.

Author Contributions

Research concept and design: B. Benmahioul
Acquisition and/or assembly of data: D. Kholkhal
Data analysis and interpretation: D. Kholkhal, B. Benmahioul
Drafting the article: D. Kholkhal
Critical revision: B. Benmahioul
Final approval: D. Kholkhal, B. Benmahioul

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