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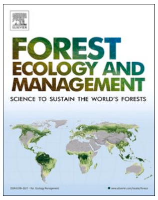

Diversity and enrichment of breeding material for resilience in European forests

Sanna Olsson [a,*], Benjamin Dauphin [b], Véronique Jorge [c], Delphine Grivet [a], Anna Maria Farsakoglou [d], Jose Climent [a], Paraskevi Alizoti [d], Patricia Faivre-Rampant [e], Sara Pinosio [j], Pascal Milesi [h,i], Simone Scalabrin [g], Francesca Bagnoli [j], Ivan Scotti [k], Giovanni G. Vendramin [j], Santiago C. Gonzalez-Martinez [f], Bruno Fady [k], Filippos A. Aravanopoulus [d], Catherine Bastien [c], Ricardo Alia [a,*]

[a] *Institute of Forest Sciences (ICIFOR-INIA), CSIC, Avda A Coruña km 7.5, ES-2840, Madrid, Spain*
[b] *Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland*
[c] *INRAE, ONF, BioForA, 45075 Orléans, France*
[d] *Aristotle University of Thessaloniki, School of Forestry and Natural Environment, 54124 Thessaloniki, Greece*
[e] *Université Paris-Saclay, INRAE, EPGV, F-91057 Evry, France*
[f] *INRAE, Université de Bordeaux, BIOGECO, F-33612 Cestas, France*
[g] *IGA Technology Services, IT-33100 Udine, Italy*
[h] *Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, SE-752 36, Uppsala, Sweden*
[i] *Science for Life Laboratory, SciLifeLab, Uppsala, Sweden*
[j] *Institute of Biosciences and Bioresources, National Research Council, Division of Florence, I-50019 Sesto Fiorentino, FI, Italy*
[k] *INRAE, URFM, Ecology of Mediterranean Forests, Avignon, France*

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ABSTRACT

Delivering material selected for breeding purposes into the wild in the context of sustainable forest management might reduce the levels of genetic diversity of future forests in comparison to that of natural populations. Another consequence might be a reduction of their resilience under uncertain future climatic and socio-economic conditions if these new populations lack adaptability. Despite the long tradition of breeding activities in Europe, there is still a need to assess the impact of genetically enriched material on forests' resilience. In this study, we address (1) the genetic diversity of selected material compared to its wild ancestors, and (2) how to enrich breeding material to support forests' resilience under changing socio-environmental conditions. We analysed 16 study cases of selected material delivered from breeding activities in four European forest tree species (*Pinus halepensis* Mill., *Pinus nigra* J.F. Arnold, *Pinus pinaster* Ait. and *Populus nigra* L.) with different levels of breeding. To answer these two questions, we first assessed and compared the genetic diversity of selected material versus natural populations using both putatively neutral and adaptive (based on diverging selection) Single Nucleotide Polymorphisms (SNPs). We then suggest how to enrich these populations for resilience under future climatic conditions by defining a core collection for each species including material from populations that will likely disappear under future conditions. Thanks to the large SNP datasets available for our focal species, we were able to detect some trends in our data. Expected and observed heterozygosity values for selected populations were almost always identical. The selected material showed small but significant genetic differentiation from their original population and their inbreeding coefficient was generally lower. However, the level of genetic improvement (i.e. low vs high) was not correlated with the observed genetic differences between selected material and natural populations.The genetic characterization of natural populations distributed across the species

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* Corresponding authors.
E-mail addresses: sanna.olsson@inia.csic.es (S. Olsson), benjamin.dauphin@wsl.ch (B. Dauphin), veronique.jorge@inrae.fr (V. Jorge), dgrivet@inia.csic.es (D. Grivet), climent@inia.csic.es (J. Climent), alizotp@for.auth.gr (P. Alizoti), patricia.faivre-rampant@inrae.fr (P. Faivre-Rampant), sara.pinosio@ibbr.cnr.it (S. Pinosio), pascal.milesi@scilifelab.uu.se (P. Milesi), sscalabrin@igatechnology.com (S. Scalabrin), francesca.bagnoli@ibbr.cnr.it (F. Bagnoli), ivan.scotti@inrae.fr (I. Scotti), giovanni.vendramin@ibbr.cnr.it (G.G. Vendramin), santiago.gonzalez-martinez@inrae.fr (S.C. Gonzalez-Martinez), bruno.fady@inrae.fr (B. Fady), aravanop@for.auth.gr (F.A. Aravanopoulus), catherine.bastien@inrae.fr (C. Bastien), alia@inia.csic.es (R. Alia).

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range, and the future projection of their range stability, made it possible to identify core-collections that would significantly enrich breeding populations under uncertain future environmental conditions.

1. Introduction

Natural forests are important reservoirs of genetic diversity and are essential for biodiversity and genetic conservation (Alberto et al., 2013; EUFORGEN, 2021; Lefèvre et al., 2012; Schueler et al., 2014). With the current, unprecedentedly rapid climate change, the future of forests, their resilience as well as their potential for the continued delivery of ecosystem goods and services, depend largely on their adaptive capacity (Lindner et al., 2014, 2010) and on the sustainable management and use of their genetic resources (Fady et al., 2016). These genetic resources also play a pivotal role in forest plantation as a major lever for adaptation (Alfaro et al., 2014; Loo et al., 2014), constituting a resource for breeding programs for improving target traits and resilience to changing environmental conditions.

Tree breeding programs deliver plants obtained from specific basic material (e.g. seed orchards, clones of clonal mixtures), after the selection of a limited number of genotypes and targeting a limited number of specific traits (Skogforsk, 2013). Tree breeding activities have been performed in Europe in operational programs since the mid-twentieth century, and in comparison to other organisms, forest trees are in a very early phase of domestication (Bradshaw, 1998). Selected material for breeding purposes (*selected material* thereafter) obtained from these programs exists for a limited number of commercially important species (Skogforsk, 2013), differing in levels of genetic diversity and genetic gain for the traits under selection. In addition to *traditionally intensive breeding* strategies, based on successive cycles of selection and crossing to maximize genetic gain over time (e.g. *Betula pendula* Roth., *Picea abies* L. Karst, *Pinus sylvestris* L. in Nordic countries, *Pinus pinaster* Ait. in France, or *Populus* spp. in Belgium, France and Italy), *low-input strategies* have also been applied for seed production and breeding in southern European conifers (e.g. *Pinus halepensis* Mill., *Pinus nigra* J.F. Arnold), and in specific regions (e.g. *Pinus pinaster* in the Iberian Peninsula, *Pinus sylvestris* in Southern Europe), based on pedigree reconstruction and testing, as well as seed orchard establishment (Bouffier et al., 2019; El-Kassaby and Lstibůrek, 2009; Skogforsk, 2013).

One major concern when delivering selected material into the wild, to maintain the adaptability of the natural populations in the context of sustainable management, is a reduced level of global genetic diversity (Koskela et al., 2013). Different studies in seed orchards or at early stages of domestication showed neither large effects on the genetic diversity nor a drastic reduction in rare alleles (Chaisurisri and El-Kassaby, 1994; El-Kassaby and Ritland, 1996; Ivetić et al., 2016; Jones et al., 2006; Sønstebø et al., 2018), but see the work of Rajora (1999). Long-term assessments of plant breeding programs have confirmed that genetic diversity decreases over time. For example, a 26 to 35 % reduction in genetic diversity was reported for maize after 12 to 15 cycles of selection (Lu and Bernardo, 2001; Hagdorn et al., 2003), while this reduction was of 40 % after 40 years of selection in barley (Condón et al., 2008). Simulations carried out in breeding programs of forest trees revealed that genetic diversity can be maintained in the long run while increasing genetic gain, if new material is included at each selection step in the breeding population (Danusevicius and Lindgren, 2005;

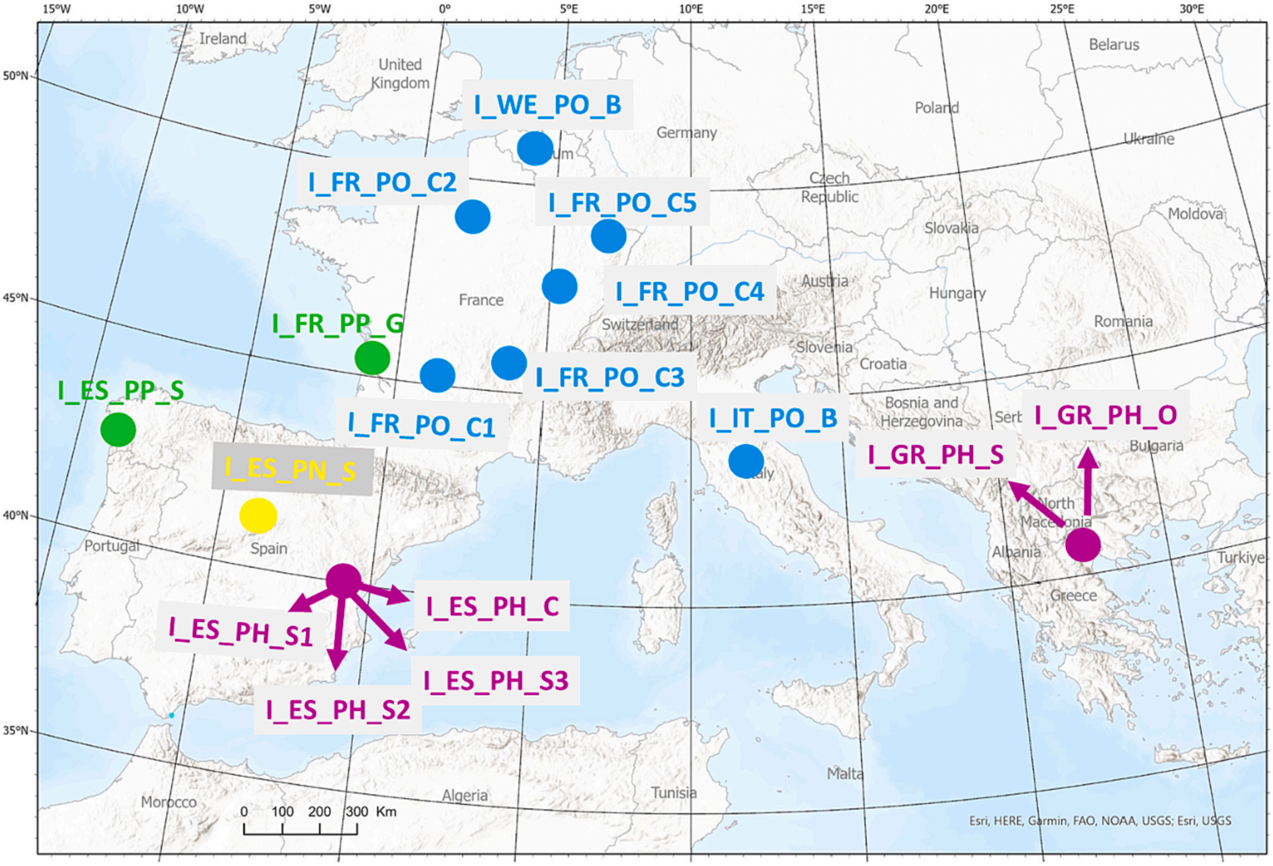

Fig. 1. Location of the selected material (codes as in Table 1, purple- *Pinus halepensis*, yellow- *P. nigra*, green- *P. pinaster*, blue- *Populus nigra*). [The location of *Populus nigra* material is approximate]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Description of the selected material for the different species.

Code[1]	Species	Area	N[2]	Description	Breeding Intensity	Objective	Natural population
I_GR_PH_S	PH	GR	49	Seed collected in a fist-generation clonal seed orchard	High	Height	GR_PH_12
I_GR_PH_O	PH	GR	66	Components of a first-generation clonal seed orchard	High	Height	GR_PH_12
I_ES_PH_S1	PH	ES	19	Seedlings from a subline (30 individuals)	Low	Height	ES_PH_04
I_ES_PH_S2	PH	ES	22	Seedlings from a subline (30 individuals)	Low	Reproduction	ES_PH_04
I_ES_PH_S3	PH	ES	20	Seedlings from a subline (30 individuals)	Low	Drought	ES_PH_04
I_ES_PH_C	PH	ES	21	Seedlings from a control subline of 30 individuals	None	Control	ES_PH_04
I_ES_PN_S	PN	ES	32	Seed collected in a first-generation clonal seed orchard	Low	Height	ES_PN_P71 ES_PN_P72
I_FR_PP_G	PP	FR	46	Founders individuals from the breeding population	High	Volume	FR_PP_LAND
I_ES_PP_S	PP	ES	21	Seed collected in a first-generation clonal seed orchard.	Low	Height	ES_PP_GALIC
I_WE_PO_B	PO	WE	20	Clones selected in Belgium and France (WE-1–5)	High	Volume	WE_PO
I_IT_PO_B	PO	IT	26	Clones selected in Italy (IT1-5)	High	Volume	IT_PO
I_FR_PO_C1	PO	FR	16	Multiclonal variety from Garonne river (GAR-1–7)	Low	Restoration	FR_PO_GAR
I_FR_PO_C2	PO	FR	24	Multiclonal variety from Loire river (LOI-1–6)	Low	Restoration	FR_PO_LOI
I_FR_PO_C3	PO	FR	25	Multiclonal variety from Rhone river (RHO-3 1–4)	Low	Restoration	FR_PO_RHO
I_FR_PO_C4	PO	FR	25	Multiclonal variety from Saone river (SAO-1–2)	Low	Restoration	FR_PO_SAO
I_FR_PO_C5	PO	FR	23	Multiclonal variety from Rhin river (RHI-1–3)	Low	Restoration	FR_PO_RHI

[1] The code refers to **I**: selected for genetic improvement, **Area** (GR-Greece, ES-Spain, WE-Belgium and France, IT-Italy, FR-France), **Species** (PH- *Pinus halepensis*, PN- *P. nigra*, PP- *P. pinaster*, PO- *Populus nigra*), and **type of material** (S: seeds, O: components of a seed orchard, G: genitors of the breeding population, Ci: Clonal Mixture i). [2]N: number of genotypes used in the study.

Ingvarsson and Dahlberg, 2019). Another consequence of delivering selected material for specific traits into the wild could be their low resilience under uncertain future climatic and socio-economic conditions as these new populations could be no longer adapted to future conditions (Aitken et al., 2008; Serra-Varela et al., 2017; Wang et al., 2010).

An option to reduce the risks of losing genetic diversity after selection and/or to improve the resilience under social and climatic uncertainty is to enrich breeding populations with existing genetic variation from natural populations (Faivre-Rampant et al., 2016; Jaramillo-Correa et al., 2015; Ruiz Daniels et al., 2018; Scotti-Saintagne et al., 2019). Based on knowledge of the genetic variation within species, it is possible to select "core" collections (i.e. a subset of accessions, capturing the genetic diversity of the original set of accessions) (Odong et al., 2013), and identify material harbouring genetic variation that likely would be of adaptive relevance under projected climate change scenarios (Ingvarsson and Bernhardsson, 2020). Different strategies have been defined to manage these collections (*e.g.* by defining sublines, *ex situ* gene-banks), or by incorporating into breeding populations material from specific populations depending on the future trajectories of climate (*e.g.* assisted migration or climate-adjusted seed sourcing method (Breed et al., 2013)).

In this study, we address two main questions for the future of forest tree improvement in Europe: (1) what is the effect of breeding on the genetic diversity of selected material compared to its wild ancestors, and (2) how to enrich breeding material for increasing its ability to produce resilient forests under changing socio-environmental conditions. We analysed 16 study cases of selected material representing the material delivered from breeding activities in four European forest tree species, three conifers (*Pinus halepensis* Mill., *Pinus nigra* J.F. Arnold, *Pinus pinaster* Ait.) and one broadleaf (*Populus nigra* L.) with different levels of breeding. We expected that the most improved material would show the lowest genetic diversity compared to the wild types. To answer these two questions, we first assessed and compared the genetic diversity of selected material versus natural populations using both putatively neutral and adaptive (based on diverging selection) Single Nucleotide Polymorphisms (SNPs). We then suggest how to enrich these populations for resilience under future climatic conditions by defining a core collection for each species and by including material from populations that will likely disappear under future conditions.

2. Material and methods

2.1. Study range and sampling design

We analysed the genetic diversity of 16 selected materials: six in *Pinus halepensis*, one in *P. nigra*, two in *P. pinaster* and seven in *Populus nigra* (Fig. 1). The selected material differs in its level of selection and breeding (Table 1) and includes: founder trees from the base breeding population (G), components of clonal seed orchards (O), seeds collected from seed orchard or selected populations (S) and clones from breeding population (B) or from clonal mixture (C).

The genetic diversity of each selected population was compared, first to the natural population from which it was originally selected (Table 1), and second, to available genotyped populations covering most of the natural range of the species (14, 18, 32 and 15 locations for *Pinus halepensis*, *P. nigra*, *P. pinaster* and *Populus nigra*, respectively (see Faivre-Rampant et al., 2016; Opgenoorth et al., 2021; Ramírez-Valiente et al., 2021; Rodriguez-Quilon et al., 2016; Santos-del-Blanco et al., 2012) (Figure S1).

2.2. Genotyping

SNPs for *Pinus halepensis* and *P. nigra* were obtained using the Single Primer Enrichment Technology (SPET) genotyping (Scaglione et al., 2019). For both species, a specific design was developed targeting 12,000 polymorphic positions available from previous studies. In particular, for *P. halepensis* we used SNP data in Olsson et al. (2021) and the reference transcriptome in Pinosio et al. (2014), while for *P. nigra* we used SNP data and the reference transcriptome in Olsson et al. (2020) complemented with thirteen transcriptomic contigs corresponding to genes of interest used in Scotti-Saintagne et al. (2019). Libraries were prepared following the standard protocol for the 'Ovation® Target Enrichment' (NuGEN Technologies). Sequencing was performed at the IGA Technology Services (IGATech, Udine, Italy) facilities using a NextSeq500 platform (Illumina, San Diego, CA, USA) in single-end mode (150 bp). BCL files from the instruments were processed using the manufacturer's pipeline software to generate FASTQ sequence files. Short reads were deposited into NCBI Short Read Archive as BioProject accessions PRJNA791282 (*P. halepensis*) and PRJNA791288 (*P. nigra*). Adaptor sequences and low-quality 3′ ends were removed from short reads using cutadapt (Martin, 2011) and ERNE-FILTER (Del Fabbro et al., 2013), respectively, with default parameters. After trimming, reads longer than 50 bp were aligned to the respective reference transcriptomes using the short read aligner BWA-MEM (Li, 2013) with default parameters. SNP calling was performed on uniquely aligned reads using the software package GATK v4.0.10.0 (DePristo et al., 2011), following the software best practices for germline short variant discovery. Briefly, we ran *HaplotypeCaller* in GVCF mode to call potential variant sites at single-sample level, followed by *GenomicsDBImport* and *GenotypeGVCFs* to perform joint genotyping on the entire cohort of samples. We then ran *SelectVariants* and *VariantFiltration* (filter expression used: QD < 2.0 || MQ < 40.0 || MQRankSum < -12.5) to select and filter SNPs. VCF files are available at: https://data.inrae.fr/dataset.xhtml?persistentId=doi:10.15454/7COC1A. Additional filtering applied in this study is described in Appendix 1. The Illumina Infinium SNP array used to genotype *Populus nigra* was developed by Faivre-Rampant et al. (2016) together with genotyping data of 888 individuals, complemented with genotyping for this study, the entire data set being available at: https://doi.org/10.57745/PQBHSP. Metadata on *Populus* genotypes can be found at https://urgi.versailles.inrae.fr/faidare/?crops=Forest%20tree&germplasmLists=BLACK_POPLAR_COLLECTION&sources=https:%2F%2Furgi.versailles.inrae.fr%2Fgnpis&page=1. The genetic data of *P. pinaster* were obtained from an Illumina Infinium SNP assay (de Miguel et al., 2022; Hurel et al., 2021) developed by Plomion et al. (2016).

2.3. SNP datasets

We generated three SNP datasets. We defined an **overall SNP** dataset (including all individuals and applied to each species) by removing monomorphic sites, SNPs with>30 % missing calls or a global minor allele frequency below 0.01 (MAF_0.01), as well as individuals with>50 % missing data using either *vcftools* v0.1.13 (Danecek et al., 2011) or function *missingno* from R-package *poppr* v2.9.3 (Kamvar et al., 2015, 2014). The **common SNP** dataset was defined from the overall SNP dataset as the one with MAF above 0.05.

The **outlier SNP** dataset included SNPs presenting signals of diverging selection in either one of the two tests described thereafter. Only individuals from natural populations were included in the outlier analyses. A threshold of MAF 0.05 (as in the common dataset) was applied to exclude poorly supported outlier loci. First, we performed an F_{ST} outlier test to detect overly-differentiated loci among populations using the island model implemented in BayeScan (Foll and Gaggiotti, 2008), which is based on an extension of the *F* model (Beaumont and Balding, 2004). BayeScan was run for each of the four species with standard parameters, i.e., 5,000 iterations, a thinning interval of 10, 20 initial pilot runs with a length of 5,000, and a burn-in length of 50,000. We set a prior odd value of 10,000 to be conservative in rejecting the neutral hypothesis. Then, we applied a false discovery rate (FDR) of 5 % and retained loci with a positive α value.

Second, we used a principal components analysis (PCA) using PCAdapt, (Luu et al., 2017). This method decomposes genotypic variation into principal components (PCs) to incorporate population genetic structure, after which loci exhibiting atypical associations with the PCs are identified as putative outliers. PCAdapt was initially run with 10 PCs and the proportion of variance explained by each PC was visually inspected. We retained the optimal number of PCs ($K = 2$ for *P. halepensis* and *P. nigra*, and $K = 5$ for *P. pinaster* and *Populus nigra*), while controlling for population genetic structure as diagnosed in Q-Q plots. We then applied a false discovery rate (FDR) of 5 % to control for false positives using the Benjamini-Hochberg procedure implemented in the *p.adjust* R function. The combination of the outlier SNPs identified in both analyses was retained for downstream analyses (*outliers*).

2.4. Core collection of genotypes

For each species, based on the overall SNP set, we selected a core

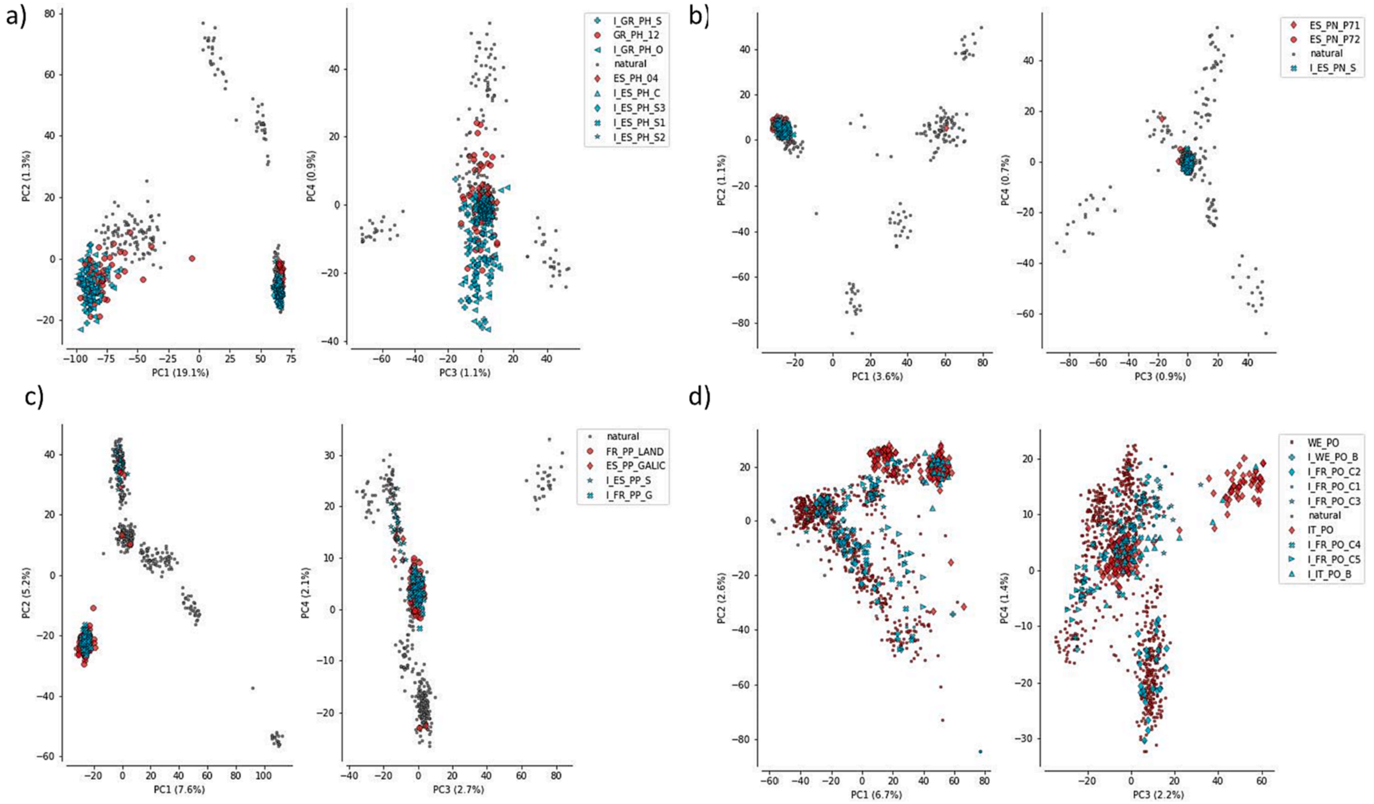

Fig. 2. PCA of selected vs natural populations for the Overall SNPs datasets. a) *Pinus halepensis*, b) *Pinus nigra*, c) *Pinus pinaster*, d) *Populus nigra*. (codes of the populations as in Fig. 1 and Table 1). [Red is used for natural and light blue for breeding populations]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Data sets and material used in the case studies. The number of SNPs (#) and percent of outlier SNPs in respect to the Common SNP dataset (%) are given for the three datasets (overall, common and outlier). Nat. Pops = natural populations, Sel. Pops = selected material for genetic improvement.

Species	Individuals #	Nat. Pops #	Sel. Pops	Overall #	Common #	Outlier #	%
Pinus halepensis	598	14	6	10,278	6,342	411	6,5
Pinus nigra	375	18	1	19,082	6,579	370	5,6
Pinus pinaster	566	32	2	5,047	4,659	166	3,6
Populus nigra	1,118	15	7	5,981	5,969	40	0,7

Table 3
Pairwise genetic differentiation (F_{ST}) between selected populations and their most closely related natural ones and inbreeding coefficient (F_{IS})). (codes of populations as in Table 1, Significance levels at the 0.05 % - based on the bootstrap confidence intervals). In F_{IS} comparisons, < indicate a significant lower value and > a greater value than the reference natural population. *ns*: non-significant).

		Overall SNP dataset			Common SNP dataset			Outlier SNP dataset		
Selected material	**Natural population**	**F_{ST}**	**F_{IS} selected**	**F_{IS} natural**	**F_{ST}**	**F_{IS} selected**	**F_{IS} natural**	**F_{ST}**	**F_{IS} selected**	**F_{IS} natural**
I_GR_PH_S	GR_PH_12	0.008 *	−0,059 * <	0,044	0.004 *	−0,079 * <	0,043	0.0187*	−0,004 * <	0,051
I_GR_PH_O	GR_PH_12	0.003 *	−0,040 * <	0,045	0.004 *	−0,058 * <	0,043	0.005 *	−0,006 * <	0,057
I_ES_PH_C	ES_PH_04	0.024 *	−0,059 * <	0,115	0.025 *	−0,072 * <	0,126	0.031 *	−0,044 * <	0,176
I_ES_PH_S1	ES_PH_04	0.029 *	−0,050 * <	0,117	0.030 *	−0,063 * <	0,128	0.009 *ns*	0,015 * <	0,177
I_ES_PH_S2	ES_PH_04	0.024 *	−0,053 * <	0,117	0.025 *	−0,064 * <	0,126	0.039 *	0,004 * <	0,176
I_ES_PH_S3	ES_PH_04	0.023 *	−0,042 * <	0,116	0.023 *	−0,051 * <	0,125	0.052 *	−0,012 * <	0,169
I_ES_PN_S	ES_PN_P71	0.005 *	−0,036 ns	−0,036	0.006 *	−0,060 * <	−0,058	0.003 *ns*	−0,021 * <	0,084
	ES_PN_P72	0.010 *	−0,037 * >	−0,056	0.011 *	−0,061 * >	−0,087	0.009 *	−0,028 * >	−0,051
I_FR_PP_G	FR_PP_LAND	0.003 *	−0,010 * <	0,015	0.003 *	−0,010 * <	0,015	0,009 *	−0,038 * <	0,019
I_ES_PP_S	ES_PP_GALIC	0.005 *	−0,018 * <	0,012	0.005 *	0,012 *ns*	−0,019	0,019 *	−0,034 * <	0,120
I_WE_PO_B	WE_PO	0.016 *	−0,029 * <	0,051	0.016 *	−0,029 * <	0,051	0.012 *	0,001 * <	0,157
I_IT_PO_B	IT_PO	0.008 *	0,052 * <	0,069	0.008 *	0,052 * <	0,069	0.009 *ns*	0,077 * <	0,203
I_FR_PO_C1	FR_PO_GAR	0.039 *	0,005 * <	0,032	0.039 *	0,005 * <	0,032	0.067 *	0,041 *ns*	0,032
I_FR_PO_C2	FR_PO_LOI	−0.001 *ns*	0,005 * <	0,017	−0.001 *ns*	0,005 * <	0,016	−0.004 *ns*	0,026 *ns*	0,017
I_FR_PO_C3	FR_PO_RHO	0.017 *	0,060 * >	0,018	0.017 *	0,060 * >	0,018	0.025 *	0,094 * >	0,035
I_FR_PO_C4	FR_PO_SAO	0.016 *	0,019 * >	−0,003	0.016 *	0,018 * >	−0,003	0.057 *	0,072 *ns*	0,081
I_FR_PO_C5	FR_PO_RHI	−0.002 *ns*	0,003 * >	−0,014	−0.002 *ns*	0,003 * >	−0,014	−0.006 *ns*	−0,037 *ns*	−0,047

collection of samples from the total samples included in the natural range of each species, by using the iterative procedure implemented in the GenoCore software (Jeong et al. 2017). For each step, GenoCore selects a sample that is the most representative of the dataset by using two statistics, the coverage (i.e., the representativeness of the sample defined) and the diversity scores (i.e. the variability of the sample). The process is repeated until the coverage reaches 99 % of the total diversity of the populations in the natural range for each species, or the coverage-increasing rate (difference between the coverage of i-th step and (i-1)-th step) reaches a value of 0.01 %.

2.5. Genetic diversity of selected and natural populations

Diversity measures were computed for the three sets of SNPs (overall, common, outlier), for each of the comparisons of selected and reference natural populations, and for the entire range of distribution of the species considered.

Allelic frequencies were estimated using *vcftools* and plotted using the R-package *ggplot2* (Wickham, 2016). We used the R-package *hierfstat* v0.5–10 (Goudet, 2005) to estimate the per-site observed heterozygosity (H_o), per-site expected heterozygosity (H_s), inbreeding coefficient (F_{IS}), and pairwise fixation index (F_{ST}) among pairs of selected *and* reference natural population, performing a bootstrap (1,000) to obtain confidence intervals. The H_s-test implemented in the R-package *adegenet* v2.1.5 (Jombart, 2008) was performed to test if two groups (the selected and reference natural population) had significant differences in expected heterozygosity. Expected and observed heterozygosities were compared for each population using Bartlett́s test of homogeneity of variances (Bartlett, 1937) and a T-test on the *adegenet* objects. A principal component analysis (PCA) as implemented in the Python package *scikit-allel* v1.2.1 (Miles et al., 2021) was performed to visualize population genetic structure.

In addition, to get insights into the distribution of allelic frequencies, we performed an analysis of minor allele frequencies (MAF) by comparing selected and reference natural populations against the genetic diversity assessed from the whole distribution area represented by the GenoCore collection. All three SNP data sets were used to compare allelic frequencies of each locus (obtained with *vcftools*) in these collections with the selected material to detect loss of rare alleles in the selected populations.

2.6. Enrichment of breeding populations

To select the natural populations to be used to enrich the breeding populations, we considered all the genotypes included in the GenoCore collection, representing a large part of the genetic diversity of the material analysed. Among them, we identified populations with unsuitable climatic conditions under the future scenarios (see Mauri et al., 2022). These populations may harbour variants of interest for the adaptation that will not be available in the future, if the population disappear. This approach is similar to the assisted migration and climate-adjusted seed sourcing methods (Breed et al., 2013).

We classified natural populations (Figure S1) in each species according to their predicted distribution in 2095 with respect to the actual distribution under the emission scenarios RCP 4.5 and 8.5 (Mauri et al., 2022). According to their terminology, the populations can be stable (i.e. the population is included in the predicted distribution under the two climatic scenarios), decolonized (i.e. the population is not included in the predicted distribution under any of the two scenarios), or uncertain (i.e. the population is included in the predicted distribution for one of the two scenarios). One *P. halepensis* Greek population (GR_PH_11) was not included at this step as the projections by Mauri et al. (2022) did not cover the sample coordinates.

Table 4
Median and mean values of minor allele frequencies (i.e. alleles with frequencies < 0.50) in four species for selected material and the GenoCore collection (See Table 1 for the codes, and description of the selected material). [Applied MAF filtering in the three data sets correspond to overall (SNPs with MAF > 0.01), common (SNPs with MAF > 0.05) and outlier (SNPs with MAF > 0.05) SNP data sets.].

Code	Overall Median	Mean	Common Median	Mean	Outlier Median	Mean
I_GR_PH_S	0.111	0.167	0.255	0.252	0.176	0.188
I_GR_PH_O	0.121	0.170	0.250	0.255	0.182	0.183
I_ES_PH_S1	0.000	0.059	0.026	0.086	0.000	0.059
I_ES_PH_S2	0.000	0.061	0.023	0.089	0.023	0.088
I_ES_PH_S3	0.000	0.060	0.025	0.089	0.000	0.048
PH GenoCore	0.103	0.154	0.219	0.233	0.388	0.348
I_ES_PN_S	0.031	0.067	0.125	0.159	0.094	0.161
PN GenoCore	0.031	0.071	0.123	0.163	0.221	0.238
I_FR_PP_G	0.261	0.250	0.283	0.272	0.293	0.247
I_ES_PP_S	0.190	0.212	0.214	0.228	0.095	0.116
PP GenoCore	0.276	0.269	0.293	0.287	0.277	0.289
I_WE_B	0.321	0.297	0.321	0.298	0.071	0.158
I_IT_PO_B	0.333	0.317	0.333	0.318	0.367	0.338
PO GenoCore	0.370	0.342	0.370	0.343	0.315	0.335

The code refers to **I**: selected material for genetic improvement, **Area** (GR-Greece, ES-Spain, WE-Belgium and France, IT-Italy, FR-France), **Species** (PH-*Pinus halepensis*, PN- *P. nigra*, PP- *P. pinaster*, PO- *Populus nigra*), GenoCore: core collection.

3. Results

The number of SNPs differed among species (Table 2), with the highest number of SNPs for *P. halepensis* (10,278) and *P. nigra* (19,082). In *P. halepensis* and *P. nigra,* a large proportion of the SNPs had very low frequencies, respectively 38.3 % and 65.5 % of the SNPs had a minor allele frequency (MAF) < 0.05 (see Figure S2 where MAF distributions are presented as densities). In *P. pinaster* and *Populus nigra,* filtering for MAF at 0.05 only slightly reduced the number of markers (Table 2) due to a genotyping tool design that included selection by allele frequency.

3.1. Genetic diversity of selected and natural populations

The comparison between expected and observed heterozygosity in each selected population did not show significant differences for any of the three pine species (Table S1, Figures in S3). In *Populus nigra*, the Italian breeding population (I_IT_PO_B) differed based on common markers but not for outlier markers (Fig. S3-4). One French multi-clonal breeding population (I_FR_PO_C3) had an observed heterozygosity significantly lower than expected for all the datasets and one (I_FR_PO_C4, T-test $p = 0.034$) for the outlier SNPs (Table S2).

We detected a significant reduction in inbreeding coefficient (F_{IS}) in most of the selected material, when compared with wild material for all three sets of SNPs (70 %, 70 % and 65 % for the overall, common and outlier loci respectively), and an increment in the case of *Pinus nigra* and one *Populus* multiclonal varieties for all the datasets and in two of the *Populus* multiclonal varieties for total and common SNP datasets (Table 3). Pairwise F_{ST} values to test the divergence between allele frequencies of the breeding and reference populations were significant for most of the study cases and datasets, except in two *Populus nigra* multiclonal varieties (Table 3). The level of differentiation is low (<1%) in most of the cases, except for some *Pinus halepensis* and *Populus nigra* selected material (>2.5 %).

The pattern of variation can be observed in the genotype-based PCA (overall SNPs dataset) showing that genetic variation in selected material was generally close to that of natural populations, except in *Populus nigra* and *Pinus halepensis* where Spanish genetic material displayed higher genotypic variation (Fig. 2); the patterns were similar when using the outlier loci (Figure S4).

3.2. Core collection of genotypes

The core collections, covering 99 % of the total diversity of each of the four species, varied in their number of components (157 genotypes for *Pinus nigra*, 155 for *P. halepensis*, 58 for *P. pinaster* and 28 for *Populus nigra*, see Table S3), with samples originating from different countries (Table 4) and each of the main PCA groups of the species (see Fig. 3 for

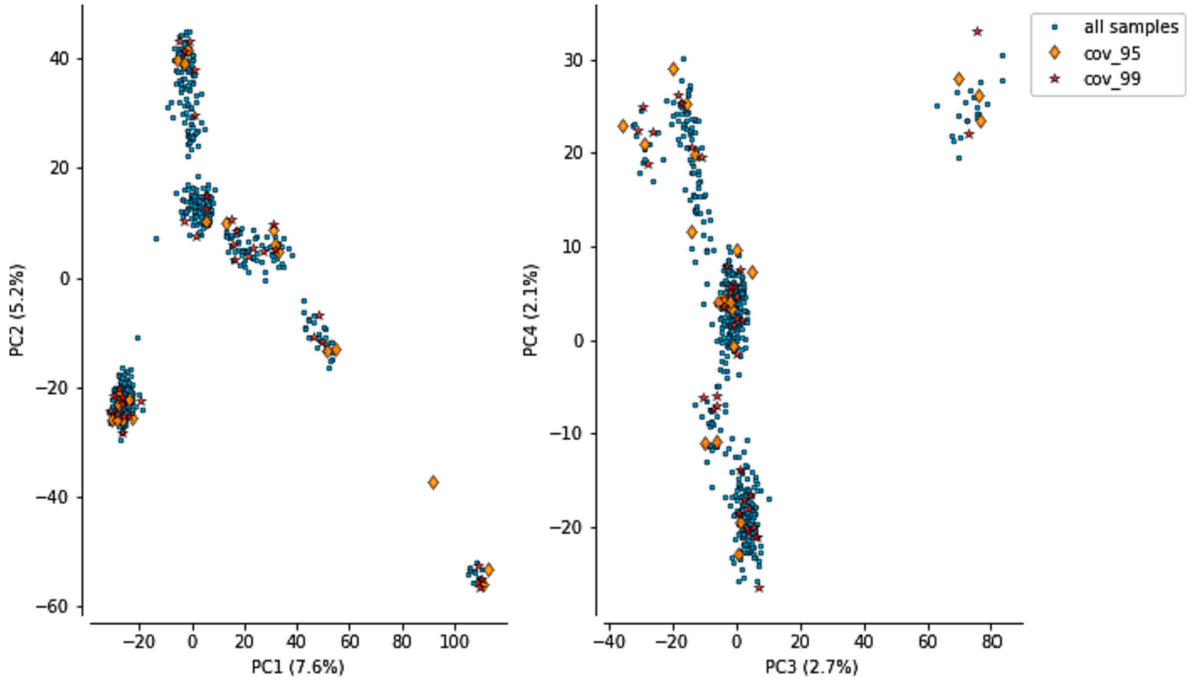

Fig. 3. PCA of natural and GenoCore-collection in *Pinus pinaster* (Blue is used for all the samples. Orange diamonds for cov_95, i.e. core collection to cover 95% of the total diversity, and orange stars for cov_99, i.e. core collection to cover 99% of the total diversity). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

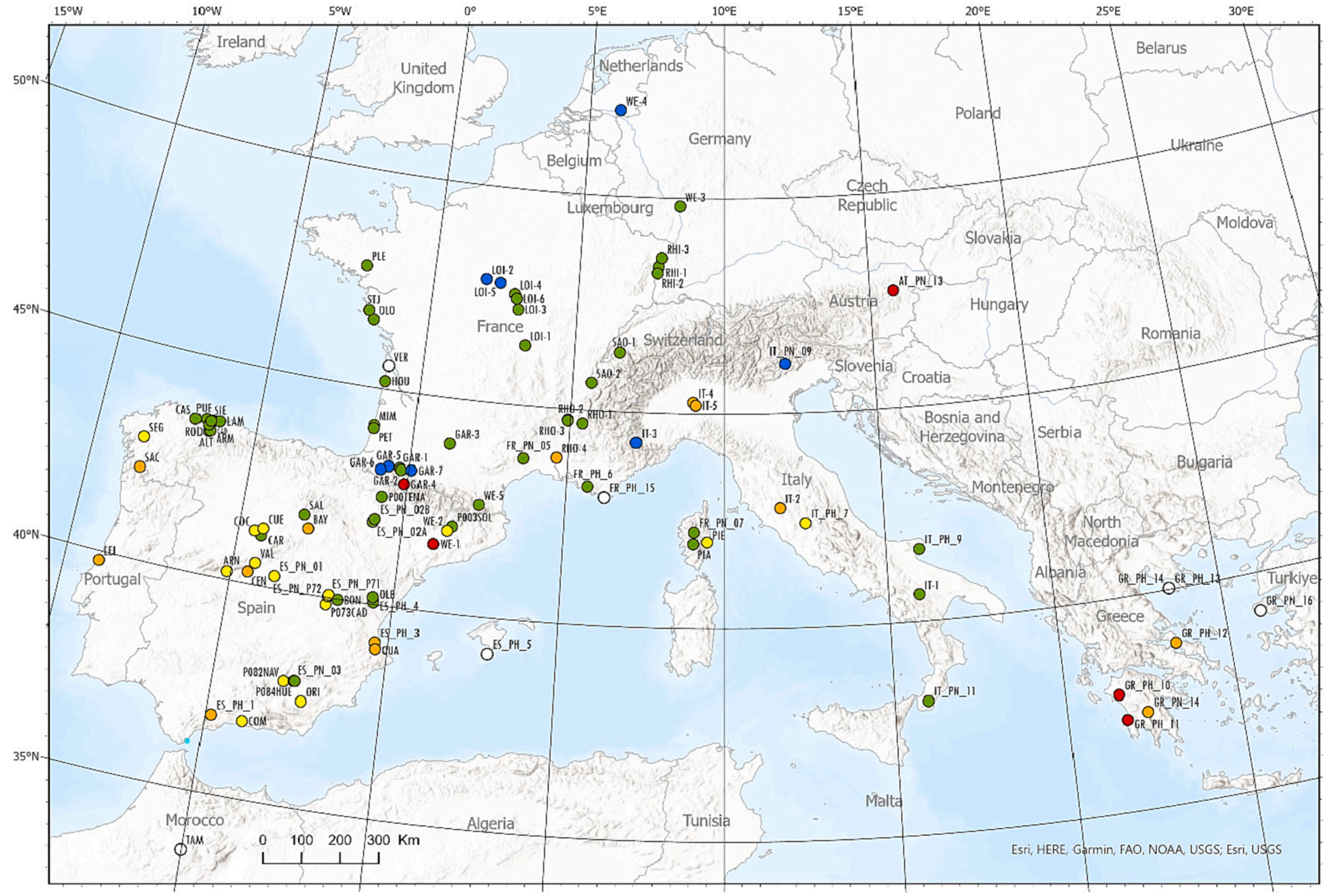

Fig. 4. Stability of natural populations under different climate scenarios by 2095 (based on Mauri et al., 2022), for the four species of the study. In green: stable populations according to the two scenarios, in yellow: populations decolonized under one of the two scenarios, in orange: populations decolonized under the two scenarios, in blue: unsuitable currently but becoming suitable in one or both of the future scenarios, in red: populations with an unsuitable climate in the present and future scenarios according to Mauri et al. (2022). In white: not included in the analyses because of inaccuracy in the raster layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the *P. pinaster* collection depicting samples covering 95 % and 99 % of the diversity).

We analysed the distribution of alleles with frequency < 0.5 in the selected material vs the GenoCore reference collections in all three data sets (Table 4; Fig. S2). A noteworthy result for the total and common SNPs, is the similar median and mean values among the selected populations and GenoCore collection, except for the Spanish *P. halepensis* selected populations, with a reduction in these values. For the outlier SNPs dataset, there is a reduction in the mean for most of the selected populations, except the Landes *Pinus pinaster* selected population, and the Italian poplar selected material.

3.3. Collection for enrichment of breeding populations

The climate projections by 2095 predict which populations will be stable according to their spatial locations and climate scenarios considered (Fig. 4). Number of non-stable populations varies between 1 and 4, the highest number being for *P. pinaster*. The collection to enrich breeding populations would include individuals from the GenoCore collection and some additional individuals from the non-stable populations for each species (Table 5) as they cover most of the genetic variation in the range of the species.

Table 5
Number of individuals of the GenoCore collection (GC) and of non-stable populations (NS) to be used for enrichment of the breeding populations. Number of the non-stable populations are indicated in brackets. *In *Populus nigra*, the number of locations refers to sampling points.

	P. halepensis		*P. nigra*		*P. pinaster*		*Populus nigra*	
Country	GC	NS	GC	NS	GC	NS	GC	NS*
AT	–	–	9	–	–	–	–	–
DE	–	–	–	–	–	–	1	–
ES	34	50 (2)	57	–	27	100 (4)	2	–
FR	11	–	30	–	24	–	15	1 (1)
GR	95	25 (1)	25	25 (1)	–	–	–	–
IT	15	–	24	–	–	–	9	7 (2)
NL	–	–	–	–	–	–	1	–
RU	–	–	12	–	–	–	–	–
MO	–	–	–	–	6	–	–	–
PT	–	–	–	–	1	25 (1)	–	–
TOTAL	155	75	157	25	58	125	28	8

4. Discussion

Our study cases examined the differences of 16 selected materials with respect to their natural populations in terms of levels of genetic diversity and genetic differentiation, using three SNP datasets. The **overall SNP** dataset provided a robust estimation of genetic diversity of selected materials (Bartholomé et al., 2016; Pégard et al., 2020), as compared with previous studies using a more limited number of markers (Sønstebø et al., 2018). The **common SNP** dataset (excluding rare alleles with MAF < 5 %) allow robust detection of changes in a reservoir of genetic variation of the species relevant in the face of uncertain climatic conditions, as most of the traits relevant for adaptation have a polygenic architecture (de Miguel et al., 2022). The **outlier SNP** dataset allowed the estimation of changes in markers presenting signals for diverging selection in natural populations, and could therefore provide information on changes in SNPs with adaptive value (Foll and Gaggiotti, 2008). The difference in number of outlier SNPs for the different species (0.7 % in poplar, and > 3 % in the pines) is related to the differences in SNP bait design and genotyping methods in the original data sets: while more common variants were targeted in the SNP arrays, SPET technology partly overcomes this bias by including *de novo* SNPs in addition to the initially identified ones (Scaglione et al., 2019). Although the present study uses SNP datasets considered as large for forest trees, they still capture a limited portion of the genome and this has to be taken into account when interpreting the output. Moreover, even though the SNP datasets are not directly linked to functional genetic diversity, the outlier SNPs provide an indirect way of accessing diverging selection and thereby adaptation of the populations, even in the absence of phenotypic information.

The main changes detected in the selected material are slight genetic differentiation (0.01 ± 0.01) in the three SNP datasets, with similar levels of diversity and a reduction of F_{is} with respect to the original population. It is also important to note the reduction of MAF in outlier loci for most of the selected populations (except the French *Pinus pinaster* and Italian poplar populations). This slight reduction could be explained by the reduced number of selection cycles considered here, and may be regarded as an expected outcome of the selection process during domestication (Aravanopoulos, 2018), and it is in accordance with simulations in more advanced programs (Ingvarsson and Dahlberg, 2019) and commercial plantations (Steinitz et al., 2012). However, there is no clear relationship between the intensity of selection and the loss of rare alleles detected in our study (see also Willing et al., 2012), as some of these effects were also observed in low-input strategies used in Aleppo pine in the Spanish populations. It is interesting to notice that our genetic estimates, based on a sufficient number of SNPs, provide a good estimator of the coefficient of differentiation for the sampling size used in the different samples (Willing et al., 2012).

Forest plantations may alter the genetic structure of native populations, via Forest Reproductive Material (FRM) transfer. This was shown in several species where the native genetic population structure was significantly altered as a consequence of intense seed exchanges (Jansen et al., 2017) and transfer of material among different regions or countries (Chen et al., 2019; Jansen et al., 2019; Raffl et al., 2018), which can pontentially affect adaptation (Milesi et al., 2019). By contrast, despite the use of FRM in afforestation, a clear structure of the genetic diversity of wild populations is still evident in others species (Bucci et al., 2007; Faivre-Rampant et al., 2016; Olsson et al., 2021; Scotti-Saintagne et al., 2019). In our study, the overall genetic diversity of the selected material slightly differed from that of the reference natural population, as in the study by Eklöf et al. (2021). However, these observations should not be extended to more advanced breeding programs and be limited to specific natural populations, as the effects on marginal or peripheral populations with interest for conservation, and different population size and demography (Cagelli and Lefevre, 1995; Ramirez-Valiente and Robledo-Arnuncio, 2014; Ramírez-Valiente and Robledo-Arnuncio, 2015), are still open questions that need further investigations.

One important aspect of our research is that the slight differentiation and reduction in heterozygosity poses an essential question for the adaptability of the populations in the long term under future conditions. Despite these slight changes in diversity, phenotypic values and quantitative differentiation can change substantially even with fairly small changes in the underlying allele frequencies, as they are decoupled from that of neutral markers (Alberto et al., 2013; Latta, 1998; McKay and Latta, 2002). It has been shown that even with low to medium SNP density panels, genomic evaluation could be comparable to well-optimized pedigree-based evaluation under certain standard conditions (Isik et al., 2016; Pégard et al., 2020). Our results suggest that selected material includes most of the diversity of the populations where they were selected, but, as expected, do not cover the full diversity range of the species. These results are based on a SNP panel that targets a subset of the genome, and in spite of not observing major changes in genetic diversity, some changes may exist in other parts of the genome. In order to counterbalance the small effect in the reduction of allelic richness, and to prepare the populations for uncertain climatic and socio-economic conditions, we used a strategy that includes new variation to enrich the breeding populations for the future. We used a low-input strategy based on maximising the allelic richness of the future collection (Gouesnard et al., 2001) by using the genetic diversity of the natural populations not included in the breeding populations. In the present study, we defined for each species core collections of a limited number of genotypes (<160 in all the cases) covering most of the genetic diversity of the species that can be managed as *ex-situ* germplasm collections to include valuable material for the future. The use of genetic measures related to allele coverage and diversity (e.g. Thachuk et al., 2009), or a core subset selection as defined by GenoCore (Jeong et al., 2017), have been implemented and also could be applied to the material in the existing clonal archives and seed orchards of the different species to evaluate and complete the core collection. A complementary approach was considered, related to expected changes under future climate scenarios. The identification of populations that will be unstable in future conditions follows the climate-adjusted seed sourcing strategies (Breed et al., 2013), and the assisted migration recommendations. It relies on identifying populations that currently grow under extreme conditions and therefore may harbour variants relevant to abiotic stress tolerance. Conserving these genetic variants from populations that may disappear in the future could therefore be of interest to enrich other populations that currently grow in milder environments but will experience a habitat change towards more arid conditions according to the models (Notivol et al., 2020; Serra-Varela et al., 2017). Selecting unstable populations as in the current study is a more conservative approach than focusing on geographical groups as for instance for *P. pinaster* and *P. halepensis* (Serra-Varela et al., 2017).

Overall, the genetic diversity of selected material currently available in the four focal species does not differ significantly from the analysed natural populations. However, the allelic coverage of selected material could be enhanced to secure the future needs for resilient forests under global change scenarios.

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CRediT authorship contribution statement

Sanna Olsson: Formal analysis, Data curation, Methodology, Writing – original draft. **Benjamin Dauphin:** Data curation, Formal analysis, Writing – review & editing. **Véronique Jorge:** Methodology, Resources, Data curation, Writing – review & editing. **Delphine Grivet:** Methodology, Resources, Data curation, Writing – original draft. **Anna Maria Farsakoglou:** Data curation, Resources, Writing – review & editing. **Jose Climent:** Funding acquisition, Resources, Writing – review & editing. **Paraskevi Alizoti:** Resources, Writing – review & editing. **Patricia Faivre-Rampant:** Resources, Data curation, Writing – review & editing. **Sara Pinosio:** Resources, Data curation, Writing – review & editing. **Pascal Milesi:** Resources, Writing – review & editing. **Simone Scalabrin:** Data curation, Writing – review & editing. **Francesca Bagnoli:** Resources, Writing – review & editing. **Ivan Scotti:** Data curation, Writing – review & editing. **Giovanni G. Vendramin:** Resources, Writing – review & editing. **Santiago C. Gonzalez-Martinez:** Resources, Writing – review & editing. **Bruno Fady:** Funding acquisition, Conceptualization, Project administration, Writing – review & editing.

Filippos A. Aravanopoulus: Methodology, Resources, Writing – review & editing. **Catherine Bastien:** Conceptualization, Writing – review & editing. **Ricardo Alia:** Methodology, Conceptualization, Resources, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available in public repositories (links in the paper)

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Appendix A. Supplementary data

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