Conifer Biotechnology

Subjects: Forestry

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Conifers are a group of plants that encompasses the oldest living trees and shrubs on our planet. They have existed for more than 300 million years, starting from a common ancestor of gymnosperms and angiosperms. Conifers comprise two-thirds of gymnosperms and include species of high forest interest, such as pines, spruces, cypresses, or sequoias. Conifers constitute the largest and most diverse group of gymnosperms. Conifers and other gymnosperms were the dominant trees during the Mesozoic Era, which is also known as the Age of the Conifers, although they posteriorly declined and were replaced by angiosperms as the dominant group.

biotechnological research in conifers | genomics | micropropagation techniques | transgenesis

1. General Traits, Distribution, and Diversity

It is very complex to gather all the characteristics of conifers into one definition; they typically have simple needle-shaped or scale-shaped evergreen leaves, even though some deciduous species have been described. In general, conifers are large woody plants with strong apical dominance, although there are shrubby species too. Its main characteristic is to develop cones or strobil es, which are primitive reproductive structures. The highly variable fruiting structures reflect strong selective pressures associated with modes of seed dispersal [1]. Regarding the mating system, conifers are predominantly allogamous. This fact, together with the long-distance pollen dispersal by wind, is responsible for the high gene flow among distant populations leading to the low levels of genetic differentiation between them and the great genetic diversity observed in multiple species [2].

Like many other green plants, they have a diplohaplonic life cycle with the particularity that the dominant diploid sporophyte phase and the annual gametophyte phase occur on the same plant (for a complete revision, see [3]). Three phases can be distinguished in the sporophyte: the juvenile stage, during which they are not reproductively competent; the reproductive onset stage, where cones are only produced in response to certain external stimuli; and the reproductive competence stage, during which cones develop annually under almost any conditions. Most conifers produce woody cones, and seeds are dispersed mainly by wind and gravity, although some species have developed edible fleshy structures for favoring animal seed dispersal.

Conifers are distributed worldwide in a great variety of ecosystems, especially in the boreal and temperate forests of North America and Eurasia. This fact reveals great adaptability to variable environmental conditions, although they are practically absent in deserts, steppes, the Arctic tundra, and some tropical rainforests [1].

Although there are some discrepancies about their taxonomic classification, it is currently accepted based on morphological and molecular studies that conifers include the Class Coniferae or Pinosida, the subclass Pinidae, and three different taxa at the level of order: the Pinales, which only includes the family Pinaceae; the Araucariales, which includes the Araucariaceae and Podocarpaceae families; and the Cupressales, which comprises the Sciadopityaceae, Cupressaceae, and Taxaceae families, although some authors include in this order the two additional families Cephalotaxaceae and Phyllocladaceae [4][5].

The family Pinaceae is the largest in terms of species, as it includes about 232 species distributed in 11 genera (*Abies*, *Cathaya*, *Cedrus*, *Keteleeria*, *Larix*, *Nothotsuga*, *Picea*, *Pinus*, *Pseudolarix*, *Pseudotsuga*, and *Tsuga*). *Pinus* is the largest genus in the family, with about 119 recognized species (The Gymnosperm Database: https://www.conifers.org/) (accessed on 28 May 2022).

Conifers comprise a probably monophyletic group of highly branched trees or shrubs with simple leaves, this being a possible apomorphy of the group. Phylogenetically they are a paraphyletic group with respect to Gnetales (taxon Pinidae, Coniferophyta or others). Different genera such as *Picea* are believed to have originated in North America and then dispersed across the Bering land bridge, showing how the place of origin does not determine the center of diversity [6]. Some results even suggest that, before the division between angiosperms and gymnosperms occurred, a functional specialization had already taken place. The current conifer species map is not yet fully known. It has been reported that the discovery of new genuine conifer species is unsettled, especially in varied and inaccessible ecosystems in the southern hemisphere [4].

Scientific analysis of phylogenetic relationships at various taxonomic levels, mechanisms of evolution at the molecular level of lineages and genomes, and biogeographic dispersal in the development of intercontinental disjunctions or patterns of species diversification [6] are challenges at this point. Understanding the genetic basis of biological processes, evolution, or variation in certain traits is also key to the molecular improvement of specimens [7]. Comparative transcriptomics and genomics of conifer developmental studies are currently being used for these purposes [8], and a database of research related to the study of conifer genera and species [4][9] will be useful for further studies on a more comprehensive and solid basis. These aspects are not unique to conifer studies, and they also have great importance in other fields [10].

2. Ecological and Economic Importance of Conifers

From an ecological and economic point of view, conifers are the most important group of gymnosperms [6]. Coniferous forests account for 31% of the world's total forest plantation area (FAOSTAT), covering vast areas in the Northern hemisphere, and constitute one of the largest terrestrial carbon sinks and play an important role in climate change mitigation. Conifers, in addition to being widely used for ornamental purposes, have an enormous economic importance, as they are a renewable source of timber, both for the elaboration of manufactured product, and to produce energy (50% of the global timber obtained is supplied by conifers, mainly by *Pinus*, as they generate higher and faster economic yield than angiosperms [4]), paper pulp, and other non-wood products such as resins, natural oils, edible seeds, and products with medical use (for example, the anti-cancer drug Taxol).

It has been reiterated that coniferous forests have a relevant role as carbon sinks [11][12] and are expected to increase their prevalence in the current century [12]. However, soil respiration in coniferous forest systems also releases greenhouse gases. Understanding gas release will allow scientific and efficient management of coniferous forests and maintaining forest reserves [13] without neglecting care in urban environments [14]. In addition, being able to accurately monitor the intensity of conifer burning on a large scale would be key to a good analysis of climatic and biological changes in ecosystems [15]. The challenge of understanding all the mechanisms governing the plant biomass and organic carbon stocks behavior will subsequently allow for improved soil organic carbon projections [16] and for combating air pollution and its consequences on health and the economy. The results of this research will, in turn, lead to the sustainability and efficiency of biotechnological conifer forestry.

Habitat deterioration, particularly fires, is depleting conifer ecosystems, a situation aggravated by ineffective conservation methods [17]. Certain conifer groups are already seriously threatened with extinction due to this combination of factors [4]. For example, *Araucaria angustifolia* is listed by the International Union for Conservation of Nature as a critically endangered species [17]. Not only this, but the preservation of conifers has a direct impact on other species so that a decrease in their numbers can have repercussions on the global functionality of the ecosystem, causing changes in trophic cascades and even the loss of biodiversity [4]. For all these reasons, the discipline of restoration ecology has arisen, which, due to the growing importance of the above, is requiring great efforts in current research [4]. Knowing the mechanisms that allow the fastest and most efficient reconfiguration of each deteriorated ecosystem, coniferous forests, will require a very complex diagnostic analysis that will involve different fields: genetic, molecular, tissue, organic, etc. It is a great challenge for the international scientific community to coordinate its efforts in ecological restoration processes, in which bioinformatics and technological innovations will play a crucial role.

Globally, conifers are currently suffering increased mortality due to recent droughts [18][19]. Understanding their ability to adjust their physiology to adapt to drought is another research challenge. Studies in *Pinus sylvestis*, *Pinus halepensis*, or *Pinus pinaster* have shown that in drier and warmer conditions, there is reduced growth and a higher mortality rate, leading to a loss of tree productivity. This can also be used in order to identify forest regions, such as those under Mediterranean conditions, with increased vulnerability so that the responses of different forest ecosystems to ongoing aridification can be monitored [18]. One of the first studies addressing the physiological and biochemical dynamics under extreme drought stress, and subsequent recovery of a gymnosperm species, *Pinus massoniana*, has now been carried out [19]. It is highly likely that, as the climate continues to warm, forest ecosystems will increase their susceptibility to severe drought, leading to community deterioration, death, or reduced growth of individuals. It is, therefore, essential to conduct different studies that assess the response peculiarities of different conifer species [20].

To face the new challenges brought about by climate change and increased pressure due to land use for agriculture, livestock, or urban development, actions must be taken to improve the use and conservation of genetic resources [21]. The seeds have great importance at this point. In this regard, experiments in *Pinus sylvestrys* have been carried out to evaluate the effect of humidity reduction on the quality of seeds obtained from cones in order to observe a faster and more intensive scale opening of cone scales [22]. Research has also been initiated on the performance of seedlings from color-sorted seeds of Scots pine [23]. In addition, the impacts of seed source in pine, and the possibility of selecting provenances to improve growth rates and physical and anatomical wood quality attributes related to tracheid dimensions, have also been analyzed in *Pinus banksiana* [24].

On the other hand, the sustainability of forest ecosystems is seriously altered by pine plantation forestry [12], this being one of the most interesting challenges in the immediate future. Furthermore, understanding ecological relationships in forestry could also prevent the proliferation of diseases in conifers in the face of increasing pests [13] and facilitate research on the suitability of seed and plant production systems, leading to better use of their breeding programs. Due to the increasing wood demand, the establishment of high-yield plantations with enhanced biomass production is necessary. For that purpose, breeding programs for the identification and selection of superior genotypes with improved production traits, such as growth rate, wood quality, and tolerance to biotic and abiotic stresses, have been developed. However, the domestication of conifer species through traditional genetic improvement techniques is much more difficult than in other crops due to their long generation times and the fact that some traits that are important for production cannot be evaluated during the juvenile stage.

Reforestation and conifer plantations sometimes have to deal with poor, eroded, or degraded soils; it is, therefore, necessary to understand the factors that facilitate the reabsorption of nutrients despite the existence of a shortage or limitation of nutrients [25], which could open interesting perspectives for the articulation of the productive system, and the obtaining of resilient conifers. Soil fertilization and the type of containers used in nurseries have been shown to improve yield and crop quality [26][27]. Future studies should address the different macronutrients present in soils at different depths and their variations in order to understand their relationship with conifer growth and development, which will contribute to sustainable *Cunninghamia lanceolata* plantations [25]. One of the factors that has allowed conifers to survive in suboptimal conditions has been the establishment of symbiotic relationships with fungi [4]. In this way, they manage to increase up to ten thousand times the area that allows them to absorb water and nutrients. The effect of inoculated native ectomycorrhizal strains and compatible fungus–conifer combinations for inoculation in seedling nurseries should be increased, even under real field conditions. This would ensure root colonization before transplanting to the field, thus, reducing seedling mortality due to water stress in *Pinus hartwegii* and *Abies religiosa* [28]. Current studies also address the usefulness of mycorrhizae to biologically control different diseases, such as pine wilt [29]. Alternatively, to improve sustainable pest management in the field of conifer bioprotection, and obtaining safe and highly effective insecticides, numerous benefits of soil fumigation for forest conifer seedling production have been described [30][31]. It is foreseeable that new and promising lines of research in this field will open up in the near future. This holistic study will require multidisciplinary and integrative research in order to encompass all interacting microbial communities [32].

At the same time, it is essential to preserve the genetic diversity of native conifer forests, which is essential to conserve the capacity for stress resilience and adaptation to variable environmental conditions of an ecosystem [11][33]. Thus, sustainable forest management requires the development of efficient breeding programs and alternative strategies for the conservation of conifer’s genetic diversity.

A summary of the main ecological problems faced by conifers is listed in **Table 1**.

Table 1. Summary of the main ecological problems faced by conifers.

Problem	References
Diseases and pests	[13]
Habitat deterioration	[17]
Drought	[18][19][20]
Climate change and human pressure	[21]

Biotechnology would have a strong impact on conifer breeding, propagation programs, and their adaptation to the environmental settings that support their development, such as soils, light, or temperature. New biotechnological tools, such as genomic, micropropagation, and genetic engineering, would also offer the possibility to overcome these problems. Nevertheless, the application of these techniques requires a better knowledge of conifer biology. For that purpose, it is necessary to better understand the molecular basis of traits and processes that are important for production and adaptation and the development of reliable experimental systems for their study.

References

1. Neale, D.B.; Wheeler, N.C. The Conifers. In The Conifers: Genomes, Variation and Evolution; Springer: Cham, Switzerland, 2019; pp. 1–21.
2. O'Connell, L.M.; Mosseler, A.; Rajora, O.P. Extensive long-distance pollen dispersal in a fragmented landscape maintains genetic diversity in white spruce. J. Hered. 2007, 98, 640–645.
3. Williams, C.G. Conifer reproductive biology. In Conifer Reproductive Biology; Springer: Dordrecht, The Netherlands, 2009.
4. Farjon, A. The Kew Review: Conifers of the World. Kew Bull. 2018, 73, 8.
5. Christenhusz, M.J.M.; Reveal, J.L.; Farjon, A.; Gardner, M.F.; Mill, R.R.; Chase, M.W. A new classification and linear sequence of extant gymnosperms. Phytotaxa 2011, 19, 55.
6. Wang, X.Q.; Ran, J.H. Evolution and biogeography of gymnosperms. Mol. Phylogenet. Evol. 2014, 75, 24–40.
7. Diao, S.; Ding, X.; Luan, Q.; Jiang, J. A complete transcriptional landscape analysis of Pinus elliottii Engelm. Using third-generation sequencing and comparative analysis in the Pinus phylogeny. Forests 2019, 10, 942.
8. Onyenedum, J.G.; Pace, M.R. The role of ontogeny in wood diversity and evolution. Am. J. Bot. 2021, 108, 2331–2355.

9. Smith, S.A.; Beaulieu, J.M.; Donoghue, M.J. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. Proc. Natl. Acad. Sci. USA 2010, 107, 5897–5902.
10. Li, Z.; Jiao, Y.; Zhang, C.; Dou, M.; Weng, K.; Wang, Y.; Xu, Y. VvHDZ28 positively regulate salicylic acid biosynthesis during seed abortion in Thompson Seedless. Plant Biotechnol. J. 2021, 19, 1824–1838.
11. Bueno, N.; Cuesta, C.; Centeno, M.L.; Ordás, R.J.; Alvarez, J.M. In vitro plant pegeneration in conifers: The role of WOX and KNOX gene families. Genes 2021, 12, 438.
12. Vogel, J.G.; Bracho, R.; Akers, M.; Amateis, R.; Bacon, A.; Burkhart, H.E.; Gonzalez-Benecke, C.A.; Grunwald, S.; Jokela, E.J.; Kane, M.B.; et al. Regional assessment of crbon pool response to intensive silvicultural practices in loblolly pine plantations. Forests 2021, 13, 36.
13. Zhang, X.; Zhao, Z.; Chen, T.; Zhao, T.; Song, L.; Mei, L. Fertilization and clear-cutting effects on greenhouse gas emissions of pinewood nematode damaged Masson pine plantation. Ecosyst. Health Sustain. 2021, 7, 1868271.
14. Hoppa, A.; Sikorska, D.; Przybysz, A.; Melon, M.; Sikorski, P. The role of trees in winter air purification on children's routes to school. Forests 2022, 13, 40.
15. Singh, S.; Singh, H.; Sharma, V.; Shrivastava, V.; Kumar, P.; Kanga, S.; Sahu, N.; Meraj, G.; Farooq, M.; Singh, S.K. Impact of forest fires on air quality in Wolgan valley, New South Wales, Australia; A mapping and monitoring study using Google Earth engine. Forests 2022, 13, 4.
16. Terrer, C.; Phillips, R.P.; Hungate, B.A.; Rosende, J.; Pett-Ridge, J.; Craig, M.E.; van Groenigen, K.J.; Keenan, T.F.; Sulman, B.N.; Stocker, B.D.; et al. A trade-off between plant and soil carbon storage under elevated CO2. Nature 2021, 591, 599–603.
17. Dorigan de Matos Furlanetto, A.L.; Kaziuk, F.D.; Martinez, G.R.; Donatti, L.; Merlin Rocha, M.E.; Dos Santos, A.L.W.; Floh, E.I.S.; Cadena, S. Mitochondrial bioenergetics and enzymatic antioxidant defense differ in Parana pine cell lines with contrasting embryogenic potential. Free Radic. Res. 2021, 55, 255–266.
18. Valeriano, C.; Gazol, A.; Colangelo, M.; Camarero, J.J. Drought drives growth and mortality rates in three pine species under mediterranean conditions. Forests 2021, 12, 1700.
19. Shao, C.; Duan, H.; Ding, G.; Luo, X.; Fu, Y.; Lou, Q. Physiological and biochemical dynamics of Pinus massoniana Lamb. Seedlings under extreme drought stress and during recovery. Forests 2022, 13, 65.
20. Qi, C.; Jiao, L.; Xue, R.; Wu, X.; Du, D. Timescale effects of radial growth responses of two dominant coniferous trees on climate change in the Eastern Qilian mountains. Forests 2022, 13, 72.

21. Flores, A.; López-Upton, J.; Rullán-Silva, C.D.; Olthoff, A.E.; Alía, R.; Sáenz-Romero, C.; Garcia del Barrio, J.M. Priorities for Conservation and Sustainable Use of Forest Genetic Resources in Four Mexican Pines. Forests 2019, 10, 675.
22. Aniszewska, M.; Gendek, A.; Tulska, E.; Pęska, P.; Moskalik, T. Influence of the duration of microwave irradiation of Scots pine (Pinus sylvestris L.) cones on the quality of harvested seeds. Forests 2019, 10, 1108.
23. Novikov, A.; Sokolov, S.; Drapalyuk, M.; Zelikov, V.; Ivetić, V. Performance of Scots pine seedlings from seeds graded by colour. Forests 2019, 10, 1064.
24. Mvolo, C.S.; Koubaa, A.; Beaulieu, J.; Cloutier, A. Effect of seed transfer on selected wood quality attributes of jack pine (Pinus banksiana Lamb.). Forests 2019, 10, 985.
25. Wu, H.; Xiang, W.; Ouyang, S.; Xiao, W.; Li, S.; Chen, L.; Lei, P.; Deng, X.; Zeng, Y.; Zeng, L.; et al. Tree growth rate and soil nutrient status determine the shift in nutrient-use strategy of Chinese fir plantations along a chronosequence. For. Ecol. Manag. 2020, 460, 117896.
26. Sung, S.-J.S.; Dumroese, R.K.; Pinto, J.R.; Sayer, M.A.S. The persistence of container nursery treatments on the field performance and root system morphology of longleaf pine seedlings. Forests 2019, 10, 807.
27. Liu, Q.; Xu, H.; Yi, H. Impact of fertilizer on crop yield and C:N:P stoichiometry in arid and semi-arid soil. Int. J. Environ. Res. Public Health 2021, 18, 4341.
28. Flores-Rentería, D.; Barradas, V.L.; Álvarez-Sánchez, J. Ectomycorrhizal pre-inoculation of Pinus hartwegii and Abies religiosa is replaced by native fungi in a temperate forest of central Mexico. Symbiosis 2018, 74, 131–144.
29. Vicente, C.S.L.; Soares, M.; Faria, J.M.S.; Ramos, A.P.; Inacio, M.L. Insights into the role of fungi in pine wilt disease. J. Fungi. 2021, 7, 780.
30. Stejskal, V.; Vendl, T.; Aulicky, R.; Athanassiou, C. Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. Insects 2021, 12, 590.
31. Balla, A.; Silini, A.; Cherif-Silini, H.; Chenari Bouket, A.; Moser, W.K.; Nowakowska, J.A.; Oszako, T.; Benia, F.; Belbahri, L. The threat of pests and pathogens and the potential for biological control in forest ecosystems. Forests 2021, 12, 1579.
32. Rama, T.; Quandt, C.A. Improving fungal cultivability for natural products discovery. Front. Microbiol. 2021, 12, 706044.
33. Potter, K.M.; Riitters, K. A national multi-scale assessment of regeneration deficit as an indicator of potential risk of forest genetic variation loss. Forests 2021, 13, 19.