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Review

Quercus suber L. Genetic Resources: Variability and Strategies for Its Conservation

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Abstract: *Quercus suber* L. is an evergreen cork oak tree that can produce cork, one of the most important valuable natural bioresources in Portugal, with a high impact for the bioeconomy. Given its socio-economic relevance and the upcoming biotic and abiotic threats cork oak faces, it is of extreme importance that genetic conservation of its genetic variability occurs so that cork oaks can adapt to new conditions. This work represents a review of the current knowledge on *Quercus suber* genetic resources, focusing on the existing genetic variability and the strategies for its conservation. Furthermore, we highlight genetic knowledge on tolerance and response to abiotic and biotic stresses and cork quality, which are useful for further studies on stress response pathways and mechanisms and improvement regarding stress tolerance.

Keywords: biotic stress; climate change; cork oak; cork quality; genetic conservation; genetic variability

1. Introduction

1.1. Characterization

Quercus suber L., commonly known as cork oak, is an evergreen tree that belongs to the *Quercus* genus and the *Fagaceae* family. The *Quercus* genus is divided into the subgenus *Quercus* and subgenus *Cerris*. Subgenus *Cerris* is exclusively Eurasian, with some species from North Africa, and is composed of three sections: section *Cyclobalanopsis*, section *Ilex*, and section *Cerris*. *Quercus suber* belongs to the subgenus *Cerris*, section *Cerris* [1]. Cork oaks can growth up to 25 m in height and have a stem diameter at breast height of more than 2 m [2,3]. Cork oak trees have a long life cycle of 200–250 years. They have a rounded crown with thick branches, while their leaves are sclerophyllous and live for about one year [4].

Cork, the harvested outer bark from the cork oak tree, is one of the bioresources with major relevance for the Portuguese bioeconomy. Cork is mostly used for wine stoppers and other products, such as agglomerates of granulates for insulation, corkboards, and cork powder [5]. Cork also has the potential to be used as activated carbon and in ecoceramics [5,6], as well as in industries where its antioxidant and aromatic properties may be useful [7,8]. While *Phellodendron amurense* Rupr. and *Quercus variabilis* Bl. can also produce cork, only *Q. suber* cork has the quality needed for industrial use that is profitable [9].

Cork oaks grow in humid and sub-humid regions such as the Mediterranean region and can survive hot and dry seasons along its distribution range. Stomatal closure, leaf shedding, and deep roots are physiological and morphological adaptations that can help the trees to maintain their water status, thus limiting water loss through transpiration and enhancing groundwater access [10]. During the development of the root system in the early

ages of the tree, the replenishment of groundwater is essential for the survival of the trees, especially as drought is a major threat to oak decline [11].

Cork oak trees are monoecious, having male and female flowers on the same individual. They are self-incompatible, the development of the flowers is asynchronous, and they are wind-pollinated [4]. Acorns, the fruit, are produced both annually and biennially and are composed mainly of starch (50%) and are high in lipids and low in proteins [12], with dual purpose for food and feed.

1.2. Distribution and Ecology

Cork oak trees are native to the Mediterranean region and are found along the coast areas of Algeria, France, Italy, Morocco, Spain, Tunisia, and in Portugal [13]. For a regional and national distribution map of *Q. suber,* kindly refer to resources developed under the scope of the chorological maps for the main European woody species [13]. In the Iberian Peninsula, *Q. suber* is found in a peculiar agrosilvopastoral system called "montado" in Portugal and "dehesa" in Spain [4]. In North Africa, these systems are called "azaghar" [4]. These systems have a high diversity of fauna and flora and are essential for environmental sustainability [14]. In addition, "montados" and cork oaks help to protect the soil, retain CO_2, and regulate the water cycle [14]. These systems are managed by men and have a socioeconomic interest to produce goods and services in a sustainable manner [4].

Cork oaks have an acidophilous and calcifuge character; therefore, they are mainly present in soils derived from siliceous substrates [15]. This need for siliceous soils is a limiting factor for their distribution on the territory. Although they can be found in moderately acidic soils with a pH of 3.4 to 7.8, they are mostly found in soils with a pH of 4.7 to 6.5. In terms of texture, cork oaks prefer loamy and sandy soils, well-aerated, over-flooded, and compact soils [15]. Occasionally, cork oaks can also be found in decarbonated soils [15].

Low temperatures are also a limiting factor for cork oaks' distribution, with $-10\,°C$ being the minimum temperature tolerated by the trees; thus, they are only found in areas below 1000 m. Cork oaks grow in regions with an average temperature of $15\,°C$, ranging from $13\,°C$ to $18\,°C$, and an average annual precipitation of 600 mm, ranging from 479 mm to 2400 mm [2,16]. Although cork oaks can be found along the whole area of Portugal, it is in "Alentejo" (south) and "Trás-os-Montes" (north) that the largest areas of cork oaks are found [17].

The cork layer produced by *Q. suber* has a dual role as protection against forest fires, protecting the tree itself and also the ecosystems and habitats where these trees are. Being a good insulating material, the bark protects buds and stem tissues [4]. Bark thickness is very important for tree recovery after a fire [4]. The thicker the bark, the more protected the stem buds are; thus, the tree can recover quickly as it can resprout from stem and crown buds [4]. On the other hand, cork-harvested trees and young trees, with thinner bark layers, are more susceptible to damage induced by fire scenarios [4,18,19].

2. Socio-Economic Relevance

2.1. Products and Sub-Products

The development of cork is an exceptional characteristic of *Q. suber* trees. The first cork harvest begins when the trees are about 25 years old and each harvest cycle takes approximately 10 years [5,20]. The cork resulting from the first harvesting is called virgin cork and is very irregular, while that originating from the following harvestings is called reproduction cork [5]. Only the second reproduction cork is suitable to produce bottle stoppers [5,21]. The quality of the cork must be carefully assessed to determine its uses and economic value. The main quality factors evaluated are macroscopic porosity, cork thickness, and inclusions [22]. For example, virgin cork and first reproduction cork are suitable for insulation purposes using agglomerates but not suitable for bottle stoppers [5].

Cork is mainly composed of suberin (33%–57%), lignin (13%–35%), cellulose and hemicellulose (6%–25%) [5,23]. Several valuable by-products can be extracted from cork,

such as waxes, tannins, and phenolic acids [5,23]. Cork is a biobased material with a multitude of interesting properties since it is highly compressible, flexible, and resilient, characteristics ideal for wine stoppers [5]. Moreover, the suberin and waxes found in cork are responsible for its impermeability to liquids and gases, an important quality for the cork stoppers to prevent wine oxidation. Because cork cells are small and hollow, thus with a high gas content present, cork has low density and high porosity. Cork has reduced conductivity of heat and sound, which is useful for agglomerate production used for the manufacture of insulation and construction products [5].

2.2. Cork Production and Industry

In 2015, Portugal had 720 thousand hectares of cork oak, which represent 22% of the national forest and 34% of the global area of the country [17]. Regarding the total area occupied by cork oaks woodlands, 71.7% is in "Alentejo", while 2.2% is in the North of Portugal. In "Trás-os-Montes", cork oak woodlands represented 10.1% of the forest area of this region (14.56 thousand ha) [18]. More recent data available showed a slight decrease in the area occupied by cork oaks [24].

The cork industry in Portugal is of relevance for the bioeconomy of the country as Portugal is the world's leading cork producer, and the international markets are profitable. This industry is also responsible for the employment of thousands of people. In 2018, there were more than 10 thousand cork companies in Portugal, which represents 0.79% of the total number of companies in Portugal [25,26]. Indeed, the cork industry represents 5% of the gross domestic product in Portugal according to the information available [25,26].

Portugal leads the global cork production with 85 thousand tons, representing 46% of the global production [17,27]. Cork exportations, in 2021, rendered EUR 1127 million, of which EUR 824 were from bottle stoppers, which are the major cork product exported [28,29].

Given its relevance for the Portuguese economy, the cork oak tree was classified as a Protected Species by Law in 2001 [30] and was considered a national tree by the government in 2011 [31].

3. Genetic Diversity

3.1. Genetic and Trait Variation

In oaks, both mitochondrial and chloroplast DNA are maternally inherited [32]. The genetic diversity of a population and its structure can be assessed by using different markers, ranging from nuclear microsatellites and chloroplast DNA to ribosomal DNA [33].

Chloroplast DNA can be useful for phylogeographic and introgression studies. Using various types of markers, it is possible to elucidate the possibility of cytoplasmic introgression in the nuclear genome and data concordance between these markers. Several studies used chloroplast DNA to assess genetic diversity, resulting in the grouping of various haplotypes into genetic lineages [34–36]. PCR-RFLP was used to study chloroplast DNA variation in *Q. suber* [34]. Outcomes of this study evidenced a great cpDNA variation and 81 haplotypes were found. These were grouped into three lineages: *suber*, *ilex-coccifera* I, and *ilex-coccifera* II. Haplotypes from the *suber* lineage were found in an Iberian–Moroccan distribution and Middle Mediterranean. Lineage *ilex-coccifera* I was found in the Iberian Peninsula, southern France, and Morocco, while lineage *ilex-coccifera* II was found in the Mediterranean Basin, southeastern Spain, and the Balearic Islands [34]. Magri et al. [35] used cpDNA microsatellites in 110 *Q. suber* populations to assess putative relationships between their geographical distribution and the palaeogeographical history of the western Mediterranean domain. Their results showed that five haplotypes were found, whose distribution is clearly geographically structured: H1 and H2 are found in Italy, H3 is found in Provence, Corsica, Sardinia, Algeria, and Tunisia, H4 is found in Portugal, western Spain, southwestern France, and northern Morocco, and H5 is found in the rest of Spain and the Balearic Islands, and eastern Morocco. Interestingly, H5 shares a lineage with *Quercus ilex* L., possibly resulting from introgression [35]. Overall, the results demonstrated that

cork oak populations have undergone a genetic drift geographically consistent with the Oligocene and Miocene break-up events of the European–Iberian continental margin [35].

Lumaret and Jabbour-Zahab [37] analyzed cpDNA and nuclear microsatellite (nSSR) polymorphisms in the same individuals, aiming to evaluate the range-wide geographical variation in gene flow between *Q. suber* and *Q. ilex*, whose geographical distributions partly overlap. Hybridization between these species occurs in nature but at a low frequency since it is constrained by the flowering time of both species and the occurrence of embryo abortion [33,38]. These authors compared hybridization and cytoplasmic and nuclear interspecies gene flow levels between populations located within and outside the areas of total replacement of *Q. suber* cpDNA by that of *Q. ilex*. Among other things, their data for cpDNA indicated clear geographical chlorotype patterns in both species and higher chlorotype variation in *Q. ilex* than in *Q. suber* [38], as evidenced in previous studies [35]. Nevertheless, the results did not support geographical variation evidence in interspecies gene flow [37]. Interestingly, F1 *Q. suber* x *Q. ilex* hybrids do not produce a corky bark [39]. Hybridization and introgression with *Q. ilex* has been proposed as the reason why *Q. suber*, which is acidophilous, was able to colonize calcareous regions since soil nature is not a limiting factor for *Q. ilex* growth as it is for *Q. suber* [39,40]. A review of the taxonomy of Portuguese oaks and their hybrids can be found in Table 1 [41].

Table 1. Portuguese oak hybrids found in the Iberian Peninsula. Information included in this table was retrieved from [41].

Hybrid	Parental Species	Reference	Observations
Quercus × *avellaniformis*	*Quercus rotundifolia* Lam. × *Q. suber*	[42]	Present near cork oak and round-leaf oak trees, where their flowering and pollination periods overlap
Quercus × *capeloana*	*Quercus pseudococcifera* Desf. × *Q. suber*	[41]	Present in Portugal in coaster hyperoceanic areas. Has totally free cup scales, while *Q. coscojosuberiformis* has more appressed scales
Quercus × *celtica*	*Quercus lusitanica* Lam. × *Q. suber*	[41]	Present in the undergrowth of *Q. suber* in Portugal
Quercus × *coscojosuberiformis*	*Quercus coccifera* L. × *Q. suber*	[43]	Present in Spain; can exist in nature as a great area of parents overlap
Quercus × *pacensis*	*Quercus faginea* Lam. × *Q. suber*	[44]	Rare in Southern Portugal, pure *Q. faginea* can develop a corky bark thanks to secondary growth

More recently, Sousa et al. [45] studied the population structure patterns of *Q. suber* by using 13 nuclear microsatellite markers in individuals from 17 distinct locations across the entire range of the species. The samples were collected from individuals of natural stands and provenance trials. Structure analyses results showed that *Q. suber* contains three major genetic clusters that correspond to a rough division of east–west areas, with Corsica belonging to a third cluster [45]. This cluster arrangement suggests that differentiation occurred by isolation in refugia, and then, when populations expanded, there may have been contact between isolated species and introgression from other *Quercus* species [45]. These authors also suggested that the high levels of genetic variation found in southern populations may have contributed to the resilience of *Q. suber* in a context of environmental change and adaptive pressure. Nevertheless, more studies are needed to corroborate such a hypothesis.

The recent developments of high-throughput sequencing technologies have facilitated the sequencing of entire genomes, supporting the development of new genomic resources and identifying single-nucleotide polymorphism (SNP). Mendes et al. [46] investigated the potential of performing kinship prediction and pedigree reconstruction by SNP genotyping in a natural regenerated cork oak population. Kinships were successfully identified, and some families' pedigrees were established, which support that pedigree information can be a valuable tool for future management strategies of cork oak populations, including future cork oak breeding schemes [46].

Overall, the studies herein described highlighted that *Quercus* species, and namely *Q. suber*, has a scattered distribution in which each region has different environmental conditions; therefore, each population is likely already adapted to its distribution area. Indeed, these studies show that genetic variation in *Q. suber* is geographically structured [33–35,47]. For a better understanding of historical events and their impact on *Q. suber* distribution range and differentiation, more studies are needed. Among the several approaches available, they may focus on further characterization of the population structure using more molecular markers and larger samples from more sample sites and characterizing populations from hypothetical glacial refugia areas as they are likely more genetically diverse [35,45,48]. It is also relevant to understand if the genetic diversity of populations is in fact due to the glacial refugia and the isolation and differentiation that presumably occurred or if it is an earlier process [35].

While genetics play a part, it seems that some trait variability found among lineages and among populations can be due to environmental conditions [34,47,49]. Cork oak populations have significant levels of phenotypic differentiation, as indicated by their morphological traits [50].

Leaf traits seem to show a certain degree of variability within the *Q. suber* populations [47,51]. Indeed, variability in leaf size, specific leaf area, and carbon isotope discrimination were found among populations of common garden trials, and this variability correlates with the annual rainfall of the habitat [51]. For example, specific leaf area varies in years in accordance with the annual rainfall and is higher when there is higher rainfall [51]. There is a correlation between water deficit and stomata morphology and carbon isotope discrimination, thus suggesting that cork oak seems to be able to adapt to different habitats and has variability in drought tolerance traits [47].

Interestingly, population variability is associated with the cpDNA lineages in some features of the plant's architecture or traits [35,47,49]. Between lineages, it was found that populations from the eastern lineage, corresponding to Magri et al. [35] haplotypes 1 and 2, showed the highest branching values, the lowest apical dominance, and the lowest values of vigor [49]. Within lineages, temperature is a key factor for adaptation and variability, with population from the same lineage but from warmer sites presenting branchier individuals and apical dominance [49]. In another work, trees from the lineages *suber* and *ilex-coccifera* of the Iberian Peninsula were analyzed along a climate gradient [47]. The leaf traits appear to be conserved between the two genetic lineages, with the existing variability in these traits being attributed to environmental conditions [47]. It would be desirable to have information of the genetic basis to support this assumption.

The results from provenance trials show variations among populations in relative height growth and mortality [52]. The Moroccan populations show the highest relative height growth in general, at age 9 years old. The mean trial mortality was 37.5%, and more than half of the Portuguese populations studied had a mean mortality lower than the trial mean. These provenance trials show that cork oak can survive and grow when the precipitation is reduced by 50% and 60% [51,52].

Genetic variation and environment adaptation are responsible for the variability observed within populations and within and between lineages in *Quercus suber*. Still, the use of approaches in which trait variation is associated to genetic variation would bring more informative tools to plant breeding, but this still constitutes a gap of knowledge in this species. Indeed, the identification of quantitative trait loci (QTLs) and candidate genes constitutes a promissory tool for marker-assisted selection programs underlying future cork oak breeding schemes.

3.2. Genetic Conservation Programs

Quercus suber shows genetic variation among populations in adaptive traits, possibly because of adaptation to climate and environmental changes along its geographical distribution [33]. It is of utmost importance to conserve this diversity as a base resource for the improvement and management of genetic resources and stands, as well as for

breeding strategies and conservation [46]. In this context, conservation strategies should ideally include populations from all the different abiotic and biotic conditions. While cork oaks have no threats regarding its extinction due to their economic value, some marginal populations are at risk of disappearing. The most common threats identified were low natural regeneration and overgrazing, particularly considering that *Q. suber* individuals are relevant components of the agropastoral systems, such as the "montados" [33].

To tackle the conservation challenges, numerous projects and initiatives have been implemented for the genetic conservation of forest resources. Among them, the EUFORGEN program [53], created in 1994, focused on the conservation of the forest genetic resources in Europe while promoting sustainable use and promotion of knowledge as a vital part of sustainable forest management [53]. This program has developed a Pan-European strategy for the genetic conservation of forest trees [54]. In 2011, and aligned with the EUFORGEN premises, Bioversity International developed a new project, the European information system on forest genetic resources (EUFGIS) [55]. The project aims to develop an information system on the forest genetic resources that can connect the data and inventories across Europe, constituting a relevant tool for understanding changes through time in genetic variations [55].

In situ and ex situ gene bank repositories have a crucial major role in the conservation of genetic resources. Presently, there are 27 genetic conservation units registered in EUFORGEN for *Q. suber*. These units consist of a "forest stand or area that contains tree populations nationally designated for the conservation of Forest Genetic Resources" [53] and can be in situ or ex situ. There are twenty-one units in Spain, one in Italy, and five in Portugal. Portuguese units are located in "Minho", "Lisboa", "Alentejo", "Trás-os-Montes", and "Algarve" [55]. These units follow a dynamic genetic conservation strategy that allows the evolutionary processes needed for the populations to be able to adapt to new conditions [33,53,56]. As adaptation to the environmental conditions is essential for population variability, conservation of this variability may be crucial for the viability of the species when facing current and future climate changes [55]. Another approach to conserve genetic resources is the cryopreservation of living plant explants. As one example, Vital et al. [57] described the successful cryopreservation of 51 cork oak genotypes. Cork oak somatic embryos were able to withstand the freezing procedure without losing their regenerative ability. Importantly, plants regenerated from these frozen embryos are plants morphologically identical to plants that have not been cryopreserved [57], supporting the use of the method for safe and long-term conservation of genetic resources.

3.3. Genome Sequencing

As with many other crops, the availability of a fully sequenced and annotated genome for cork oak is an essential resource to address fundamental questions about this species' biology, cork formation, and interactions with the environment [58].

The cork oak is a diploid ($2n = 24$) species, with an estimated genome size of 934 Mb, by using flow cytometry [59]. Until 2010, no draft sequenced genome nor a physical map were available for this species [9]. To increase the *Quercus suber* genomic resources available, the Cork oak ESTs Consortium (COEC) was created and involved 12 projects nationwide in Portugal with the aim of obtaining and characterizing Expressed Sequence Tags (EST) for this species [9]. The information obtained from EST constituted a first approach for gene discovery and a way to start elucidating its physiology and functional genome. In 2014, this consortium released the first reference transcriptome for cork oak, based on Expressed Sequence Tags (ESTs) [9]. Approximately 159,000 unigenes were annotated as result of the pyrosequencing of 21 normalized cDNA libraries derived from multiple tissues and organs, developmental stages, and physiological conditions studied in *Q. suber*.

Subsequently, in 2018, the first draft version of the cork oak genome was developed [58] under the scope of the research activities and outcomes of the GENOSUBER consortium [60]. This draft genome, comprising 23,347 scaffolds and 953.3 Mb in size, was obtained by using a de novo assembly strategy using high-throughput sequence data. Under the scope

of this study, a total of 79,752 genes and 83,814 transcripts were predicted, including 33,658 high-confidence genes [58]. A portal Cork Oak Genome (CorkOakDB; available at www.corkoakdb.org, accessed on 28 June 2023) has been developed, which allows public access to search and explore the curated genomic and transcriptomic data on this species [61].

4. Molecular Mechanisms of Stress Responses and Cork Formation

Developing genomic resources available for cork oaks is of paramount importance for future breeding approaches. Model projections predict an increase in annual temperature of more than 2 °C by 2080 and a decrease in precipitation in the Mediterranean region. Given the long life span of forest trees, climate change is a major threat as adaptation to environmental changes takes considerable time [62]. The "cork oak decline", observed in Mediterranean areas since the 1980s, seems to be caused by a combination of different factors, including abiotic, such as drought and increased temperature, biotic, such as pathogens, and anthropogenic, such as incorrect management practice that leads to overgrazing [63].

Given its economic value and long life cycle, more detailed knowledge of the cork oak genome will provide access to more detailed information on candidate genes underlying biotic and abiotic stress tolerance or cork quality. This information will support the deployment of new strategies to improve the responses of this species towards environmental constraints or, as an example, modulate the cork quality. Some of these approaches are described in the following sub-sections.

4.1. Pests and Diseases

As cork oak forests face climate change and physiological stress, this can weaken trees and predispose them to colonization by pathogens and pests, which may contribute to the oak decline [64].

Insect pests are one of the biotic factors that can contribute to oak decline. The most harmful insects can be sorted as defoliating insects and bark-/wood-boring insects [65]. *Periclista andrei* Konow are one of the most important defoliating insects in Portugal [66]. The attack of defoliators prompts trees to the attack of pathogens [67]. As for bark-boring insects and wood-boring insects, the most harmful belonging to the Coleoptera order, the boring of the wood of weakened trees can cause the death of the tree [64,65,68]. For example, *Platypus cylindrus* Fab. belongs to the Coleoptera order and *Curculionidae* family and entails borer insects that attack the trunk and branches [69]. The boring activity and gallery excavations of these insects combined with the inoculation of ambrosia fungi, such as *Ceratocystiopsis quercin* sp. nov., weaken the host trees, leading to tree mortality, thus contributing to the cork oak decline [69]. Although there are a considerable amount of works describing the damaging effects of pests in cork oak physiology, no publicly available reports describing the genetic or molecular mechanism by which cork oak cope with pests' attacks is described. Future studies should address this existing gap in knowledge.

Cork oak suffers from few major disease problems since very few are primary pathogens able to attack healthy tree tissue [64]. More than 300 species of fungi and oomycota are reported on cork oak, of which at least 100 are pathogenic [69]. Among these, a relatively high number are considered opportunistic pathogens since they colonize oak tissues when they have been previously weakened by abiotic or biotic factors [64,70].

One of the most destructive root pathogens is the oomycete *Phytophthora cinnamomi* Rands, which has been associated with decline in *Q. suber* and *Q. ilex* populations in the Mediterranean region [71]. This oomycete infects roots and collars and spreads through the phloem and cambial tissues and survives dry periods. Conditions for the growth, survival, and spread of *P. cinnamomi* are likely to become more favorable as the weather is getting warmer and there is increased drought [71].

Q. suber seems to be more susceptible to *P. cinnamomi* infection and more rapidly colonized than *Q. variabilis*, generally described as more resistant to *P. cinnamomi* infection [72].

Upon infection, *Q. variabilis* also displayed an up-regulation in stress-related proteins in the leaves when compared to *Q. suber*. Peroxidases, superoxide dismutases, and glutathione S-transferases, together with proteins related to jasmonic acid metabolism, were found differentially accumulated. The authors suggest that these differences in the defense response could be responsible for the higher susceptibility of *Q. suber* to *P. cinnamomi* attack. In another study, cDNA-AFLP was used to identify candidate genes differentially expressed in *Q. suber* roots infected with zoospores of *P. cinnamomi* at different time points [73]. Among the results, six candidate genes were selected for qRT-PCR validation based on their expression patterns and homology with genes known to play a role in defense. The results evidenced that, following root infection with *P. cinnamomi*, there is an up-regulation in the expression of the genes *QsRPc* (encoding for a CC-NBS-LRR RESISTANCE PROTEIN), *QsCAD2* (encoding cinnamyl alcohol dehydrogenase2), *QsPDI* (encoding protein disulphide isomerase), and *QsCHI* (encoding a chitinase) and a down-regulation in the gene *QsTLP* (encoding a thaumatin-like protein) [73].

4.2. Temperature and Drought Stress

Cork oak is well-adapted to the Mediterranean climate and shows great plasticity to the characteristic hot and dry summers of this region [51]. This tree has a high thermotolerance and can acclimate to drought, even showing acclimation and survival at 55 °C [74,75]. However, the knowledge about the molecular mechanism underlying such adaptive response remains scanty.

Young cork oak trees that were exposed to temperatures of 10 °C and 28 °C show different metabolism responses, with different accumulation of compounds and transcripts involved in metabolites biosynthesis, which are important for stress survival and cell wall composition [76]. At low temperatures, there is a higher concentration of sucrose and transcripts levels of enzymes involved in the biosynthesis of flavonoids, while, at high temperatures, there is a higher concentration of quinic acid and transcript levels of enzymes involved in the shikimate pathway [76].

It is generally well accepted that DNA methylation and histone posttranslational modifications play a major role in regulating developmental and stress responses in plants. Correia et al. [74] investigated if epigenetic modifications could be related to heat stress tolerance in cork oak. DNA methylation and acetylated histone H3 (AcH3) levels were monitored during cumulative high-temperature stress from 25 °C to 55 °C. Among others, the results indicated that elevated temperatures can lead to an increase in DNA methylation and a decrease in histone H3 acetylation [74]. This study pointed the putative role played by epigenetic regulatory mechanisms on the modulation of the expression of genes implicated in heat responses in cork oak.

Some members of the *R2R3-MYB* family of transcription factors (TFs) have been linked to drought stress responses in *Arabidopsis thaliana* (L.) Heynh., such as AtMYB96 [77], AtMYB60 [78], and AtMYB68 [79]. QsMYB1 is a member of this TF family and is possibly involved in cork biosynthesis [80]. Almeida et al. [81] investigated how QsMYB1 TF was regulated at the transcriptional level by heat and drought stresses, validating the hypothesis that this TF is a player in the transcriptional regulatory network of cork oak under abiotic stresses. The results evidenced that both stresses modulated the expression pattern of the two *QsMYB1* splicing variants. Indeed, high temperature has more influence on the regulation of the spliced *QsMYB1.1* transcript, while drought affects mainly the un-spliced one, *QsMYB1.2*. This study highlights the need for further studies to support the use of the QsMYB1 to improve drought or thermotolerance or drought tolerance in this species [81]. As one example, the influence of other members of the *R2R3-MYB* family on thermotolerance should be explored with more detail.

Taking advantage of new genomic resources developed for *Q. suber*, Magalhães et al. [82] investigated the transcriptome changes observed in *Q. suber* roots exposed to long-term drought. Using an RNA-Seq approach, these authors identified over 500 differentially expressed unigenes, including a significant number of different TFs and other

signal transduction components. One of the most relevant findings of this study was the identification of a complete canonical ABA/ABF-dependent signaling pathway. This pathway, which is activated in *Q. suber* roots responding to long-term drought stress, has been described as one of the main key mechanisms in this species' response to drought [82].

Despite the advances reported herein, we are still far from having a global picture about how *Q. suber* copes with drought and adapts to different climate conditions to understand the impact of climate changes on *Q. suber* forests and productivity [45,47]. The information on the molecular mechanisms activated in response to abiotic stresses, as well as regarding the genes controlling such responses in cork oak, is scarce. Further studies focusing on the characterization of drought tolerance morphological traits, such as variability in rooting depth and xylem organization in cork oak populations in areas impacted by drought, are needed [45,47]. This knowledge may lead to an improvement in conservation programs for this species and the understanding of the impact of population variability in those programs [83].

4.3. Salinity Stress

Soil salinity is an abiotic factor that can limit cork oak tree growth and productivity [84]. Despite the relevance of soil salinization in many Mediterranean ecosystems [85], the knowledge about cork oak plasticity and resistance in response to high salinity also remains limited.

The effects of a high-salinity episode (300 mN NaCl) on PSII activity and oxidative status were studied after 24 h and six days exposition in leaves of young plants of *Q. suber* [85]. The results evidenced that *Q. suber* displays features typical of salt-tolerant species, such as increased NPQ and carotenoid production, accumulation of primary metabolites, such as soluble sugars and starch in leaves, and up-regulation of the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase, and catalase. However, the same study also provided evidence of sensitivity to tested saline conditions, particularly after six days exposure, as noticed by lipid peroxidation, damage to photochemical capacity, and decreased chlorophyll production.

As a follow-up to the above-mentioned study [85], Dias et al. [84] studied the molecular mechanisms behind the salt stress response in cork oak roots. Two-month-old plants were watered with 300 mM NaCl. Enzymatic and non-enzymatic antioxidants, lipid peroxidation, and the relative expression of genes related to stress response were investigated in *Q. suber* roots eight hours and six days after salt treatment. The results showed that, at an early stage of salinity exposure by high doses of NaCl (300 mM), there is an up-regulation of genes related to dehydrins synthesis (*QsLTI30*), membrane protection (*QsFAD7*), and activation of antioxidant enzymes (SOD and CAT). With more prolonged exposure to salt stress, oxidative damage occurs and up-regulation of genes related to the defense against oxidative stress (*QsCSD1* and *QsAPX2*) and antioxidant defenses and an increase in the activities of antioxidant enzymes (SOD and APX) were noticed [85]. Indeed, the overall results support that *Q. suber* roots adopt protective complementary strategies to deal with salt stress, the response being dependent on salinity exposure.

Overall, all these works left open questions regarding how cork oak copes with salinity. More studies either addressing other experimental conditions (e.g., NaCl concentration, exposure time, sampled tissues), methods (e.g., shot gun metabolomics), and/or cork oak accessions or populations should be conducted to elucidate the responses to this abiotic constraint [85]. Understanding the molecular mechanisms behind salt stress tolerance is essential for selection of more tolerant trees, conservation of those traits, and management of this tree productivity [85,86].

4.4. Cork Quality and Sustainability

As mentioned previously, the natural ability of cork oak to produce a valuable thick bark (cork) in a sustainable manner is the basis for one of the most relevant industries in Portugal. Given its importance, numerous works have addressed the molecular mech-

anisms underlying secondary growth in cork oak trees, as well as the identification of candidate genes implicated in cork formation and quality.

Almeida et al. [80] have characterized a *R2R3-MYB* TF, named QsMYB1, previously identified as related with cork formation and differentiation [79]. These authors showed that QsMYB1 is mainly active in organs and tissues, with secondary growth resulting from the activity of phellogen. The role of QsMYB1 in the regulatory network of cork oak's response to abiotic stress was also pointed out [81], but its real function remains not well understood. To overcome this gap in knowledge, Capote et al. [87] hypothesized that QsMYB1 may be regulating metabolic pathways involved in cork formation, namely the biosynthesis of lignin and suberin, which constitute the major biopolymers present in cork. To corroborate this assumption, these authors conducted a chromatin immunoprecipitation (ChIP) followed by high-throughput DNA sequencing (ChIP-Seq) to identify the binding sites of a target protein across a genome. The results showed that QsMYB1 targets genes coding for key enzymes responsible for the biosynthetic pathway of lignin and suberin, essential for cork formation. Moreover, QsMYB1 targets also include genes encoding for ATP-BINDING CASSETTE PROTEINS TYPE G (ABCG) transporters and LIPID-TRANSFER PROTEINS (*LTPs*) implicated in the transport of monomeric suberin units across the cellular membrane. Thus, this work confirmed the specific role of QsMYB1 in regulating cork formation and development [87].

Teixera et al. [88] conducted a comparative RNA-Seq study on phellogenic tissue of good- and bad-quality cork samples. The overall aim was to identify candidate genes responsible for production of cork of superior quality that could be used as biomarkers of cork quality. Among others, the results evidenced an up-regulation of genes encoding for heat-shock proteins in good-quality cork, which are essential during heat stress, and allow proper phellogen development. On the other hand, genes involved in stress response, such as the ones encoding for ribosomal protein genes and ubiquitin-related genes, were up-regulated in bad-quality cork.

Another interesting study to unveil candidate genes implicated in cork formation was conducted by Boher et al. [89]. These authors conducted a transcriptome study between two oak species, *Q. suber* and *Q. ilex*, that differs in the thickness and organization of the external bark. *Quercus ilex* outer bark contains sequential periderms interspersed with dead secondary phloem, which constitutes the rhytidome, which differs from the cork oak outer bark, which only contains thick layers of phellem (cork) that accumulate until reaching a considerable thickness [89]. The results showed that some similitudes can be observed in both transcriptomes, but the *Q. suber* external bark transcriptome stood out, with up-regulated genes related to suberin, the main polymer responsible for the protective function of periderm. On the other side, the up-regulated categories found enriched in *Q. ilex* were abiotic stress and chromatin assembly. Overall, this study revealed possible candidates accounting for the exceptionally thick and pure *Q. suber* phellem, such as those involved in secondary metabolism and phellogen activity.

5. Conclusions

Quercus suber is an evergreen oak tree with the outstanding ability of producing outer bark that contains thick layers of phellem (cork) that accumulate until reaching a considerable thickness for its harvesting. The cork sector in Portugal places special attention on the important role of the forest, construction, and wine sectors, protected by the law and population. Cork is a natural, renewable, and sustainable resource, mainly used to produce bottle stoppers for the wine sector and materials for construction sector. The cork industry is one of major relevance for the Portuguese bioeconomy. Cork wastes and by-products for other industries are relevant in terms of the circular economy and sustainability as the waste is reduced and the products obtained are valuable [90].

Given its distribution along the Mediterranean basin, cork oak has adapted to the local environmental conditions, showing genetic variability in adaptive traits. Further studies

using different DNA markers would be beneficial to elucidate the origin and population structure of cork oaks.

The variability found in different cork oak populations must be conserved in dedicated gene banks, with the potential to be used in future breeding programs, tackling adaptation to climate change, tolerance of emerging pests and diseases, and, obviously, cork quality. Although drought seems to have no impact on cork's chemical composition, with characteristics such as suberin-to-lignin proportion not being affected by this condition [91], more studies are needed to elucidate how abiotic stress affects cork biosynthesis and quality. It is worth highlighting that European countries should continue to join efforts for the conservation of genetic resources of this important tree.

With the recent release of a draft genome of cork oak and the availability of numerous high-throughput methodologies (Omics), it is now possible to characterize the multiple layers of genome expression and unveil the epigenome in this species. More studies that would link relevant morphological characteristics and phenotypic responses to genomic elements are needed. They would be useful to further understand the genetic and molecular mechanism underlying the response of cork oaks to abiotic and biotic stresses. Importantly, the identification of candidate genes implicated in cork quality can support the development of new strategies for improvement or breeding for this important species.

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