B4EST

Adaptive BREEDING for productive, sustainable and resilient FORESTs under climate change

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1 Summary

Breeding activities with many economically important forest tree species in Europe have continued for decades and genetically improved forest reproductive materials resulting from these efforts are now widely available for reforestation. Genetic gains from tree breeding not only result in larger quantities of high-value end-products, but they can also make forests more efficient sinks of carbon, helping to mitigate the impacts of the ongoing climate change. The impact of long-term breeding activities ultimately depends on the speed of breeding progress and the scale at which the genetic gains are delivered to operational forestry. Both breeding operations and mass reproduction of FRM are limited by various constraints related to, e.g., unfavourable biological traits of trees and production capacity. Such limitations obviously need to be addressed to exploit the full potential of tree breeding. This may require integrating new innovations into tree breeding programs.

In this report, we review the current state of breeding activities in Europe as well as analyse, for the first time, prominent technological and societal evolutions which are expected to shape the ways tree breeding is conducted in the coming decades. Our transversal analysis focuses on eight economically important tree species in Europe with a variable history and intensity of breeding, ranging from low-input breeding programs (stone pine) to more advanced programs (eucalyptus, maritime pine, Norway spruce, poplar, Scots pine). The analysis is in a great debt to the enclosed species case studies which offer a more detailed eucalyptus, maritime pine, Norway spruce, poplar, Scots pine and stone pine (Appendix).

We observed a large variability in the organisation of European tree breeding and the number of actors participating in breeding activities in each tree species. Owing to the generally slow return of breeding investments, the breeding programs of most tree species are run fully or partly on public funding. Fast-growing eucalyptus is the only case where breeding is exclusively funded and managed by private companies. We foresee a need to increase collaboration among the various parties involved in breeding. This trend is driven by the fact that the complexity of tree breeding is increasing with the introduction of new knowledge and advanced technologies, requiring new types of expertise not found in any single institution.

The ongoing climate change is likely to lead to emergence of new insects and pathogens as well as increased frequency of extreme weather events such as early frosts and long periods of drought. Such stresses will result in decelerated growth and mortality, affecting the productivity of most tree species in a negative way. These threats are difficult to predict and effectively respond by time consuming breeding measures. Most breeding programs modify their list of selection criteria to consider adaptive performance, tolerance to various environmental stresses and stable performance over environments. Testing breeding materials in multiple environments and possibly contrasted (warmer and drier) climates will become more important. As climate change progresses, breeding programs may also have to look for new supplementary breeding materials from other climates. Decision-support tools which assist landowners to choose the most productive FRM for a certain site offer another potential effective way to mitigate harmful effects of climate change.

Technological advances have the potential to benefit operational breeding in multiple ways. New technologies can reduce the costs of breeding or amplify genetic gains by speeding up the breeding process or by increasing the accuracy of genetic evaluation. They also enable more accurate monitoring of the genetic diversity and conserving it for advanced breeding. We expect, however, that

the disruptiveness in forest tree breeding will probably not emerge from a single technology but will require the combination of technologies.

2 Introduction

Forests have provided human societies with a multitude of goods and services for thousands of years. Many European economies rely on forestry and industrial production of pulp, paper, fuel, construction-grade timber, and other engineered forest products which substitute more carbon-intensive products. Forests are also efficient sinks of carbon emissions, mitigating the impacts of climate change. The global forest area is declining, increasing pressure on the conservation of natural forests as well as increasing the productivity of managed forests. We can safely assume that planted stands, which grow much faster than natural forests, play an important role in finding a balance between conflicting demands. Most importantly, artificial reforestation is a prerequisite for the deployment of the results of genetic improvement. Many economically important tree species in Europe have been subject of breeding programs for decades, and following these long-term investments, genetically improved FRM is currently widely available for reforestation in many countries (Tables 1,2).

Forest tree breeding programs aim at developing varieties which increase the quantity and quality of harvested forest products. Although the progress from breeding of long-lived forest trees is relatively slow compared to breeding of farm animals and annual crop plants, there is already firm evidence showing that forest trees are no different from other plant species in their positive response to repeated cycles of selection, field testing and controlled mating. Substantial genetic gains in wood productivity of genetically improved FRM have been demonstrated (Jansson et al. 2017). Apart from the improved performance, the genetic makeup of the currently available genetically improved FRM is still very close to that of the original natural stands.

Among all the plants, forest trees are demanding breeding subjects due to their long lifespan, large area required for field testing, vast genome size, multiple traits of low heritability, and unfavorable biological characteristics such as late fecundity or difficult sexual or vegetative propagation. For decades, tree breeding programs have tested and implemented various tools and technologies to alleviate such constraints in the hope of increasing genetic gains, promoting the pace of breeding, or reducing costs of breeding activities. Some of them, such as the promotion of flowering by plant hormones and top-grafting and vegetative propagation of rooted cuttings, turned out to be operationally feasible and were quickly adopted for breeding, whereas some others, such as genetic engineering techniques, remained far removed from real breeding programs and soon vanished from discussion. In some cases, a technology may require a considerable time to evolve before it becomes useful for breeding. The advent of DNA markers occurred already in the 1980's, but the early applications suffered from a number of shortcomings and never made their way into mainline breeding programs. Only the more recent advances in high-throughput sequencing of the DNA have quickly expanded the range of potential breeding applications and resulted in renewed interest in genetic markers in many forest tree species.

The ability to integrate new innovations as well as to adapt to changes in the environment of operation is critical for tree breeding programs and is becoming even more important considering demands for increased wood supply, carbon fixation, and adaptation to the climate change. The technological novelties we analyse in this report fall into two main categories, 1) the technologies which reduce the costs of breeding by facilitating the collection and analysis of massive amounts of phenotypic data, and 2) the tools which capitalise on the progress in genomics and may be used to increase the accuracy of genetic evaluation or the speed of the breeding process by replacing or supplementing phenotyping information by information on SNP markers. Our working hypothesis is that, discussing the accurate

integration of new technologies in breeding programs requires having a rather broad view of breeding activities. More precisely, some attention should also be paid to the type of FRM that are used and the potential bottlenecks related to the production of FRM, the breeding objectives and their possible evolution, and the actors that are involved in breeding activities.

The objective of this transversal analysis, performed for the first time for some of the major European tree species, is to review potential evolutions which may shape the ways tree breeding is conducted in the foreseeable future programs. The breeding programs addressed represent various intensities of breeding, ranging from low-input breeding programs (ash, Douglas-fir, stone pine) to more advanced programs (eucalyptus, maritime pine, Norway spruce, poplar, Scots pine). The breeding programs are listed in Table 1, which also provides a summary of the current use of genetically improved FRM delivered by these programs. Our synthesis is in a great debt to the enclosed species reports offering a more detailed analysis of the ongoing status of breeding programs and potential evolutions in eucalyptus, maritime pine, Norway spruce, poplar, Scots pine, and stone pine.

Table 1. Main features of European forest tree breeding programs

Species	Country	Starting year	# of improved generation	Cumulated trial area (ha)	Av. trial area establish per year (ha)
Maritime pine	France	1960	3	400-600	6-10
	Spain *	1998	1	10	1.5
	Spain	1990	1	100	3
Stone pine	Spain	1989	1	20	0
Scots pine	Finland	1947	3	2250	3
	Sweden	1940-1950	3	900	10-12
	Norway	1947,2020	0	0	5
Poplar	Italy	1980	2	280-330	8-10
Norway spruce	Finland	1947	2	420	8
	Sweden	1939	2	1000	20
	Norway	1947	2	224.64	8-10
Eucalyptus	Portugal	1964	3	250	2

Data were gathered by researchers and experts in tree breeding in various European countries, involved in the B4EST project. Specific reports were written for each of the tree species, following the same structure. This transversal analysis has thus been established primarily on the basis of the report

per tree. Additional data collection was made to provide comparative figures such as those presented in Table 1 and 2. At last, for the section on technological opportunities, experts completed a specific survey to provide their more specific knowledge on the degree of maturity and application of the various technologies that will be mentioned.

The first section covers the current situation concerning the deployed forest reproductive material, breeding objectives, strategies and organisation. The second section addresses the evolution perspectives concerning the technologies, and the evolution related to climate change and societal expectations.

3 Current situation

3.1 Forest Reproductive Material (FRM)

The share of improved forest reproductive material has been increasing in Europe over the past decades. For most species and countries, the share of improved FRM used in plantations has risen above 90% (see last column of table 2 for more details). One exception is the use of forest trees for protection instead of production in Mediterranean countries (e.g. maritime or stone pine in Spain and Portugal).

In this section, we first present the different types of FRM that are deployed depending on the tree species, and the way they are produced. Even if the core of this analysis is the breeding activity, it is important to address these issues for at least two reasons: first, the type of FRM constrains the breeding program and its possible evolution that will be discussed later; second, in terms of priority setting, the interest for improving the genetic gain is limited if there are some possible bottlenecks in the deployment of improved FRM. Note, at last, that all the FRM are not deployed with the same end, and these different ends are discussed in the section “breeding objectives”.

Different types of FRM: seeds, seedlings, and clones

The impact of breeding activities depends on the scale of deployment of improved FRM in forest regeneration. Two main channels are used for the mass propagation of improved material/germplasm created by breeding programs: vegetative propagation utilising different types of basic material such as clones, clone mixtures or parents of families, or seed production in seed orchards through sexual reproduction of selected or tested parents. Seed orchard seedlots are ordinarily used by nurseries to raise seedlings, but in some cases they are directly used in regeneration (e.g., direct seeding of Scots pine in Finland). In specific situations where the production capacity of seed orchards is limited, seed can also be harvested from plantations established with improved material of the previous generation. Table 2 synthesises the type of FRM used for different tree species and countries as well as the magnitude of the deployment.

Two main reasons explain the choice of seed production from seed orchards instead of other types of FRM. First, seed orchards enable the mass propagation of improved material for a reasonable cost whereas the cost of vegetative mass propagation on a large scale is prohibitive for many species. Second, seedlots produced from seed orchards are genetically more diverse compared to clonal mixtures, which could mean improved resilience in variable and uncertain climates, as well as under conditions with unpredictable pathogenic pressure. This argument related to resilience is particularly important because of the long duration of rotation for tree production. There are two types of seed orchards: clonal seed orchards contain grafted propagules of multiple (often more than 20) selections; seedling seed orchards consist of families of selected parents. The latter option is observed for Norway spruce, Scots pine, maritime pine, and eucalyptus.

Vegetative propagation is the primary option for tree species in which clonal plants from elite breeding materials can be produced on a large scale for a reasonable price. Vegetatively propagated FRM is both more homogenous and potentially more productive than sexually reproduced FRM from seed orchards. Furthermore, the deployment of the most advanced improved materials takes place much faster vegetatively than by seed orchards for which there is a lengthy time lag before the planted orchard starts to produce seed. Clonal FRM is mainly deployed in poplars, stone pine and eucalyptus.

For Stone Pine, scions are grafted to make clones, while for eucalyptus and poplars, clones mainly come from shoot cuttings. For eucalyptus, commercial clones can also be based on tissues that are multiplied and manipulated in vitro. The cloning is made from the progeny of an intra-specific crossing or, as it is the case for poplar, interspecific crosses (*P. deltoides* x *nigra*). It should be observed also that, with clones, breeding programs can provide a rather large range of varieties with different properties.

Table 2. FRM and their current deployment European countries

Species	Country	Type of FRM	# seedling/clones sold per year	% of FRM in new planted area per year
Maritime pine	France	Seedlings	37M seedlings	>95%
	Spain *	Seedlings	20K seedlings	< 5%
	Spain	Seedlings	0.5M seedlings	<15%
Stone pine	Spain	Clones	< 1K clones	< 1% (still starting)
	Portugal	Clones	> 10K clones	< 5%
Scots pine	Finland	Seedlings and seeds	41M seedlings (plus 20 000 ha regenerated by direct seeding)	96%
	Sweden	Seedlings	236 M seedlings	98%
	Norway	Seedlings	2M seedlings	99%
Poplar	Italy and France	Clones	140K - 180K clones	10 - 15 % in Italy 40 - 50 % in France
Norway spruce	Finland	Seedlings	75M seedlings	70%
	Sweden	Seedlings	140M seedlings	70%
	Norway	Seedlings	40M seedlings	95%
Eucalyptus	Portugal (Altri Forestal)	Clones and Seedlings	2.7M seedlings and 1.5M clones	62%

In eucalyptus, the rooting ability varies greatly among species and genotypes. Although vegetative propagation provides higher genetic gains, sexual reproduction through seed orchards remains the only option for rooting recalcitrant genotypes. In Portugal where eucalyptus is widely cultivated, the

two main companies are specialised on one type of FRM: Altri mainly uses the seed orchard option while Navigator mainly uses the clone option.

Finally, notwithstanding the relevance of artificial regeneration in planted forests, a short mention is due that many European forest systems are still managed by natural regeneration techniques. This method is based on gene pools of locally adapted tree populations, often in continued covered forests or under shelterwood systems, to avoid extended clear cut areas or for achieving desired species compositions in mixed forests. As natural regeneration involves no genetic improvement effects, this issue is not further treated in the present synthesis.

FRM production and related issues

FRM production activities include establishment, maintenance and harvesting of seed orchards, as well as production of seedlings and clonal plants. These activities are most often made by private companies.

As mentioned at the beginning of this section, not all new surfaces are necessarily regenerated with improved FRM. Moreover, it is often the case that forest owners cannot use the most advanced FRM. Shortage of FRM is mainly explained by production capacity, but it can be the consequence of specific problems like pest problems in the seed orchards for maritime pine. For example, the share of improved Norway spruce seedlings from nurseries has fluctuated between 20% and 75% in Finland over the past decade. The temporary shortages have been mostly due to several consecutive poor crop years in seed orchards. This shortage is typically more pronounced for the most recent improved material that embed highest genetic gain.

FRM production is constrained by production capacity and there is some inertia for adjusting these capacities. This is particularly the case for conifer seed orchards which show at least 8 to 10 years lag between the establishment and the first harvest. In addition, the production time of seed orchards is generally much longer compared to the generation interval in breeding. Because investments in seed orchards are more easily covered when seed harvests are continued in existing orchards, seed producers may have a low incentive to replace their old but still productive seed orchards by new and genetically more advanced ones. Seed shortage problems could obviously be avoided by oversizing production capacity with a frequent turnover. However, such a strategy requires high investment that cannot usually be covered by the sales of rather moderately priced seed.

The main capacity constraints in the production of clonal FRM are related, for example, to the number of mother plants that can provide scions, productivity of scions per mother plant and rooting rate of cuttings. For example, commercial tree nurseries in Spain are still struggling to upscale the supply chain for grafted stone pine treelets, this being for the moment the main bottleneck for the deployment of selected clones as grafted trees.

At last, there are also constraints to production capacities for seedling production in nurseries but these constraints are not specific to tree species. They can also be adjusted more easily compared to the other capacity constraints mentioned before.

3.2 Breeding objectives

Breeding objectives mainly focus on the quantity and quality of wood or other end-products (resin, cones). In Scots pine and Norway spruce, the overall objective of breeding is the increase of wood production and of the economic value of end-products (Rosvall 2011, Ruotsalainen & Persson 2013; Jansson et al., 2017; Skogfrøverket, 2017). As Scots pine and Norway spruce are mainly used for sawn timber, stem quality (knot size, stem straightness and absence of defects) is an important objective. For Norway spruce, the economic value of wood is strongly related to structural uses that require stiffness and strength. Besides the emphasis on reducing stem defects, e.g., by culling against the most early flushing materials that are prone to spring frost, there is an increasing emphasis on wood density in the most productive regions. For maritime pine at the beginning of the breeding program, only growth and stem straightness were considered: plus-trees were selected on height, diameter and visual scoring of stem form. Traits related to wood quality (wood density, branch quality, twisting-rust resistance) were introduced to breeding later. In Castille, maritime pine is still selected for resin production. Douglas-fir in France is now selected on a set of more diversified traits, like phenology, architecture and branching habits and wood density, reducing correspondingly the weight on growth which was the main focus at the start of the breeding program. In the case of eucalyptus, breeding objectives have been aligned since the beginning with the needs of pulp and paper companies with trees selected for growth and wood quality (wood density and cellulose content). In poplar, vegetative propagation ability, vigour, stem form and wood quality are major breeding objectives. Even in the case of stone pine, which in forests is used primarily for land reclamation and forest restoration, the main objective is to improve cone and kernel production. In ash, genetic improvement through selection and breeding is mainly aimed at improving growth and wood quality aspects. Ash can be used for high value veneer, saw-log for furniture, floors and tool handles. This makes wood quality characteristics such as straightness of the stem, absence of forking and low branchiness important traits in selection programmes (Pâques, 2013).

Those criteria linked to production are followed by the objectives of resistance or tolerance to biotic and abiotic stresses, for all species. These criteria deal with the adaptability of the species to their growing environment, and the stability of their performances over a large range of growing sites to promote the general adaptation, including hardiness or avoidance to frost in fall and spring. Scots pine and Norway spruce are tested for vitality and growth across environments. This testing should promote adaptation in general, maintaining or improving frost hardiness and tolerance to the winter conditions (autumn frost for Scots pine, autumn and spring frost for Norway spruce). Maritime pine is evaluated for cold tolerance - as temperatures can reach -15 or -20 degrees in Aquitaine - and is also tested for its tolerance to drought. Resistance to various pathogens and pests is becoming one of the most important goals for all species: root rot for Norway spruce, *Fusarium circinatum* and nematode for maritime pine, *Phoracantha semipunctata*, *Gonipterus platensis* and fungi from genus *Mycosphaerella* for eucalyptus, ash dieback in ash populations. These genetic resistances are even more important for pests that can’t be controlled by phytosanitary measures, as it is the case for nematodes in maritime pine with the only effective way being to cut down infected trees once a disease outbreak is identified.

For all tree species, because of long commercial rotations, these breeding goals have to be generic enough to maintain their relevancy under different future scenarios, that can be climatic scenarios as well as commercial scenarios with evolving industrial processes and needs. As an example, a Maritime pine variety specially dedicated to biomass production was created, but forest producers were

reluctant to adopt this variety because they considered that it was too risky to produce for a unique market.

3.3 Breeding strategies

For all the B4EST species, breeding programs were initiated during the second half of the XXth century (Table 1) by the selection of phenotypically superior plus trees as candidates in natural stands. One or several cycles of breeding has then been initiated or completed. In general, a breeding cycle is based on two steps:

- Genetic evaluation and selection of the best candidates as parents based on progeny testing (backward selection), or information on the candidates and their sibs (forward selection) Currently, this evaluation is based solely on phenotypic information.
- Creation of the next-generation recruitment population by intermating the selected parents maintained in clonal archives (breeding arboretums).

The highest-ranking breeding material, independently of generation, is deployed as parents in seed orchards or to families used for vegetative propagation. Beyond these general common features, the precise organisation of breeding programs differs among the tree species for several aspects.

Breeding programs generate genetic gains by ranking and selecting individuals for their genetic merit. Two modes of selection are often distinguished, “backward” and “forward”. Backward selection is performed among the current-generation candidates based on progeny testing which assembles performance data in multiple environments. Progeny testing increases accuracy of genetic evaluation at the expense of the time needed to reproduce the test materials and to run the progeny trials. Forward selection is performed in the next-generation recruitment population for untested trees which are evaluated using information on their own phenotypic performance as well as the performance of their relatives (applying genetic evaluation models that incorporate a full relationship matrix). In tree species that enable vegetative propagation, the accuracy of both forward and backward selection can be improved by establishing progeny trials with multiple clonal replicates of the candidates. In principle, all long-term programs are based on forward selection. Backward selection is applied in specific instances, usually in connection with the deployment (clone selection for seed orchards) and in the first breeding cycle where a large number of plus tree candidates are ranked and selected as founders for the long-term breeding population.

Crosses have a marginal but still important impact on gain. A new recruitment population can be generated in different ways, using controlled crosses (pair matings or polycross) or open pollination with a possible recovery of paternity. Controlled crosses give a tighter tuning of gain compared to open pollinated schemes that lack the full control of pedigree. The choice of a crossing design is most of the time driven by biological and operational (economical) constraints. The time needed to reach reproductive maturity and uneven cone setting (in case of, e.g., Norway spruce) are important constraints for the progress of managing these crosses.

Phenotypic data are collected at different stages of tree growth, the trait observed at each stage being possibly different. For most of the species, the selection is made in one step, once all phenotyping is collected and jointly analysed. For other cases like poplar in France, selection is made at multiple stages following independent culling levels, focusing on different sets of traits at each step. As vegetative

propagation is easy for this case, the selected candidates at each stage are multiplied, so that evaluation at the following steps can be made more precisely using a larger number of clones.

In regions such as the Nordic countries which are characterised by a wide range of climatic conditions, breeding materials are divided into multiple independent subpopulations promoting differentiated adaptation following the large-scale climatic clines/gradients.

At last, in the case of poplar, the improved material corresponds to an interspecific crossing (*Populus deltoides x nigra*). As a consequence, the selection is made for both "own" value and cross value (semi-reciprocal selection).

Breeding strategies have been little impacted so far by the development of molecular markers. Their use for various applications will be detailed below in the section "technological opportunities" as none of the breeding programs considered here has yet routinely implemented molecular markers.

3.4 Organisation of breeding

The type of actors involved in breeding is quite heterogeneous among species and countries. We can observe a gradient of situations, from an involvement in breeding 100% from the public sector to breeding programs totally handled by private industrial companies. In this section, we will describe the current organisation of breeding activities for the species considered and propose some drivers of this organisation as well as contemplate possible evolutions and perspectives.

Breeding activities are carried out by public or semi-public research institutes in the case of stone pine in Spain, Scots pine and Norway spruce in Finland, poplar in France, Douglas-fir and maritime pine in France and ash in the Netherlands. In the case of stone pine in Spain, breeding activities result from rather isolated initiatives developed by forest administrations in several Spanish regions, assisted by diverse universities, the national research institute INIA and the Ministry of Agriculture. Scots pine and Norway spruce breeding programs in Finland are publicly funded and managed by a public research institute (Luke). In Maritime pine in Spain, breeding activities are covered by public funds and handled by regional institutions in regions where maritime pine is cultivated. The French breeding program for Maritime pine is managed by two institutions (INRAE – National Research Institute for Agriculture, Food and Environment – and the technical institute FCBA), and coordinated by a common structure (GIS *Pin Maritime du Futur*). It mainly relies on public funding, private funding being very limited. A similar scheme is present for Douglas-fir in France, involving 3 main actors INRAE, FCBA and ONF, within an embryonic GIS with public funding. In Norway, the breeding programs are carried out by the non-commercial foundation Skogfrøverket (Norwegian Forest Seed Center), which relies on 80% public funding and on 20% income from seed sales. In the Netherlands, Wageningen Research, a not-for-profit research organisation, is the main actor engaged in tree improvement of ash, whose activities mainly rely on funding from the Ministry of Agriculture, Nature and Food Quality.

The cases of Norway spruce in Sweden, Stone pine in Portugal and poplar in Italy are intermediate. In Sweden, the breeding activities for Norway Spruce are managed by Skogforsk, a private foundation with partly public and private funding. It provides improved breeding material for seed orchards owned by private companies. In Portugal, stone pine breeding is managed by the national research institute (INIAV) and supported by large private landowners associations (UNAC, ANSUB,...). Mother tree orchards are owned and managed by regional private forest owners' associations. Poplar breeding

programs in Italy are independently managed by the public organisation CREA and private companies, with no stabilised collaboration between public and private research groups. The Italian national register of poplar clones includes 46 clones from CREA and 39 from private companies.

The two Eucalyptus breeding programs in Portugal represent a unique case where breeding activities are exclusively carried out by the private sector. The programs are managed by two main pulp and paper companies, Altri and The Navigator Company. Collaborations with public institutes and universities exist but often depend on private funding.

The main driver of the organisation is the profitability of breeding activities. Indeed, the private rate of return on breeding is very low for several reasons: the long delay from the initiation of breeding to the first releases of improved FRM, the required inputs (in time, money, surfaces, human resources), the limited market size (the annual regeneration area) and the low willingness of landowners to pay extra for improved FRM. Public funding is thus essential for tree breeding, this involvement being possibly impacted by the negative societal perception of planted populations.

The important breeding investment by pulp and paper companies in the case of eucalyptus in Portugal and Norway spruce in Sweden is part of an overall investment in upstream activities (breeding, seed, seedling and wood production) to secure the supply of the mills. These mills represent huge investments that require a regular supply of good quality wood in sufficient quantity. The use of improved varieties, both in the forest stands owned by the companies and in the private forest owner's stands – who supply the mills – address this specific purpose.

4 Evolution perspectives

4.1 Technological opportunities

The level of development of new technologies varies between species. In this section we will give details on the ongoing and planned developments in terms of phenotyping, genotyping, data analysis and biological tools.

Phenotyping tools

For all the species, the current evaluation of multiple traits (tree height and diameter, branch traits, stem sinuosity, wood quality, disease resistance…) is expensive and time-consuming. Furthermore, genotype-environment interactions have to be considered due to the increasing impact of climate changes on tree growth and health. This requires multiplicated measurements on contrasting sites. Development of more efficient phenotyping platforms and new phenotyping technologies then represent a great challenge for the advances in forest tree breeding. More effective methods would increase the number of trees evaluated (higher selection intensity, exploration of more sites to better characterise GxE effects, enlargement of the breeding population to capture extra diversity) for the limited budgets.

Several new phenotyping technologies have been developed in recent years or are under development. The use of unmanned aerial vehicles (UAV) for high-resolution aerial photography (3D photogrammetry) or scanning with light detection and ranging (LIDAR) are powerful methods to

phenotype field trials for growth and survival, and to evaluate seed orchards or clonal archives for cone and pollen productivity. It should replace, in the short term, the use of telescopic pole or Vertex for height measurements for most species, particularly after canopy closure in stands that are taller than 10-12 m. This technology is already at its early application stage for maritime pine, Norway spruce and Scots pine. UAV associated with a sawing system is currently used for Norway spruce and Scots pine to collect twigs (for grafting), needles (for DNA extraction) and cone samples. UAV associated with LIDAR is under rapid development and it appears to have even more disruptive potential as collection of information on traits which are laborious to assess by conventional methods, such as (stem form, tree crown volume, leaf area index, etc.) might be possible.

For wood quality evaluation, sophisticated technologies based on X-ray measurements of increment cores (Jacquin et al., 2019; Steffenrem et al., 2014) are being replaced in most species (Norway spruce, maritime pine, Scots pine…) by the use of the Resistograph which accurately measures wood density (Fundova et al., 2018, 2019). This technology is also useful for assessing annual radial growth. Finally, the use of near infrared spectroscopy (NIRS) technology appears encouraging to predict susceptibility to biotic stresses, such as *chalara* for ash. Rincent et al. (2018) went further and proposed "phenomic selection" aiming to predict complex traits based on this low cost and non-destructive tool. A proof-of-concept is currently developed on poplar.

Overall, these new technologies will contribute to high-throughput phenotyping, opening new perspectives to better characterise trees in a wide environmental range, to increase selection intensity and to explore a broader genetic variability.

Genotyping tools

SNP arrays are tools of choice for high-throughput genotyping due to high reproducibility and to the ease to process raw data in comparison to genotyping-by-sequencing technologies. However, their development is limited by the investments required for their design for which a SNP discovery preliminary step is mandatory and is biased towards the specific germplasm utilised for their development (Barabaschi et al., 2016). The European Union research project B4EST has enhanced the development of such SNP arrays for forest trees, particularly with the commercial multi-species 4TREES array designed with 50K SNPs for *Populus* sp, *Fraxinus* sp, *Pinus pinaster* and *Pinus pinea*. Two other arrays were also generated within this project: one for Scots pine and one for Norway spruce. The high throughput genotyping tools available for each species are detailed in Table 3. These genotyping tools must be associated with genotyping platforms ensuring efficient and robust protocol for samples collection, DNA extraction, genotyping and biobank storage as developed, for example, in Nordic countries for Scots pine.

Table 3: High-throughput genotyping tools available per species

Species	Genotyping tool	#markers	Reference
Scots pine	Thermo Fisher Axiom PiSy50k array	50K SNPs	Kastally et al. 2021.
Norway spruce		50K SNPs	

Poplar	Illumina ISelect Infinium	34K SNPs	Geraldes et al. 2013
	Infinium	12K SNPs	Faivre-Rampant et al. 2016
	Thermo Fisher Axiom 4TREE array		
maritime pine	Illumina Infinium		Chancerel et al. 2013
	Illumina Infinium		Plomion et al. 2016
	Thermo Fisher Axiom 4TREE array	12.5K SNPs	
stone pine	Thermo Fisher Axiom 4TREE array		
eucalyptus	Thermo Fisher Axiom Euc72K	72K SNPs	
Ash	Thermo Fisher Axiom 4TREE array		
Douglas-fir		50K SNPs	

The most immediate application of molecular markers is their use for verifying genotypic identities and checking of pedigree errors. This use avoids labelling and grafting errors, ensuring that correct genotypes are used for controlled crosses, clonal archives and seed orchards. Moreover, pedigree corrections contribute to a more accurate BLUP evaluation and thus higher genetic gains. Most breeding programs have already applied molecular markers for these purposes, however its use in routine and at large scale is still in its infancy. For instance, in the case of maritime pine in France, a low-density SNP array has been developed specifically for this purpose (Vidal et al. 2017). A first set of 6,000 trees was genotyped and several additional thousands of trees will be genotyped in a short term. This process has already enabled identification of errors in about 15% of the trees grafted in clonal archives. From now on, each new selected tree will be genotyped before grafting in the clonal archives.

Pedigree reconstruction by using DNA markers opens an opportunity for implementing less costly breeding strategies. One strategy is the replacement of bi-parental controlled crosses by polymix breeding, followed by pedigree reconstruction (Isik 2014 , Lambeth et al. 2001). The main advantage of this strategy is to generate a large number of families with a limited number of crosses, and it could be particularly attractive for breeding programs with limited resources to implement conventional breeding schemes. It has been evaluated in the frame of the French maritime pine breeding programme both for genetic gains (Bouffier et al. 2019) and for economic efficiency (B4EST deliverable D5.2). Another related strategy, considered in the case of Scots pine and Norway spruce, uses a concept called “Breeding without breeding” (El-Kassaby and Lstiburek, 2009). It treats planted stands as ad-hoc progeny trials and uses informative DNA markers for fingerprinting and reconstructing the pedigree of phenotypically forward-selected candidates. In the case of Scots pine in Norway, the plan is to skip the initial phase of plus tree selection in natural stands and instead perform the first selections in stands established from bulked seedlots of plus trees or breeding arboretums, or commercial stands known to be planted with improved materials. The stands are phenotyped by the most effective

methods available and then a sample is genotyped to obtain the pedigree of a sufficient number of top candidates and random trees. The pedigree matrix, comprising full-sibs and half-sibs is then used in the breeding value prediction (Lstiburek et al., 2015, Lstiburek et al. 2017).

Use of high-throughput genotyping allows to compile the realised relationships between individuals and thus to incorporate the genomic relationship matrix instead of the pedigree-based matrix to improve BLUP accuracy. This G-BLUP methodology or the derived H-BLUP methodology (where genomic and pedigree information are combined) should be implemented at short term in several breeding programs (Scots pine, Maritime pine, Norway spruce).

The most disruptive application of molecular markers is for genomic selection. Even if it has not yet been implemented routinely in forest tree breeding programs, this application is considered in almost all the species (Douglas-fir, eucalyptus, maritime pine, Norway spruce, poplar, Scots pine, stone pine). Its main interest seems to be the pre-selection for traits expressed at late age (e.g. traits linked to wood quality) or currently too time or cost expensive to assess (e.g. pine nematode resistance). This option, considered for Scots pine, Maritime pine and Eucalyptus, may limit phenotypic evaluation to the most promising genotypes and would potentially increase selection intensity and reduce phenotyping costs, in addition to the potential integration of new selection criteria. The use of genomic selection for shortening the duration of the breeding cycle (early selection) appears of limited interest in many conifers due to their late age of sexual maturity.

Finally, another application of molecular markers is the development of Marker Assisted Selection (MAS), particularly for pest resistance when few genes could explain a large proportion of the genetic variability. Studies are in progress to enhance MAS in poplar to select for Woolly Poplar Aphid resistance or in ash for ash dieback resistance.

Tools for data analysis

Several new or improved statistical methodologies have facilitated the deployment of high-throughput phenotyping and genotyping in the breeding strategies. For example, software, such as the package BreedR, is now available to easily handle large genomic data for GBLUP analyses. Another important challenge in long-term tree breeding is the requirement to maintain sufficient diversity for future breeding while maximising the short-term response to selection. Finding an optimal balance between gain and diversity becomes complicated when the pool of candidates for selection includes related individuals across two or more generations, which is a common case in all advanced-generation breeding programs. New tools have emerged, such as OPSEL (Mullin 2017a) and XDESIGN (Mullin 2017b), which help breeders to better manage genetic diversity in breeding populations based on pedigree and/or genomic information, either for the design of the mating plan or for the selection.

Integration of environmental data is probably a key challenge to face climatic changes. Spatial analyses are now largely used in genetic trial analyses to consider environmental heterogeneity at the stand level. More recently, random regression modelling has been considered in forest tree species to predict tree growth reaction over environmental gradients (Marchal et al. 2019). In the maritime pine breeding population, random regression is currently applied on annual growth data recorded in rings to build reaction norms under a water stress gradient.

Biological tools

Mass propagation techniques open new opportunities both to deploy at large scale clonal varieties and to establish clonal trials to increase breeding value accuracy. Somatic embryogenesis has been developed in some species (Stone pine, Norway spruce, Eucalyptus, Scots pine), but its uses are limited for cost reasons. Its automation could make it competitive in comparison with seedlings. In the case of Eucalyptus, the challenge is to develop vegetative propagation processes that overcome the limitations of poor rooting and the high costs of cloning *E. globulus*. It includes material regeneration by micropropagation, embryogenesis and organogenesis. Cuttings are being considered in maritime pine in Spain for deployment in the most productive areas.

Maximizing the number of seeds produced by cross is of first importance to generate the successive recruitment population. Techniques for flowering acceleration are sought in Scots pine and Norway spruce, as completing controlled crosses on the selected individuals is often the most tedious phase of the breeding cycle in these species. The main methods to promote flowering include top-grafting onto sexually mature inter-stocks, gibberellin treatments heat-treatments in greenhouses and various damage-causing treatments applied to grafts of the selected trees (Eriksson, Jansson, Almqvist 1998; Johnsen et al. 1994).

Optimization of seed orchard management is a challenge in the case of maritime pine, as heterogeneous parental contribution can be observed in open-pollinated seed orchards and as pollen contamination from un-improved surrounded stands induces heavy losses in seed genetic value (Bouffier et al. 2017). Moreover, seed productivity has tremendously decreased in seed orchards since 2009-2010 (Boivin and Davi 2016), probably due to a combination of biotic (seed bug *Leptoglossus occidentalis*) and abiotic factors (spring frost, summer drought). Various options are considered: chemical treatments, more intensive management for seed production in a controlled environment (greenhouse seed orchard), and mass pollination with no flower bagging under greenhouse conditions. Also, in Norway spruce, optimization of seed orchard management is a priority, as substantial losses of cone harvests are caused by cone and seed pathogens (e.g. Rosenberg, Almqvist and Weslien 2012). The treatments might be removal of *Prunus* species, known to be the main hosts for cherry-spruce rust (Kaitera, Kauppila and Hantula 2021), removal of redundant cones, etc. (summarized in Almqvist and Wennerström (2020)). There is also a challenge to increase floral induction and fruit set at eucalyptus seed orchards for some genotypes. Biostimulants and fertilisers used in orchard farming are being considered for this purpose.

Transgenesis and gene editing

Despite decades of research in transgenic technologies applied to forest trees (van Frankenhuyzen and Beardmore, 2004; Yin et al 2021), this tool was not identified as an opportunity for breeding by the European forest tree breeders. The main concern is related to ecological risks associated with the introgression of the transgene into wild genepools. This risk is probably higher for forest trees than for annual crops due to the low genetic differentiation between improved tree varieties and natural stands. However, transgenic trees are specifically excluded from PEFC and FSC certifications. Similar considerations are associated with gene editing as, for the time being, it is considered as GMO following the decision of the 2018 European Court of Justice. Another reason for the limited interest

in these technologies is the pattern of inheritance of traits of interest for forest tree breeding programs. Most of these traits have a quantitative genetic basis, i.e., they are controlled by possibly hundreds of genes each with a tiny effect on the trait, whereas genetic engineering technologies are mainly applied to traits with simple inheritance.

4.2 Effects of climate change and perspectives for breeding

For most species, climate change is associated with reduced forest productivity in the warmer regions of their distributions due to drought and more severe pathogenic stress. However, Scots pine, Norway spruce and other species in the boreal forest are today limited by temperature and the length of the growing season might increase in productivity with the change in climate (Bergh et al. 2010). This increase should be more important with the use of forest reproductive materials adapted to new conditions. Under this perspective, a decision-support tool has been introduced in Sweden and Finland to assist landowners in their choice of FRM (Berlin et al. 2019). The negative impact of climate change is linked to (i) occurrence of drought periods, (ii) emergence of new insects or pathogens, (iii) frost risk and (iv) more extreme weather events.

Drought stress results in productivity losses due to decelerated growth and increased mortality of trees. Extreme drought events occurred for example early in the 2000's and in 2018 in Central Europe and in the South of Nordic countries, and affected Norway spruce trees which showed poor tolerance to these drought episodes, particularly above its middle age of 40-50 years (Rosner et al. 2014, 2016; Hentschel et al. 2014). In Eucalyptus, drought and temperature increase are expected to be major drivers for the productivity decrease in Portugal. In maritime pine and stone pine, drought stress also affects seed productivity, decreasing the yield in seed orchards. In southern environments, drought could be a problem for Scots pine. In poplar, unfavourable changes in wood composition following a drought stress treatment have been reported (Wildhagen et al., 2018). Furthermore, it has been demonstrated recently that heat and drought stresses aggravate each other in poplar, leading to increased water loss by transpiration (Urban et al., 2017).

Emergence of new insects or pathogens: This threat is common to all species, and is recognized as difficult to predict. For example, in eucalyptus, there were almost no pests in the first plantations. The number of fungi and insects, which can become pests and relevant diseases, has grown exponentially. The predicted warming is likely to be associated with changes in biotic threats, emphasising the need to control new pathogens with new silvicultural practices (e.g. phytosanitary control). The same situation occurs with most tree species. Maritime pine plantations in Spain are affected by pine wood nematode (*Bursaphelenchus xylophilus*) causing pine wilt disease and possibly death of infected trees in a few weeks or months. This nematode was first introduced to Portugal in 1999 and it quickly spread across Spain, now representing a major threat for Maritime pine plantations in France, as there is no available phytosanitary control for this pest. For ash, the ash dieback disease, caused by the invasive fungus *Hymenoscyphus fraxineus*, is a relatively new disease, first observed in Poland in the 1990s. Since then, the fungus has spread rapidly over almost the entire natural range of ash in Europe - it has been recorded in the Netherlands since 2010 - with a devastating impact on ash forests. New pests and diseases are likely to become new challenges for ash, as ash borer (*Agrilus planipennis*), that will require adapting the current breeding approaches to include more resistance traits. The difficulty of predicting the emergence and diffusion of pests is a major constraint. For example, in the case of

poplar hybrids, there is a risk of releasing new improved clones that might not perform as expected because of insufficient resistance.

The occurrence of more extreme events is likely to increase, affecting forest species: wind and precipitation for Norway spruce; heat waves causing forest fires for Eucalyptus; drought, heat waves, floods and windstorms for poplar; cold waves; among others.

Climate change will also have effects that cannot be considered as positive or negative for forest tree species. The main effect of this type is the modification of the distribution zone, with a planted area which may stretch to northern zones than the natural species' distribution area (Maritime pine, Poplar).

The challenge of climate change calls for modifications in current breeding activities. The first one is the necessity to test breeding materials in multiple environments which may be contrasted, warmer and drier (maritime pine, eucalyptus, and poplar) to promote stable performance. For example, in maritime pine, French candidates are already evaluated in genetic trials in Spain, and the number of Spanish trials sites is expected to increase. On the other hand, as the production zone of Maritime pine is expected to expand to northern zones in France, some trials are established in these future areas of production with genetic material selected in the current breeding zone. In Norway spruce breeding programs in Sweden, Finland, and now also Norway, clonally or vegetatively propagated candidates are evaluated in field experiments established in multiple different environments. Such field experiments facilitate evaluation of phenotypic plasticity and the stability of the candidates (Karlsson & Högberg, 1998).

In several studies of Norway spruce, it has been shown that the adaptive performance is influenced by the reproductive environment (summarised in Johnsen et al. 2009). The experience is that higher temperatures during zygotic embryogenesis and seed maturation (Johnsen et al. 2005), alter the phenology so that the progenies are adapted to an extended growing season (Solvin and Steffenrem 2019), without any known genetic selection on the way (Kvaalen and Johnsen 2008). Also other conifers seem to have similar regulation, e.g. Scots pine (Dormling and Johnsen 1992), Lodgepole pine (Wei et al. 2001) and the Picea glauca x engelmannii complex (Webber et al. 2005) This plasticity, often connected with epigenetic regulation of gene expression, can be utilised to promote rapid adaptation by the means of localisation of seed orchards nearer to the future climate, or modification the temperature during somatic embryogenesis.

In the case of poplar, studies show that clones characterised by high productivity are generally less tolerant to abiotic stresses (Monclus et al. 2006, Attia et al. 2015, Viger et al. 2016). These studies also highlight that the high degree of genetic variability among poplar clones should be further explored by re-assessing, in different environments, clones that are already established for their productivity and are grown in specific areas.

The second anticipated modification in breeding activities is related to the introduction of new selection criteria linked to the emergent biotic and abiotic stresses. This challenge is common to all the species. However, breeding cannot provide immediate results to face these stresses. It is then important to anticipate them and to predict the new thresholds which have to be considered in selection criteria – for example, minimum temperatures which have to be tolerated. Breeding also has to gain in flexibility to adapt to different scenarios. Some of these new selection criteria may require

large investments, as is the case with breeding for nematode tolerance in Maritime pine which requires a quarantine greenhouse (under construction in France). These investments in time and money on these new criteria may reduce the funding available for the work on the other criteria. On the other hand, delivering plant material with a sufficient level of tolerance to relevant pests, pathogens or abiotic stresses will condition the perdurance of profitable plantation forestry in the near future - two topical examples are the abandonment of active maritime pine silviculture in the north-western Iberia due to the threat of pine wood nematode prevalence, and the factual collapse of private spruce silviculture in lowlands regions of Central Europe.

To better meet these new goals, breeding programs need to look for new sources of genetic variability. For example, in the case of Norway spruce, it is necessary in some regions to supplement the existing breeding population or to establish new populations, especially when new breeding objectives are identified. In the case of Maritime pine, climate change and nematode threat also require an introduction of new genetic variability, considering non-local provenance (e.g. Corsican provenance which seems to be more tolerant to nematode; Spanish, Portuguese and Moroccan provenances that should be more resistant to drought). New hybrids are also considered, in Maritime pine by crossing *Pinus pinaster* with *P. brutia*, *P. halepensis* or *P. canariensis*, and in eucalyptus by crossing *E. globulus* with other eucalyptus species which have a higher tolerance to drought and cold, or with a hybrid of *E. rudis* x *saligna* which is tolerant to the pest *Gonipterus platensis*. However, some of these species considered for hybridization are not allowed to be used in Portugal. In the case of poplar, genome-wide association studies have led to the identification of gene polymorphisms that are associated with stress response traits, including stomatal density. Their introgression into commercial clones by crossing or by biotechnological methods such as cis-genesis or genome editing is considered. Moreover, hybridization of *Populus deltoides* and *P. nigra* led to the creation of new clones of P. ×canadensis which have an improved resistance to spring leaf and shoot blight (Gennaro and Giorcelli, 2019).

Climate change could also lead to a more drastic evolution if its effects made it easier to grow another species than to work in breeding to adapt the current species to the new climatic conditions.

4.3 Evolution of the needs from society

The societal concerns and demands can influence the breeding funding and the breeding objectives and may lead to an evolution of the regulations on plantations and silvicultural practices.

The main societal concern is related to the diversity of ecosystem services (ES) provided by forests, which are classified into four categories[1]: provisioning services (e.g. wood provision), regulating services (e.g. carbon sequestration, climate and air quality, waste-water treatment), supporting services (e.g. habitat for species, maintenance of genetic diversity) and cultural services (e.g. recreational value of forests). Among these services, the preservation of wildlife and biodiversity in forest stands and wildfire prevention in case of southern Europe are major concerns (Soliño et al. 2018, 2020).

Environmental associations deplore the excessive homogeneity in forest stands of improved varieties, which they considered having too low genetic diversity both in the trees themselves and underneath.

[1] See https://www.fao.org/ecosystem-services-biodiversity/background/cultural-services/en/

Preservation of genetic diversity should become a more important challenge for breeders. On their side, large forest owners tend to implement actions to increase biodiversity in their stands (conservation areas, biodiversity stations, plantation of autochthonous plants, ecosystems restauration, development of honey and mushrooms production…). New selection criteria could emerge with the development of these actions, as flowering habits for honey production and pollination services.

Furthermore, some silvicultural practices such as extensive clear-cuts are often considered harmful for biodiversity and carbon storage, advocating the use of natural regeneration where feasible in semi-natural forests. If such movements were to lead to the prohibition of clear cuts or limitations of the size of clear-cut areas, this would also affect artificial reforestation with improved FRM.

In Portugal and Spain, eucalyptus and maritime pine forest plantations are seen as responsible for forest fires. As a result, a ban for eucalyptus plantation on new lands has been implemented in Portugal in 2018 and in northwest Spain in 2021, with the objective of limiting the expansion of the eucalyptus area. This regulation constrains the wood supply for pulp and paper mills, and makes it necessary to increase even more the wood productivity of forest stands, which represents a challenge for breeders.

Public perception of the role of forests for carbon storage could also make the breeding goals evolve. If volume growth remains the main goal (as it is the main factor for increased carbon storage), wood quality traits should gain importance as they might be decisive for the durability of the forest products as long-lasting wood constructions. Furthermore, an extension of the duration of the economic rotation would be very interesting to increase carbon storage, which would put more emphasis on the forest stability criteria.

Moreover, breeding should consider the expected evolutions of silviculture towards more sustainable and extensive plantations. For instance, the expected minimization of pest-control interventions will put more emphasis on pest resistance for the new varieties.

At last, the increasing demand for raw materials for bioeconomy from forests can create new outlets or expand existing ones: food additives, structural and massive building constructions, pulp and paper products including textiles, proteins for animal and fish fodder, biomass... Nevertheless, breeders and forest owners can be reluctant to develop or adopt highly specific varieties, as it is impossible to predict the future importance of these outlets. For instance, a specific maritime pine variety for biomass production has been created, but it has been later abandoned.

5 Conclusions

In this report, we reviewed the current state of breeding activities in Europe as well as analyse, for the first time, prominent technological and societal evolutions which are expected to shape the ways tree breeding is conducted in the coming decades. Our transversal analysis focuses on eight economically important tree species in Europe with a variable history and intensity of breeding, ranging from low-input breeding programs (ash, Douglas-fir, stone pine) to more advanced programs (eucalyptus, maritime pine, Norway spruce, poplar, Scots pine).

Tree breeding and related research activities are key to leverage tree production and related services. However, time duration related to tree growth as well as cost for tree evaluation are major constraints for tree breeding and FRM production, leading to moderate or no private investment in tree breeding

and some problems related to production capacity for FRM. New technological opportunities related to genomics, phenotyping and mass propagation can reduce these constraints and may have some disruptive impact on tree breeding and deployment of FRM.

In this transversal analysis, we show that technological advances have indeed the potential to benefit operational breeding programs in multiple ways. They can reduce the costs of breeding or amplify genetic gains by speeding up the breeding process or by increasing the accuracy of genetic evaluation. They also enable more accurate monitoring of the genetic diversity and conserving it for advanced breeding with a positive effect on long-term genetic gains. We also show that disruptiveness in forest tree breeding will probably not emerge from a single technology but will require the combination of technologies.

Because of the time length of tree production, tree breeding has to anticipate the impact of climate change and, more generally, the evolution needs from society. Breeding programs are likely to shift their emphasis to emergent biotic and abiotic stresses due to the ongoing climate change. Breeding objectives may also evolve in response to the increasing public perception of forests as carbon storages.

Beyond the interest of combining different new techniques, it is important to underline the importance of keeping consistency in breeding and related activities. Investment in new techniques should not lead to deter investment from key basic breeding operations (e.g. field testing) and maintenance of breeding operations (in situ and ex situ conservation). Also, FRM production will always be a bottleneck for the diffusion of new tree varieties, so that investment in new breeding techniques should be consistent with investment in mass propagations techniques.

Because of the different evolution that we just mentioned, we expect that breeding might not be done in the same way in the coming years. The current paradigm one species-one breeder no longer holds in some institutions. Tree breeding is gradually becoming more complex with the introduction of new knowledge and advanced technologies, for which traditional breeders are not necessarily trained. This will require new types of training and expertise and possibly also changes favouring greater mutualism among the parties involved in breeding.

Collaboration among countries could also be a useful lever to ensure a stability for seed supply. Minor species, such as ash, which have high ecological value but low economic importance, depend on low-input breeding and limited funding. Therefore, these species are even more at risk if new diseases and pests emerge or if climate changes. European-wide cooperation, especially in the pre-breeding phase, e.g., screening a wide range of germplasm for resistance and exchanging the best performing genotypes (candidate parents for further breeding or for seed orchards) may increase the effectiveness of the genetic improvement of these species.

6 Dissemination plan

The previous transversal analysis, together with the species annexe, is planned to be synthetized into a publishable manuscript in the following months, as an opinion and review paper of forest tree breeding in Europe.

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Appendix

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SPECIES CASE STUDIES

Eucalyptus

Authors: Luis Leal, Luis Fontes, Jacqueline Grima-Pettenati, Jean-Marc Gion

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1 Breeding activities

1.1 Current breeding programs in Europe

The genus *Eucalyptus* includes approximately 800 species native from Oceania (Nicolle, 2016). Due to its fast growth, high productivity, wide adaptability and variety of uses (Eldridge et al., 1993) more than 20 M ha of eucalypts are planted worldwide (Booth, 2013). Some eucalyptus species were described and introduced in Europe in late XVIIIth, early XIXth century. However, their distribution in Europe happened later and Guichemont is reported as being the first, or one of the first, to bring this exotic into botanical gardens. In particular, the spread of *E. globulus* in France and Algeria is acknowledged to Prospero Ramel who also promoted the use of the species in southern Europe (Radich, 1994).

In Portugal it is known to be planted in 1850s by a 'plant lover' from a group of five known individuals. The first industrial plantations are linked to the first mill using eucalypts wood in 1926. However, eucalypts plantations expanded only in the 1960s. Eucalypts wood characteristics were particularly interesting for pulp production and the startup of Celulose Billerud (CELBI) in 1960s led to deeper investigations on *E. globulus* wood, followed by large plantations program. The first works showed a considerable degree of variability among nuclear wood characteristics for pulp production. Based on the assumption that selected wood properties (as cellulose content) could be inherited as it was known for other traits of interest, the company initiated a program for selecting trees for seed collecting based on growth, wood density and cellulose content. The selection of superior stands, identification of 'plus trees', sample collection and analysis, selection and seed collection led to a final set up with 36 mother trees and 49 half-sib individuals per family being field tested in 1966/1967. Preliminary growth tests and grafting experiences resulted in 25 genotypes (out of the 200 recommended for seed collection from 260 "plus tree" selected) planted in the first seed orchard of *E. globulus*.

Since then, work has progressed in different ways. Breeding activities have been carried out exclusively by the two main pulp and paper companies, Altri (https://altri.pt/pt) and The Navigator Company (http://www.thenavigatorcompany.com/). These companies manage less than 20% of planted eucalypts, since the remaining area is being owned and managed by private owners. However, almost all the breeding work has been driven by the industry often together with the academia. Between 1970 and 1990 they identified and used in trials more than 2000 'plus trees' being until today an important part of the existing breeding populations (Borralho et al., 2007). 'Plus trees' selection was complemented by several introductions of eucalypts (*E. globulus* and other species) from Australia as well as from other countries where they had been used in plantations (Spain, Chile, Uruguay and USA). Probably one of the most important introductions comprising more than 1000 family seed lots from most of *E. globulus* provenances came from Australia, collected by CSIRO.

The two most advanced active Portuguese breeding programs from Altri and The Navigator Company include breeding trials across the whole eucalypts planted area in the country. These programs are currently in their 3rd generation and capture the genetic gain through vegetative propagation to produce clones, as well as, through seed production both from open and controlled pollination. Altri, on an early basis, does controlled pollination on around 700 000 flowers, a unique approach worlwide for E. globulus.

Eucalyptus breeding in Spain has been carried out by Energía & Celulosa (ENCE) and collaboration exists between Spanish and Portuguese companies. There are several published works mainly in the 1970s and 1980s from Association FOrêt-CELlulose (AFOCEL), currently "Institut Technologique Foret Cellulose Bois-Construction Ameublement" (FCBA), with different Eucalyptus species with emphasis on breeding for cold tolerance in France. *E. gunnii*, the most frost tolerant Eucalyptus species is being used to confer this trait in FCBA breeding program.

1.2 Breeding objectives

The first *E. globulus* breeding activities in Portugal started early as compared to the main world eucalyptus programs. They started in 1966 in Billerud (today Altri) and in the 1980s and 1990s for the other companies: Portucel, Soporcel (both being, today, The Navigator Company) and Caima (today Altri). As breeding of eucalypts was led by the pulp and paper industry, it is not surprising that the improvement efforts of all these programs has been channeled towards the well-defined industrial objective of producing wood as raw material for pulp and paper industry rather than only growth traits.

From the beginning of the breeding programs, it was considered to select simultaneously for growth and wood quality as a main issue. Other characteristics such as stem straightness and tolerance to biotic or abiotic threats have been of secondary importance. In fact, *E. globulus* stem has already an excellent shape, with good pruning, low frequency of bifurcated trunks and a straight stem suitable for cutting, so its improvement would have little economic impact unless for wood transportation. In terms of pests and diseases, until the early 1980s, when *Phoracantha semipunctata* appeared, there were no major sanitary problems. Currently with the increase of pests and diseases, as well as, with the ongoing climate change, the importance for biotic an abiotic tolerance has increased dramatically and breeding for such traits is at the core of B4EST goals.

1.3 Breeding and deployment strategies

The existence of sustained gains in breeding programs over time implies, after an initial selection of the best parents, their genetic recombination and the production and testing of their offspring, in order to generate a new breeding cycle. The initial selection of eucalyptus 'plus trees' allowed to lay the foundations for advanced breeding programs in Portugal. Initially, selected 'plus trees' were grafted and installed in seed orchards. Whenever available, seeds were collected directly from the "plus trees" and subsequently installed in progeny trials. In some cases, 'plus trees' were cloned by cuttings from stump shoots, giving rise to several clonal tests. New Australian material from open-pollinated families was also incorporated shortly thereafter. The seed orchards as well as many progeny trials allowed the subsequent steps of recombination, testing and selection to be carried out in advanced breeding generations. The first seed orchards in Portugal were created by Altri in the 1970s and in the following years other orchards were installed by The Navigator Company. In the 1990s, Altri installed the first second-generation orchards.

The development of breeding activities over time requires a well-defined long-term strategy that allows for prioritizing, channeling efforts and justifying decisions. Portugal was associated with important developments in this domain, with the implementation of innovative forest breeding strategies. In 1987, Altri launched the D95 program, thanks to a set of field trials and seed orchards previously installed. This program was a novelty for two main reasons: it set clear selection objectives and a time horizon for achieving them (that of doubling productivity in terms of tons of pulp per hectare and per year by 1995, i.e. 10 years) and it structured the breeding population in two

hierarchical categories; i/ an elite population concentrating selection, crossing and testing efforts, and ii/ a second population encompassing a larger range of genetic variability and advancing at a slower pace. This two-tiered strategy called “Nucleus Breeding” was borrowed and adapted from the sheep breeding scheme in Australia. Within Altri’s eucalypts breeding program, the nucleus was constituted in 1988 by around 40 parents and the base population by around 300. Both selected from the existing progeny tests and according to the same selection criteria: growth and wood density. After 1995, a new breeding strategy called 3D was launched by Altri, following the same hierarchical improvement structure, but placing greater emphasis on both technological traits and genetic diversity.

The Navigator Company, instead of programming activities in discrete breeding generations (each generation with its core or base population) and in well-separated hierarchical levels, aggregated the process of analysis, selection, recombination and choice of clones into annual activities that focus on a unique population. Decisions related to breeding activities were dependent on dynamic rules to determine the crossing and cloning work, each year. The scheme, so called “Rolling Front” (Borralho & Dutkowski, 1996) is supposed to accelerate the genetic progress of the breeding program, without causing prejudice to diversity. The Navigator Company exclusively uses controlled pollination as a way to generate new families, partially cloned. Twenty to 40 genotypes of each family are propagated by traditional cuttings, generating over two years between 2 and 50 copies that will subsequently be installed in field trials.

1.4 Achievements

The contribution of breeding programs to the quantity and quality of eucalypts wood produced in Portugal depends on the genetic value of the trees used, their proportion in relation to the total of existing plantations and their behavior under field conditions. The field conditions also depend on the improvement of silvicultural practices and on continuous management efforts regarding the deployment of improved plants, the installation technique and the way in which eucalypts stands are managed and harvested. Overall, the planted area using genetic improved eucalypts has been estimated to less than 10% of the eucalyptus plantations in 2005 (Almeida et al., 2005). With the current improvement programs providing material with the capacity to increase forest productivity by 25 to 50%, there is a major opportunity for the conversion of most of the Portuguese eucalypts planted forests. Without increasing substantially, the proportion of improved genetic material within existing planted forests, the potential to increase the productivity of a fundamental resource for the forestry sector in Portugal could be lost when considering future climate change scenarios.

2 Constraints

2.1 Funding breeding activities

Forest breeding programs require long-term funding support. Existing public research funding programs do not have timeframes compatible with the long-term nature of breeding projects and their genetic improvement activities. In Portugal, only private companies have invested in eucalypts breeding projects and there is a lack of interest from public institutions. Therefore, collaboration with academia often depends on private seed money. Existing generalized negative perception of eucalypt planted forests and reduced ‘license to operate’ leads to difficulties in accessing research funds.

2.2 Vegetative propagation ability

Vegetative propagation, or cloning, either via shoot cuttings or via tissues that have been multiplied and manipulated in vitro, is a known viable process for large-scale production of improved genetic material for many tree species. However, different species of the Eucalyptus genus and their interspecific hybrids have sharp differences in rooting ability. Species such as *E. saligna* are easy to root as well as other ones such as *E. globulus* are rooting recalcitrant. The cloning of *E. globulus* 'plus trees' was tested for the first time in Portugal in late 1970s, and the first success case was reported in 1979. The interest in this propagation technique was certainly stimulated by the enormous success obtained in Congo, South Africa and especially in Brazil, with tropical eucalyptus species. However, its adaptation to the *E. globulus* programs in Portugal was more time-consuming and troubled than expected. The cutting survival and the subsequent success of rooting was quite low. Only around 10 to 15 % of selected 'plus trees' could be cloned. Therefore, for many individuals, rooting turned out to be a relevant bottleneck for the development of cloning as a propagation technique. Recent developments such as the results from Project iPLANT carried out by The Navigator Company and Altri have been improving the productivity and efficiency of the *E. globulus* cloning process, opening a new window of opportunity for producing a wider range of improved genetic material through cloning.

2.3 Ability to predict biotic and abiotic threats

B4EST is working to increase eucalyptus survival, health, resilience and productivity under climate change and natural disturbances, while maintaining genetic diversity and key ecological functions and fostering a competitive EU bio-based economy. Therefore, biotic and abiotic threats are at the center of the breeding work under B4EST perspective. Extreme events are expected to increase with climate change, for instance: droughts and heat waves in the summer; and cold spells in the winter. Since forest breeding cannot provide immediate results to face biotic and abiotic threats it is necessary to predict which will be the main threats in the future, their magnitude so that it will be possible to infer the necessary thresholds (e.g. minimum temperature tolerated) that shall be considered for breeding. In addition, fire risk demand extra care and create uncertainty on success of breeding fields experiments and installations.

3 Perspectives for future breeding

3.1 Technological factors

3.1.1 Biotechnology and genomic applications

Although the direct use of genomics is still scarce in Portugal, biotechnology applied to eucalypts has had some expression in Portugal. First areas of research in biotechnology included material regeneration by micropropagation, embryogenesis and organogenesis. These works were motivated by the desire to develop vegetative propagation processes that overcome the limitations of poor rooting and the high costs of cloning *E. globulus*.

Today, advances in molecular genetics and biotechnologies allowed the high-throughput development of molecular markers based on the polymorphism of single nucleotides (SNPs). Such SNPs can be used at a large scale along the genome to characterize the genetic diversity within populations, the relatedness between individuals, as well as the determination of the identity of a clone by "fingerprinting". The genetic mapping of these markers into linkage groups (i.e. the 11 chromosomes

that make up the eucalyptus genome), anchored on a reference genome (physical mapping), and the analyses of subsequent correlations between alleles and phenotypic characteristics allow to identify genomic regions responsible for traits variation (Quantitative Trait Loci; QTL). New breeding methodologies using such molecular information, so called “genomic selection approaches”, have been successfully tested in forest trees such as eucalypts.

The B4EST project aims to experiment such approaches in the framework of the ALTRI breeding programme for several main issues: i/ verifying the identity of the documented genetic material used in the breeding population, ii/ identify most of the native origins of the base population, iii/ testing genomic selection models thanks to different calibration and validation populations, iv/ predicting the genetic values of individual genotype for different traits of interest based exclusively on its genetic profile.

Although the immediate application of these tools in selection programs is still not implemented in Portugal, these developments will certainly improve the rigor of selection of key phenotypic variables, anticipate the age of selection, and should enable to increase the selection pressure to reduce handling of the number of genotypes before variety deployment.

3.1.2 Phenotyping

Phenotyping activities have been traditionally carried out through field trials where growth is measured, and mortality is assessed. Wood properties such as pulp yield and wood density have been carried out by laboratory procedures at Altri whereas The Navigator Company has been using indirect methods such as Pilodyn for wood density and NIR for pulp yield. Currently Altri is working towards including extensively NIR technology to assess wood properties in its breeding program as well as for other monitoring applications.

Currently, tests in controlled environment have been undertaken to evaluate possible tolerance to drought and cold under Project iPLANT: cold tolerance of eucalyptus genetic materials has been evaluated through leaf discs exposed to different target freezing temperatures (Costa E Silva et al., 2009) allowing to create a rank for cold tolerance of tested clones. Drought tolerance has been assessed in containers inside growth chambers and based on a group of drought tolerance attributes a creating a rank for drought tolerance of tested clones

In the framework of B4EST, a collaborative work with a team of Toulouse 3 University [https://www.lrsv.ups-tlse.fr/equipes-de-recherche/regulation-and-dynamics-of-wood-formation/] has been performed to evaluate the genetic diversity of eucalypts cuttings from Altri towards frost and drought. Basal frost tolerance (without prior cold acclimation) is informative on the capacity of the plants to face late spring frost events (plantation time) or early frost in autumn. E. globulus and hybrids cuttings from Altri were exposed to freezing temperatures (-7°C, -4°C, -3°C, -2°C). Relative basal tolerance to frost was estimated by combining survival rate and phenotypic damages based on visual observations on apexes, leaves, stems (more than 30 phenotypic scores) at 1 week, 3 weeks and 5 weeks after frost exposure. A normalized scoring of frost damage was set up to give a precise evaluation of overall plant damage and possible recovery (regrowth). The implementation of this frost tolerance protocol on young plants combined with the normalized scoring will be useful to select the best genetic backgrounds for this trait in breeding programs. In addition, a fine phenotypic analysis of traits known to be involved in abiotic stress resistance for a subset of Altri clones: stomatal density,

wood structure (vessel density and area, secondary cell wall thickness) and wood composition (assessed by FTIR spectroscopy) has been performed showing a large natural variability for stomatal and vessel densities, for instance. Finally, a dedicated protocol has been set up to perform a fine evaluation of drought resistance in controlled conditions of two clones using of a phenotyping platform equipped with optimolog robots and automatized imaging. Besides manual and automatic phenotyping, samples of leaves, stems and roots at different kinetic time points have been collected for RNA Seq and FTIR analysis. The RNA Seqs will be used to construct gene co-expression networks and to correlate specific phenotypic traits to gene modules allowing the identification of meaningful genes such hubs. This biological information may potentially improve the accuracy of genomic prediction.

3.1.3 Mass propagation

Since eucalypts have remarkable biological versatility, capturing the gains from its genetic improvement can use several strategies. The most traditional way has been the use of seed orchards (Eldridge et al., 1993). As *E. globulus* is a species with a mixed pollination system, one of the critical aspects to control in a seed orchard is its average self-pollination rate. For this it is important to ensure that the various genotypes present have an abundant and synchronous flowering. This aspect is critical in the Portuguese case, as the programs integrate material from different sources and this characteristic is strongly conditioned by the region of origin (Dutkowski & Potts, 1999). Another fundamental aspect is the existence of pollinators, particularly flying insect, in abundance, at a time of year (autumn and winter) where they are not frequent. The location of orchards, especially regarding frost risks, is another important aspect to consider. In Portugal, of the four orchards installed by Altri in the 1970s, three were abandoned for not producing significant amounts of seed due early flower and fruit abortion and abscission (Eldridge et al., 1993). Despite these difficulties, open pollination seed orchards are still an interesting and easy option to produce improved eucalyptus plants and are still used at The Navigator Company. However, in face of the difficulties from open pollination, mass propagation of eucalypts using seed rely on large-scale controlled pollination at Altri. Vegetative propagation, or cloning, is the other major mass propagation strategy for eucalyptus at The Navigator Company, as well as, to a lesser extent at Altri.

3.2 Environmental and socio-economic factors

3.2.1 Environmental changes

Ongoing climate change is expected to have a negative impact on most of eucalypts distribution in Portugal. Drought and temperature increase are expected to be major drivers for the expected productivity decrease in Portugal. A major unknown is the extent of the expected increase on extreme events. There is a lack of information about the magnitude of the extreme events so that it is difficult to predict what shall be the required thresholds of tolerance to them. Existing breeding programs try to respond to environmental changes by testing genetically improved eucalypts in multiple test environments, in addition to look for alternatives such as hybridization of *E. globulus* with other eucalyptus species which have a higher tolerance to drought and cold.

When eucalypts started to be planted in Portugal in larger scale there was almost no biotic threats associated with it in Portugal. Over the time the number of species of fungi and insects which can become pests and relevant diseases for eucalyptus has grown exponentially. The predicted warming

is likely to be associated with changes in the biotic threats of eucalypts which are more difficult to predict than the changes in the abiotic environment itself. Control of pests and diseases often rely on cultural practices, chemical applications, biological control and in a lesser extent using more tolerant eucalyptus genetic material. However, examples of such biotic tolerant eucalyptus exist such as: an hybrid of *E. rudis x E. saligna* from The Navigator Company which is tolerant to *Gonipterus platensis*; two of *E. globulus* clones from Altri which show tolerance/resilience to leaves diseases provoked by fungi from genus *Mycosphaerella*.

A revisited analysis of many years of collected data on growth of clones from the Altri breeding programs, associated to past climatic data should help to characterize the reaction norms of the eucalyptus genetic material. Such novel approaches specifying the genotype by environment (GxE) interaction could help to better characterize the behavior of specific clones within the breeding zones in Portugal, and by extension could help to define new selection criteria for the Altri breeding program.

3.2.2 Societal demands

Public concerns regarding eucalyptus planted forests in Portugal are often voiced. The Navigator Company and Altri has been actively working towards providing an increase of biodiversity in its managed land through specific programs. As an example, Altri Diversity Program aims to (i) double the conservation area in ten years, by reaching 16 thousand hectares in conservation areas managed by Altri Florestal; (ii) produce and plant, in its own and third-party areas, 1 million autochthonous plants; (iii) expand the network of biodiversity stations and 'biospots', increasing the sites to be shared with the community, generating conservation actions and monitoring of natural values; (iv) restore high conservation value ecosystems, preserving the habitat of threatened species, through the improvement of riparian galleries, the reinforcement of native vegetation and the removal of invaders; (v) integrate with forest management, activities of economic, social and environmental value. With local partners, develop, among others, projects to produce honey, mushrooms and tourism.

Breeding eucalypts requires new attention to maintain or increase population diversity through gene banks, high genetic diversity parks/orchards, assign compartments for future seed collection where mixtures of best full-sib families have been planted. Considering the interest in new products such as honey, its necessary to enlarge the set of selection traits for breeding, e. g. flowering habits. Eucalypts can also play a role in societal demands such as phytoremediation (Luo et al., 2018). In face of new demands for using eucalypts, breeding needs to include traits which will be crucial for its new uses.

There is today a conflict from the pressure to reduce the planted area of eucalypts in Portugal and the pressure to fulfil the opportunity to supply with raw material an important industry sector. In such context the increase of eucalyptus productivity remains a key for satisfying both conflicting paths. Low planted area with improved genetic material constraints the ability to increase forest productivity. In such context, it is critical to improve communication to stakeholders of the benefits of improved FRM in terms of increased productivity, increased income, and when it applies the improved tolerance to future threats.

3.2.3 Organizational evolutions

Current breeding programs of eucalyptus in Portugal are organized separately in two main companies Altri and The Navigator Company. Most of the breeding activities have relied on private funding. Unless

any unforeseen evolution will happen, it might be expected that available stable funding might have to rely on the companies to invest in their own breeding programs. Both these two companies have been increasing collaboration in their breeding activities in recent years. Collaborative Project iPLANT looking at improved genetic material for drought and cold tolerance. In addition, field breeding trials have been established with improved genetic materials from controlled crosses from progenitors of both companies.

In addition to existing breeding programs, companies must develop alternative management schemes to 'capture' resilience, allowing economic outcome and keeping diversity. Future breeding activities must encompass these new challenges. Companies requiring faster responses from a slow process which is breeding need to look for a 'fast track breeding' where genomic selection might play a role.

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SPECIES CASE STUDIES

Maritime pine
1. Spain

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1 Breeding activities

1.1 Current breeding programs in Spain

The maritime pine breeding programs in Europe are restricted to three main countries: first, south western France in Aquitaine, with the most advanced breeding program, second breeding activities in Spain (in different regions, though mainly in the North-West in Galicia), and third the breeding program in Portugal. The level of development, and other aspects differ significantly among these three areas. Here we describe the activities in Spain.

In maritime pine, the area of new plantations in Spain has decreased to 2,786 ha/year in the last 15 years, and only 27% of them with an objective of production, mainly in the Atlantic region. On the other hand, most of the Spanish growing area of maritime pine is under Mediterranean conditions, of lower productivity than the Atlantic region, where multipurpose forestry integrates provision of regulating ecosystem services like soil and groundwater protection, erosion control and wildlife conservation, combined with timber and resin provisioning. The production of FRM is 2.5 million of plants/year, 11% of this material from the qualified and tested categories (Alia et al. 2022)

The activities started back in the 60's of the last century by provenance research (Molina, 1966; Alía et al., 1995), in order to select provenances for use in northern and central Spain. The results of this research confirmed the large differences among the Mediterranean and Atlantic populations, and allowed to identify different regions of provenance for the species. From these studies, the breeding programme for the Atlantic region (Galicia) was based on autochthonous provenances, differing further within two breeding zones: coastal and interior, though afterwards this subdivision has been demonstrated to be unnecessary (Zas et al., 2004). At present, the breeding program in Galicia is based on new breeding objectives related to the adaptation of the species to different biotic and abiotic factors (Menéndez-Gutiérrez et al., 2018; Prada et al., 2016).

In the Castilian plateau, breeding for resin production was also an important objective, and selection of plus trees, seed orchard establishment and testing has continued at different periods and with different intensities (Tadesse et al., 2001). In addition, it is important to mention the national program for seed orchard establishment defined in the 1980s (Pardos, Gil, 1984) in which other provenances (e.g. interior mountain provenances like the Iberian range (Montañas de Soria-Burgos) or the Central range (Sierra de Gredos) were considered, based on their good performance, stem form and potential for afforestation programs. At the entire area of distribution, an evaluation program was implemented based on clonal and progeny tests from 29 populations (Jaramillo-Correa et al., 2015). The strategies followed were based on recurrent selection, with selection of plus trees in the most important regions of provenance, for establishing clonal seed orchards and progeny tests from open-pollinated progenies.

1.2 Breeding objectives

The objective of the breeding program for resin production was based on high resin yield production (in kg/tree and year). For the rest of the activities, and during the first stages, the main objective was to increase stem volume production and improve stem form (straightness). At present, the wood

quality characteristics have been included, along with tolerance or resistance to biotic and abiotic risks (drought and cold, diseases, pests) (Menéndez-Gutiérrez et al., 2018; Prada et al., 2016).

Also, in Galicia, emerging pests and diseases are now important breeding objectives, with material selected for their resistance to Fusarium circinatum, to the pine nematode, and to drought stress. For the rest of the area, drought stress tolerance is becoming of high interest (Gaspar et al., 2013).

1.3 Breeding and deployment strategies

Breeding strategies differ among the different regions. In Galicia a forward selection strategy is being applied, with establishment of four first generation seed orchards (clonal and seedling). The breeding and deployment strategies have been adapted recently to new deployment methods based on parents of families. These materials offer the possibility of improved material for different objectives (the mentioned above), as well as the production of FRM by seeds or by massive vegetative propagation of young materials.

For the Mediterranean area, two first generation clonal seed orchards have been established for obtention of seeds from crossed between selected basic materials. Nevertheless, it is necessary to stress that in most of the Mediterranean region of the species, timber growth is lower and rotation length often reach the century. There, forest managers demand and prefer for afforestation and ecosystem restoration the use of regional or local reproductive materials instead, even if they are only source-identified and selected, weighing hence more a assumed local adaptation than the superior quality of improved MFR. On the other hand, maritime pine forest regeneration at the end of rotation after final fellings is mostly based on natural regeneration systems in shelterwood or clearcutting with seed tree retention, using rarely underplanting or direct sowing in case of failure of spontaneous regeneration.

1.4 Achievements

One major achievement of the breeding program is the identification of the best material to use in different areas. Due to the large distribution range in Spain, and the large genetic and ecological diversity, it would be necessary to define the procurement and deployment areas for the species. Only some part of the areas are of interest for breeding programs, namely the NW for timber and the central plateau for resin tapping. Though even if in these areas, still more than 80% of the FRM is unimproved, from identified seed-sources, at least in the most productive areas (NW Spain), the use of improved material is being demanded by forest owners. Nowadays the main objective of the breeding program is to offer new FRM materials adapted to the new conditions, for instance resistance to the main biotic agents.

Another major achievement is the compatibility of breeding and conservation activities in natural pine forests. The species is included in the national and regional in situ conservation strategies, and different areas have been delimited for in situ conservation. Also, the management of the natural and semi-natural area favours the continuity of local gen pools by natural regeneration, adapting under selection to current conditions and ongoing climate change.

2 Constraints

One operative drawback in the main timber region of Spain, Galicia, it that due to different factors (mainly fungal diseases in seed orchards and nurseries), there are some limitations for the operative deployment of improved nursery plants, whose demand in this case is still not satisfied.

But the major constraints to breeding in Spain are rather related to external factors. First, institutional ones, as the species covers a diversity of Autonomous Regions, which keep exclusive competences of activities in the topic. Second, socio-economic constrains, as the profitability of the species is reduced in most of the areas, and breeding activities are limited to public funds focused in low intensity activities. There are also changes in the public perception of plantation forests in comparison to near-to-nature forest ecosystems. And last not least, the introduction or prevalence of some biological agents, namely emerging pests and diseases, together with the ongoing increase of climate change impacts in some regions, are limiting or modifying the breeding objectives pursued. To address these constraints, some actions for the coordination of actions have been undertaken at the national and regional level, coordinated within the National Breeding Program.

3 Perspectives for future breeding

3.1 Technological factors

There are new technological factors that would affect the efficiency in breeding of maritime pine, as they influence the evaluation and deployment of FRM.

3.1.1 Genomics

Genomics is becoming of high interest in the breeding program, mainly for disentangling the traits of interest for breeding, as well as for parentage reconstruction as some low-input strategies should be applied in different areas. We need also to reinforce monitoring of genetic resources to make compatible breeding activities with the conservation of genetic resources in the species.

3.1.2 Phenotyping

This is a major interest in developing the breeding program. Many of the traits under consideration can be easily phenotyped using different technologies (eg. NIRs for wood density and lignin content; drones for height and different abiotic factors; sonics evaluation for wood stiffness). However, we still lack phenotyping methods for new important traits (tolerance to Fusarium and pine wood nematode). We are also now developing a common phenotyping procedure in genetic tests, that is still a main limitation due to the cost of establishment and tending, for evaluating the genetic resources in different environments.

One highly relevant topic is the evaluation of materials under analogue-to-future climatic conditions, as the main climate pathways predict a possible loss or drastic reduction in feasibility of some growing area or provenances regions for the species. This broad-range common garden evaluation of is also essential for improving the transfer guidelines of improved material of the species.

3.1.3 Breeding techniques

Within this area, we need to focus on implementing low-input breeding and deployment strategies, based in the use of genomic information, high-throughput phenotyping and FRM production for production of improved material using forward selection and genomic prediction.

3.1.4 Mass propagation

The main activity is related to the implementation of massive vegetative propagation methods in Pinus pinaster, based in protocols already defined (cuttings, somatic embryogenesis).

3.2 Environmental and socio-economic factors

3.2.1 Environmental changes

In Spain as well as in Portugal, the importance of different biotic (Fusarium, pine wood nematode) and abiotic factors are affecting the use of the species and the implications for breeding (Serra-Varela et al., 2017b, 2017a). Deliverable 5.6 has analysed the risks and benefits of the improved material under this new scenario that also affect the rotation and silvicultural aspects. Based on these changes, we need a flexible breeding strategy, as new breeding objectives can arise in the future due to new demands or new emerging pests and diseases, or the importance of different abiotic factors. Tolerance is becoming an important breeding objective, as induced defenses can be a response of the Pinus pinaster genotypes to different agents. Those strategies would incorporate the advances in clonal replication of the material in maritime pine, and the use or early selection methods to reduce the interval for deployment of improved material. However, one main limitation to better implement the strategies is the lack of real data under contrasting environments in the response of the genotypes to infer changes in phenotypic plasticity, norms of reaction and trade-offs. The Clonapin collection from a previous project was a useful setting to advance our knowledge of these mechanisms at the distribution range of the species, but we lack common garden trials with a collection of genotypes derived from the breeding populations in as many contrasting sites as possible, and those mimicking the future conditions of the species.

The Deliverable 5.6 has also pointed out the limitations of the existing experimental settings to improve the transfer guidelines of improved FRM under future climate scenarios. This is a priority to define the flexible strategies under different future scenarios.

3.2.2 Societal demands

One major concern, at least in some part of the area (e.g. NW Spain) is the public perception of the use of FRM of this species, due to different factors related to the importance of forest fires, the role of Pinus pinaster in this context, and therefore the importance of breeding programs. These breeding programs are financed to a large extent with public funds by society as a whole through taxes. Based in a discrete choice experiment focused on pine forest systems in Spain (Soliño et al., 2020) have found that a breeding program that would generate greater welfare for Spanish society is one that focuses on improved seeds, quality wood, and resistance to F. circinatum, including control of genetic diversity and contributions to the biodiversity of forest systems. The preferences on the objectives related to timber and non-timber productions, it is notable to notice that society is demanding integrated production programs, but with a greater intensity in aspects related to product quality, probably as a

reflection of coordinated push that is showing the wood industry, foresters, nurseries, breeding research centers and governments (https://www.fundacionarume.gal/es/actualidad).

Therefore, we need to stress the importance of genetic diversity in the material deployed from the breeding programs, as well as the importance of these new plantations in the biodiversity of the areas. This is posing questions related to the resilience of these forests under future climatic scenarios, and the monitoring of changes to early detect some risks associated to the use of the FRM.

3.2.3 Organizational evolutions

The breeding activities in NW Spain in maritime pine are not an exception to other breeding activities in Spain. The analysis to implement a National breeding program in Spain has detected some priority actions to develop in relation to the organizations and institutions involved in breeding. Firstly, It is necessary to increase cooperation among the institutions involved. Many of them are research institutions, and therefore it is necessary to better define the strategies and objectives at the short and long term, and to revise the actions continuously (every two years). Secondly, it has been identified that the development of phenotyping, genotyping and evaluation of genetic material should be developed as a common service to the different institutions involved. And thirdly, we need to improve the dissemination and transfer of improved material to the sector and the society in general by using different mechanisms implemented by the National Committee of genetic resources. And, in the specific case of very scarce and high-value improved material, recent public-private supply cooperation inititives (e.g. obtaining and supplying of tolerant to pine nematode genetic material to nurseries to apply vegetative multiplication tecniques to plant threaten areas) must be considered and favored.

3.3 Other major factors

One major factor in the future of breeding is the consolidation of the National Breeding program, as it constitutes the framework of the actions, especially for coordinating the activities and setting priorities.

3.4 Conclusions

Breeding in maritime pine in Spain is focused on special uses and regions. We do not forecast a major role of breeding in the production of FRM in the near future but to fill different demands (resin production, adaptation to pest and diseases, wood production) in specific areas. New development (especially genomic approaches and high-throughput phenotyping methods by sensors and drones) will provide the basis for low-input breeding strategies aimed to these specific objectives.

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SPECIES CASE STUDIES

Maritime pine
2. France

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1 Breeding activities in France

1.1 Current breeding program

The French maritime pine breeding programme has been described in detail elsewhere by Annie Raffin (Mullin et al. 2011; Mullin and Lee 2013).

The breeding programme started in the 1960s, when early provenance trials had already shown the superiority of the local Landes provenance for growth and cold resistance (Illy 1966). Nouvelle-Aquitaine is the most northern region of the species' natural distribution, which is otherwise localized on the Atlantic coast of Spain and Portugal and around the Mediterranean basin (Spain, South-Eastern France, Italy, Tunisia, Algeria, and Morocco). Cold resistance was identified as an important issue, especially when lowest night temperatures in Aquitaine can reach -10°C or -15°C every few winters (-20°C in February 1985). The local provenances were thus chosen to build up the breeding population, despite their form defects: stem flexuosity and poor branching. A total of 380 plus trees were selected phenotypically in Aquitaine, based on height and diameter, and visual scoring of stem form. In addition, genetic variation among provenances and performance of crosses between provenances were explored (Harfouche and Kremer 2000). Among all tested combinations, Landes × Corsica families proved to be the best material for growth and form in Aquitaine conditions. A few hundred clones from the Corsica provenance were selected in provenance trials located in Aquitaine, based on growth, stem straightness, branch quality, pyralis resistance (Dioryctria sylvestrella), and cold resistance. The objective of this second population is to produce improved Landes × Corsica varieties for better stem straightness and branch quality.

Development of the breeding programme followed a classical intra-population recurrent selection approach, with a main population composed of the Landes plus-trees. Double-pair mating designs are used to produce material for progeny tests and the base of the next generation, while polycross testing is performed for parental ranking. Trials are replicated on several contrasting sites, usually with single-tree plots and a large number of replications per site (Groupe_Pin_Maritime_du_Futur 2005). Following this strategy, the main population has cycled through three generations, with more than 5,000 individuals selected, and 7,000 families tested over more 90 trials. Since 2012, the genetic evaluation is based on the individual model. A database gathering the measurements on more than 400,000 trees and the documented pedigree over three generations is used for BLUP evaluation.

1.2 Breeding objectives

In the early days of maritime pine breeding, the main objectives were the improvement of growth potential and stem straightness. The current selection index is still focused on volume and stem straightness but other traits related to branches (branch angle, branch diameter, ramicorns) and wood density are now included. A subset of selected trees is also evaluated for twisting-rust resistance (*Melampsora pinitorca*) with a cut-shoot assessment (Desprez-Loustau 1990). Other new selection criteria are under study as described in section 3.1.2.

1.3 Deployment strategies

Up to now, three generations of seed orchards have been produced (Groupe_Pin_Maritime_du_Futur 2014). For economic and technical reasons, the deployment strategy for maritime pine varieties in Aquitaine is based mainly on open-pollinated orchards. The first-generation orchards (entry in production in 1987) were seedling seed orchards based on a very large number of full-sib families, corresponding to the progeny tests of plus-trees, and were rogued after genetic assessment. These orchards demonstrated genetic gains of 10-15 % in volume and stem straightness at about age 15 years. Second-generation orchards were characterized by a reduced genetic basis and greater expected genetic gains, compared to those in the first generation. They were based on a few tens (usually around 30) of backward-selected genotypes, either as a classical grafted clonal orchard or as a randomized plantation of polycross families obtained by controlled pollination between selected clones (Baradat et al. 1992). The polycross-family seed orchards were planted over 180 ha and are open-pollinated (entry in production in 1999). The expected genetic gain was estimated from progeny trials at age 13 years to be 30% for both volume and stem straightness over unimproved material. Third-generation seed orchards have been established over 180 ha, and have been in production since 2011. They are either clonal or polycross-family seed orchards. This third generation also includes a Landes × Corsica variety, to be produced by controlled pollination. A new deployment strategy have been considered for the fourth generation. Seed orchards are now renewed more rapidly (every 2-3 years) so that the genetic gain is transfer smoothly from the breeding population to the deployment population.

1.4 Achievements

The current use of improved FRM for maritime pine is described more in details in the delivrable D4.9 “Guidelines for deployment and silvicultural management of improved FRM”.

2 Constraints

2.1 Genetic evaluation based only on progeny trials

Due to biological constraints, cuttings are not used for genetic evaluation which means that no clonal trials have been established as part of the French maritime pine breeding programme. Genetic evaluation is based on multi-sites progeny trials. This is a limitation for GxE estimation but reaction norms estimated on annual increment could be an alternative for growth traits (as described in section 3.1.2). It also makes more difficult the genetic evaluation for selection criteria related to disease resistance. For example, breeding values for nematode resistance will be estimated on progenies which implies extra time to screen the population in comparison with species where clonal propagation is widely used.

2.2 Deployment based on open-pollinated seed orchards

Nor cuttings or somatic embryogenesis are possible for deployment in maritime pine for technical reasons. The deployment is thus based on seed orchards (clonal or seedlings seed orchards); and for cost-effective reasons, open-pollination is considered in most of them. As a consequence, pollen contamination rate from 20 to 95% has been measured in maritime pine seed orchards (see section 3.1.3). This deployment strategy implies to focus breeding on the estimation of additive effects (SCA is no use as no control-crosses are performed).

Moreover high weight is put on genetic diversity in maritime varieties where effective size above 15 is recommended. This means to consider about 40-50 parental genotypes in the seed orchard and to maintain a large effective size (around 100) in the breeding population.

2.3 Limited number of control crosses per year

Control crosses are key to produce advanced generations combining alleles of interest. However due to limited staff and the short period when this breeding operation is possible, about 50 control-crosses are possible per year (ie. 50 full-sib families). Unfortunately, very low success rate has been observed during the last years due to biotic and abiotic reasons (see section 3.1.3). This is a strong constraint for the breeding programme, especially at a time when we need to recombine large genetic variability to face new selection criteria (climatic changes and pine wood nematode nematode).

2.4 Major biotic threat: pine wood nematode

Pine wood nematode (see section 3.2.1) is a major threat for maritime pine plantations in France. Since its introduction in Portugal in 1999, it becomes very likely that this pest will affect French plantations at short or long term. A quarantine greenhouse is under construction at Bordeaux to initiate breeding for nematode resistance. High resources will be devoted to this selection criteria which implies less resources for other activities in the breeding programme.

3 Perspectives for future breeding

3.1 Technological factors

We have selected in this section various technological novelties that could help maritime pine breeders to develop the future varieties. These novelties are currently under study at INRAE and could play a key role to i/ accelerate breeding cycles and genetic gains, ii/ breed for new selection criteria related to economic or environmental changes, iii/ explore and integrate new genetic variability into the current breeding population. For each novelty, we will indicate if its application into the breeding program is planned at short (1-3 years), medium (4-8 years) or long term (over 8 years).

3.1.1 New genomics for breeding

Two Illumina Infinium SNP arrays have been developed for maritime pine: the first one by Chancerel et al. (2013) and the second one by (Plomion et al. 2016) with 2,600 and 4,440 polymorphic SNP respectively for the French breeding population. A new genotyping tool has been developed with the project B4EST: “4TREES” is an Axiom 50K SNP array (Thermofisher) designed for four species (Populus sp, Fraxinus sp, Pinus pinaster and Pinus pinea). Based on the first genotyping results with 4TREES, about 8,000 polymorphic SNP should be available for the French breeding population. In addition to this high throughput genotyping tool, a low density SNP array (62 markers) based on the Sequenom MassArray technology has been developed by Vidal et al. (2017). The genotyping with this array is performed locally at the Genome Transcriptome Facility of Bordeaux (https://pgtb.cgfb.u-bordeaux.fr/) which offers a large flexibility for the implementation in the breeding programme.

Genotyping activities for practical breeding applications have started in 2018 with the use of the low-density SNP array in routine. The first application is to check identity and documented pedigree in the clonal archives (used as breeding arboretum). Up to now, more than 6,000 trees have been genotyped with the low-density SNP array. Several additional thousands of trees have to be genotyped at short

term to fully complete this process. From now on, each new selected trees will be genotyped before grafting in the clonal archives. This new step in the French breeding program allows i/ cleansing errors in the pedigree documented and thus improve BLUP accuracy, ii/ carrying out control crosses on trees which identity have been certified and thus establishing progeny trials theoretically free of pedigree errors. This process has enabled the identification of errors in about 15% of the trees grafted in the clonal archives. This estimation covers various types of error such as: different molecular profiles for ramets of a given clone, clones with different codes but with identical molecular profile, pedigree errors for one or two parents. The second application is the implementation of a new breeding strategies based on polycross breeding (Lambeth et al. 2001). Polycross breeding with pedigree reconstruction has been evaluated in the frame on the French maritime pine breeding programme both for genetic gains (Bouffier et al. 2019) and for economic efficiency (B4EST deliverable D5.2). Polycross trials established initially for backward selection are now revisited for forward selection. Genotyping with the low-density array is thus a key step to recover the full pedigree. At short term, selection in polycross trials after pedigree reconstruction will be considered probably for a limited part of the breeding population. The main advantage of this strategy is to generate a large number of families with a limited number of crosses. Two other application of the low-density SNP array can be mentioned here even if they are not directly related to the breeding activities, one related to the deployment population and one related to genetic resources. The former is the use of molecular markers to estimate pollen contamination and mating structure in open clonal seed orchards (Bouffier et al. 2017) in order to optimize their location and design. The latter consists in the identification of maritime pine gene pools based on a limited number of markers to better define resources to be conserved for potential infusion or to certify seedlot origin (Plomion et al. 2016).

The high throughput genotyping tools have been used for three successive genomic selection (GS) proof-of-concepts in maritime pine: Isik et al. (2016), Bartholome et al. (2016) and an ongoing B4EST PhD (Victor Papin, INRAE). First results showed that a genomic-based approach in maritime pine breeding can reach similar prediction accuracies compared to the pedigree-based alternative. However, the major limitation of our current predictive models is the difficulty to estimate the within-family variability whereas it is theoretically a major strength of GS (Bouffier et al. 2018). The design of a new training population with higher number of trees per family in comparison to previous studies should bring new perspectives for GS in maritime pine. We estimate that its implementation in the breeding program could be of first importance at medium or long term. The major perspective for GS in maritime pine is not the reduction of selection cycle length (due to the late age of sexual maturity) but the integration of new selection criteria that are currently too time or cost expensive. This is a real opportunity for adaptive traits such as pest resistance (in particular resistance to pine nematode which has to be evaluated in a greenhouse, see 3.2.1) or reaction norm based on longitudinal data (see 3.1.2). Finally, GS could be at long term a tool of choice to better monitor genetic diversity in the breeding population, either for the design of the mating plan or for the within-family selection.

3.1.2 Phenotyping

New phenotyping tools are being evaluated with the objective either to reduce the measurement time / cost (wood density estimated with the Resistograph or growth traits estimated with a drone) or to include new selection criteria (disease resistance or traits related to NoR).

Height measurements are currently realized with a telescopic pole for trees under approximately 9 years old and with the Vertex clinometer (indirect measurement based on ultrasonic sound and angles) for older trees. These measurements, which are key for growth evaluation in a breeding programme, are time consuming and sometimes inaccurate for tall trees. It is why UAV (Unmanned Aerial Vehicule) has been evaluated as an alternative tool to estimate individual tree height. A preliminary study carried out on maritime pine (Segura et al. 2021) demonstrates that the correlation between tree height measurements estimated with a pole and with the UAV-based photogrammetric methodology is very high (>0.95). These promising results have to be confirmed but tree height evaluation could be done at short term with UAV to reduce the cost of this measurement. More generally, UAV remote sensing could pave the way to the phenotyping of new selection criteria. For example, pollen and cone productivity cannot be easily evaluated from the ground whereas it is a key factor to select genotypes which will effectively contribute in an open-pollinated seed orchard. UAV opens the opportunity to evaluate these traits on hundreds or thousands of trees.

Wood density is not a major selection criterion for maritime pine but it still contributes to the selection index. Wood density used to be measured with an increment core collected at breast height but this methodology is time-consuming and it has been replaced by the Resistograph tool which is based on the measure of the drilling resistance of a needle driven with a constant force into a tree. Resistograph measurements have been shown to be highly correlated with X-ray micro-density (Bouffier et al. 2008). Several hundreds of trees per day can be evaluated for wood density with Resistograph. By going further in the Resistograph profiles analysis, it could be possible to estimate ring width over several years. If this is the case, reaction norm for growth could be obtained on a large number of trees by combining ring width and climatic data. This research issue is under study by Victor Papin (B4EST PhD student, see deliverable 3.3).

3.1.3 Intensification of seed orchard management

In the French maritime pine breeding programme, much effort has been put into the management of the breeding population and little into the deployment population. The limited number of studies on maritime pine seed orchard management can be explained by the high level of seed yields observed until few years ago even when managed extensively. However, a recent study (Bouffier et al. 2017) shows that high level of pollen contamination and heterogeneous parental contribution can be observed in maritime pine seedlots collected in open-pollinated seed orchards. Pollen contamination from un-improved surrounded stands induces heavy losses in seed genetic value: the higher is the expected genetic gain in last generation seed orchards, the worst is the negative effects of pollen contamination. Two main factors have been identified for pollen contamination: the seed orchard location (distance from the massif Landes de Gascogne, soil composition) and the year of pollination (seed orchard age, annual weather conditions). Moreover, seed productivity has tremendously decreased in seed orchards since 2009-2010 (Boivin and Davi 2016), probably due to a combination of biotic (*Leptoglossus occidentalis*, a seed bug first observed in 2008 in the Landes de Gascogne forest) and abiotic factors (spring frost, summer drought). It has already forced orchard managers to harvest former generation seed orchards to reconstitute their stocks to avoid seed shortage in the coming years.

Seed productivity decrease has become the main argument to initiate studies on intensification of seed orchard management. To date, only studies related to seed orchard health have been carried out to

evaluate efficiency of chemical treatments against biotic stress. The results are mixed and no recommendation can be made at this stage. As part of the B4EST project, a greenhouse seed orchard has been established in order to apply a more intensive management for seed production, with a good control of environmental conditions. Mass pollination is tested with no flower bagging under greenhouse conditions, to evaluate the technical feasibility of this method, the potential of seed yield per cone and the pollen contamination rate (deliverable D4.7).

However, projects are under consideration to intensify maritime pine seed orchard management through pruning, irrigation and flowering induction.

3.2 Environmental factors

3.2.1 Breeding for pine wood nematode resistance

Pine wood nematode (PWN) is a nematode (*Bursaphelenchus xylophilus*) vectored by an insect (*Monochamus galloprovincialis*). It causes pine wilt disease and infected trees can die within few weeks or months. PWN has been introduced in Portugal in 1999 and the whole country has been declared contaminated area in 2008 (quarantine disease). Few infected trees have been detected in Spain near Portugal border. No disease outbreak has been detected in France but PWN is a major threat for the Landes de Gascogne forest and for the wood industry. There is currently no effective way to control PWN, except cutting down infected trees once a disease outbreak has been identified. Genetic studies have started in Portugal and Spain to identify genetic variability for PWN resistance. In France, INRAE has obtained funding to build a quarantine greenhouse in order to carry out researches on interaction between PWN and maritime pine. The objective is also to launch a large screening program to detect sources of resistance for future seed orchards. This strategy has been successfully conducted in Japan on other species. The greenhouse is under construction and the screening program for PWN resistance should start in 2023. The perspective for the deployment of resistant varieties is unknown as it depends on the source of resistance that will be identify in the current breeding population.

3.2.2 Climatic changes: towards several breeding zones?

In France, most of the maritime pine plantations are located in the Landes de Gascogne forest. As a consequence, a unique breeding zone has been defined corresponding to this forest area. It is a flat area of about one million hectares which is relatively homogeneous with sandy podzol. However, despite similar precipitations, slight differences for soil humidity can be observed. That is why the genetic evaluation is currently carried out over two or three contrasted sites for humidity (dry, semi-humid and humid heathlands). GxE interactions over this humidity gradient are generally limited for most of the traits and selection is thus based on the mean performance over the three site types.

Climatic changes could have two main consequences on the breeding activities. The first consequence is the need to evaluate the breeding population in drier conditions which means establishing genetic trials further south. A first one has been established in Spain (Saldaña in Castilla y Leon) two years ago. The number of maritime pine trials established in Iberian Peninsula for breeding purpose is expected to increase at short term. The existing collaborations between French, Portuguese and Spanish researchers is an asset which should facilitate the management of these trials. The second consequence of climatic change is the probable expansion of the maritime pine production zone further north in France. Some trials have established north of the Landes de Gascogne forest (Poitou-

Charentes, Val de Loire, Sarthe, Bretagne) with genetic material selected in the current breeding zone. Results are quite promising. Climatic changes should push maritime breeders to extend the current breeding zone both in south for evaluation in dry conditions and in north to meet new demands for maritime pine plantations. This could have for consequences at long the design of several breeding zone to deploy varieties mores adapted to the local environmental conditions.

In addition, at short and medium term, the breeding population can be evaluated for drought tolerance with the current genetic trials if we are able to identify relevant selection trait. Studies related on the definition of reaction norms based on annual growth (detailed in section 3.1.2) should lead for applications in breeding.

3.2.3 Increase genetic variability to face climatic changes and pine wood nematode

The main breeding population is constituted with genotypes from the local Landes provenance: G0 trees selected in forest and two successive generations G1 and G2. A more limited breeding population was selected in the Corsican provenance and has been evaluated in progeny trials. Some genotypes from other provenances (Portuguese and Maroccan) have also been crossed with genotypes from the Landes provenances and established in the genetic trials. However, breeding activities (crosses and evaluation) are currently mainly focused on the Landes provenance.

Climate change and the threat of pin wood nematode support the need to enlarge the genetic variability in the breeding population. Corsican provenance seems to be particularly interesting as some Corsican x Landes families tends to be more resistant to nematode based on very preliminary results. Provenances located more south of the distribution area such as Spain, Portuguese and Moroccan provenances should be more resistant to drought than the local one. In a near future, more breeding effort will be devoted to these non-local provenances, either to breed them as a pure provenance or to cross them with the Landes provenance.

Finally, genetic variability can also come from other species. Inter species hybrids involving maritime pine could be tried, for example by crossing Pinus pinaster with P. Brutia, P. Halepensis or P. camariensis. However, it seems that these hybrids are difficult to obtain.

3.3 Socio-economic factors

The maritime pine forest is the most productive component of the French forest: 7% of the national surface for 18% of the national timber production and 27% of the pulpwood (FCBA 2020). Almost all maritime pine plantations are realized with improved varieties which represents more than 35 million seedlings produced per year. This is a favourable context to carry out a breeding program for maritime pine in France as it has been detailed in section 1. However, evolution of social expectations and the organization of the forest breeding sector could call into question the current breeding programme.

3.3.1 Social acceptation of plantations and clear cuts in France

In recent years, a protest movement against clear cuts and plantations has been organized by environmental associations at the national level and has found an echo in the society. The legislation has not evolved yet but the context is clearly less favourable for clear cuts which worries forest sector in the Landes de Gascogne forest. This protestation again clear cuts is generally associated with the

promotion of natural regeneration even if the feasibility in the context of maritime pine has not been established. It is difficult to quantify these protestations and to predict the evolution of the legislation. However, it could have consequences on the demand of improved varieties at long term.

3.3.2 Organizational evolutions

The French maritime pine breeding programme is managed by the consortium GIS Groupement Pin Maritime du Futur since 1995 and is mainly funded by public money from the French Ministry of Agriculture and the Region Nouvelle-Aquitaine. The staff is mainly provided by INRAE (public research institute) and to a lesser extent by FCBA (technological institute of the forest sector partially funded by taxes on wood products). There is only very limited investment from the private sector in the breeding programme and this organization is not expected to change in the coming years. The main consequence is the limited staff dedicated to the breeding operations (control crosses, field measurements, grafting…). This a clearly a limitation to extend the breeding programme to new issues in the sense that if resources are devoted to new selection criteria (PWN resistance for example), some current activities will have to be realized with less resources than today.

3.3.3 Towards specialized varieties?

Deployment is current based on polyvalent varieties improved for growth, wood quality and adaptation. This strategy should be maintained for the future as the main seed demand is for the Landes de Gascogne forest which is quite homogeneous from an environmental point of view. However, some specific needs for wood uses or for specific environments (plantations outside the Landes de Gascogne forest) could justify the development of specialized varieties but probably for limited quantities.

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SPECIES CASE STUDIES

Mediterranean stone pine

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1 Breeding activities

1.1 Background

Mediterranean stone pine (*Pinus pinea* L.) is an emblematic tree species of mixed Mediterranean woodlands. Its natural distribution is associated with edaphically poor sites, namely bare rocky slopes or, more often, loose sands, outcompeting other trees especially in coastal sand deposits with active dune dynamics, estuary spits, tombolos and silted former marshes (Martínez & Montero 2004; Manzano et al., 2019; Fernández et al., 2021). In a wider ecological context of mixed open Mediterranean woodlands dynamics, its role is often limited as a by-species: scattered trees overtopping lower main strata of oaks, junipers and shrubs, regenerating scarcely and in patches (Mutke et al., 2012; Pardos et al. 2021). Despite its presence as native or naturalised archaeophyte,, which can be found on the described habitat types along virtually the whole northern Mediterranean shore and beyond, pure stand and especially old-growth stone pine forests are rare and locally limited except on the Iberian Peninsula, and more often they have been established by historic forestation, especially for land reclamation in coastal areas at least since 18th c., and more widely during 20th c., viz. in the Maghreb, Balkans, and Near East (Mutke et al., 2019).

Motivated by the high prices paid for Mediterranean pine nut kernels as gourmet nuts, that can exceed 100 €/kg in retail, private landowners have expanded in the last thirty years stone pine plantations for the interest of cone production in agroforestry systems, particularly in southern Portugal and western Turkey. As consequence, total area of the species has expanded since 1900 threefold from less than 0.3 million hectares [Mha], and some 0.6 Mha in 1990, to currently more than 1 Mha, though employed forest reproductive materials -seeds or seedlings- have been mostly unselected or even of unknown origin (Mutke et al., 2012, 2019).

On the other hand, the species has been found to be noteworthy genetically depauperate, both in neutral markers and in quantitative traits, putatively due to a severe population bottleneck occurred in a local refugium during the Last Glacial Maximum, when its continued presence so far has been confirmed only for southernmost Iberia (Vendramin et al., 2008; Mutke et al., 2019; Jaramillo Correa et al., 2020). Range expansion might have been recent afterwards and even only anthropic, along seafaring trade networks at least since ancient Phoenician and Classic times, when the species was being used in gardening and landscaping for its singular umbrella-shaped crown, its esteemed edible kernels, and associated cultural-spiritual ecosystem services, gaining the classic Latin name of pinus sativa, the domestic pine, devoted to the magna mater goddess Cybele - but also, more mundane, because its closed cones , smoothed with clay, were used as stoppers for shipped wine amphorae, implying long-distance oversea transfer of the enclosed seeds that hence might have been planted abroad afterwards (Twede, 2002; Sabato et al., 2018; Mutke et al., 2019; Moricca et al., 2021).

Stone pine timber production cannot compete with other more profitable forest trees, due to its natural presence restricted to only the poorest soil classes, and also due to its open candelabra crown owed to its strong polyarchic branching habit that leads to poor stem forms (Mutke et al., 2005a). But the collection of the big cones, in Mediterranean stone pine a biomass sink similar in magnitude to annual stem growth, does more than compensate the forest owner for the reduced commercial revenues from timber, given the high price paid for the kernels (Mutke et al., 2012; Shestakova et al., 2021).

Consequently, in the last decades activities on genetic improvement of this species has centred on cone and seed production rather than aiming towards increased timber growth and yield (Guadaño & Mutke, 2016; Mutke et al., 2019). Additionally, its relevance for, and extended use in, forest ecosystem restoration on degraded and eroded soils has also withheld selection criteria related to adaptation and tolerance to abiotic and biotic agents when delimiting selected seed stands for supply forest reproductive materials (FRM) used for forestation, following the European scheme for identified and selected FRM from seed stands. Both uses of forest reproductive materials, seeds for restoration and regeneration versus elite clones selected for cone production in grafted orchards, are strictly separated (Gordo et al., 1999).

On the other hand, cone production from forests is often limited and irregular in time and space due environmental vetoes in nutrient-poor highly drained sites under erratic Mediterranean climate, displaying a strong masting habit, and more so under pressure of ongoing climate change (Mutke et al., 2005b; Calama et al., 2011, 2016, 2019; Pardos et al., 2015; Shestakova et al., 2021). This limited supply, as well as the costly collection and processing, had made Mediterranean pine nut kernels traditionally a rare and expensive gourmet food: mean yields in forests can be a few hundred kilograms of cones per hectare and year, and kernel yield is less than 5% of cone weight.

But nowadays, cone harvesting and processing mechanisation have allowed for starting profitable pine nut production from orchard plantations where annual harvest can be several tonnes of cones, offering a profitable alternative land use or in agroforestry on rain-fed farmland or pastures (Mutke et al., 2007a; Loewe & Delard, 2019; Mutke, 2020; Guàrdia et al., 2021).

1.2 Current breeding programmes in Europe

In the 1950s and 1960s, first explorations of plus trees prospection and mass propagation by grafting had been started in Italy and Spain, though experiences were not continued (Cappelli, 1958; Magini & Giannini, 1971; Balguerías, 1971; Gil & Abellanas, 1989; Prada et al., 1997). Only since the 1990s, several hundred plus trees for high cone yields were prospected in Spain and Portugal and their scions collected and grafted for evaluation in common garden trials. As results, the best performing genotypes have been determined as elite clones, registered in both countries as qualified or tested basic materials for vegetative FRM, used as scion donors for grafted plantations (Iglesias, 1997; Gordo et al., 2000; Mutke et al., 2005c, 2007b; Carrasquinho et al., 2010; Iglesias et al., 2014).

Nevertheless, rather than coordinated formal breeding programmes, these activities were quite isolated initiatives developed by regional forest administrations in several Spanish autonomous regions, namely in Castile-Leon, Andalusia and Catalonia, assisted by diverse universities, the national research institute INIA and the Ministry of Agriculture, while in Portugal, the research institutes INIAV and ISA (U. Lisbon) have supported private forest owners associations in this development (Mutke et al., 2000, 2017). Recently, the Catalonian research institute IRTA has joined INIA in expanding the Spanish network of clonal test sites, aiming at a better characterisation of genotype by environment interactions, i.e. performance of different clones in contrasted agroclimatic zones (Guadaño & Mutke, 2016; Mutke et al., 2017; de la Mata et al., 2019; Guàrdia et al., 2021).

1.3 Breeding objectives

The purpose of genetic selection in stone pine is an improved commercial cone and kernel production, a deployment clearly separated from the use of the species as genuine forest tree that is mainly deployed in land reclamation and forest restoration at harsh sites, where selected seed stands provide sufficient MFR for the praxis. The potential of stone pine as orchard crop for cones is as alternative land use in agroforestry systems on higher site qualities or even farmland, or mixed with cork oak for providing incomes during the decades before the oaks will reach minimum size for cork stripping (Correia et al., 2018b; Mutke, 2019; Correia, 2020).

1.4 Breeding and deployment strategies

Given that seed-grown, not-grafted stone pines do not reach reproductive maturity before 15-20 years, and therefore no precocious juvenile selection can be applied for seed production, sexual breeding has been only a secondary strategy in this species. Progenies trials have been set up a decade ago, but they will still need years for allowing evaluation of their cone production, heritabilities or breeding potential.

In consequence, the main efforts have been made in characterisation of clones tested in grafted common-garden trials, where mature scions conserve the capacity to produce cones from the beginning, offering data series after a few years allowing for selection of elite clones as basic materials for scions. For the same reason, the fruticulture of this tree is based on grafted trees on farmland, in analogy to most fruit and nut species, thus it's essentially the "domestication of the domestic pine" (Mutke et al., 2019).

The observed above mentioned gradient in different ranking of best performing clones at trial sites along climate zones or altitudes has motivated the extension of the first reciprocal trial network to new regions, before delivering refined deployment recommendations differed for for agroclimatic zones (Iglesias et al., 2014; Guadaño & Mutke, 2016). For the time being, clonal tests have been established only in Mediterranean climate zones, lacking in consequence data about experiences for cone production in Atlantic regions where the introduction of stone pine as crop had been proposed by the B4EST project outline, and where cones are indeed harvested successfully from old local stone pine afforestations, for instance at the Île de Ré next to La Rochelle, France (cf. https://www.realahune.fr/pillage-industriel-ressources-naturelles-lile-re/).

1.5 Achievements

In 2015, in Spain fifteen clones were formally registered as basic materials supplying scions for grafting, based on more than a decade of evaluation in grafted common gardens: five clones tested at different sites in the category tested, and ten clones as qualified based on information from only one trial site, while these and more clones are under ongoing evaluation in a wider network of trials. Ongoing research is comparing also the performances of stone pine and Aleppo pine rootstocks in calcareous and alkaline soils (Guadaño & Mutke, 2016; Guàrdia et al., 2021).

In Portugal, where the benign maritime climate allows for in situ grafting on sapling as rootstocks, selection and breeding have relied on propagating a mixture of 64 clones from plus trees, that had been selected in stone pine stands for cone production and legally registered in 2009 as qualified basic

material (clone mixture), without any further experimental evaluation. They have been multiplied by grafting, establishing two scion-donor orchards in the Alentejo region, the heartland of Portuguese pine nut production. Both mother tree orchards are owned and managed by regional private forest owners' associations, the APFC in Coruche and the ANSUB in Alcácer do Sal. Since 2009, ten thousand of scions have been delivered to farmers for transforming recent stone pine plantations into pine orchards as cone crop (Silva & Mutke, 2020).

No breeding activities on stone pine have been developed in any other EU member state with presence of stone pine, namely France, Italy, Croatia or Greece, and few information has been published internationally from Turkey as another of the leading pine nut producing countries. In new-coming stone pine growing countries on the southern hemisphere, tree growers have achieved to establish a few several thousand hectares of commercial plantations for producing pine nuts, namely in New Zealand and Chile (Loewe-Muñóz et al, 2021, cf. also https://pinoli.co.nz/pages/our-orchards).

2 Constraints

In 2022, in commercial tree nurseries in Spain are still struggling to upscale the supply chain for grafted treelets to operativeness, the lack of plants being for the moment the main bottleneck for the deployment of the selected clones as grafted trees, which are asked for by innovative lands owners as novel tree crop for marginal rainfed farmland. On the other hand, also plantation tending and management practices are still to be further developed and optimised, though first experiences indicate that potential cone yields from grafted orchards on farmland are more than tenfold per hectare compared with non-grafted mature pine forests on drought-prone sandy sites (Guàrdia et al., 2021).

Noteworthy, strong, autonomously operating forest owners associations like those that set up the supply chain for graft scions in Southern Portugal, supported by large private properties of several thousand hectares each, are less present in Spain, where stone pine forests are mostly either public or smallholdings if private. But recently, several operational group under the EIP Agri has been set up led by regional forest owners' associations in Spain (https://www.fafcyle.es/gopinon/) and Portugal (http://www2.icnf.pt/portal/florestas/prag-doe/go/gopinhao;
https://www.iniav.pt/projetos/fertipinea) that are tackling these practical issues, the same as the ongoing Spanish-French Interreg POCTEFA project Quality Pinea (https://es.qualitypinea.eu/).

3 Perspectives for future breeding

3.1 Technological factors

3.1.1 Differing FRM for multipurpose forests and for orchard cone production

As said before, the use of *Pinus pinea* in natural forest systems or for afforestation and land reclamation does not require improved forest reproductive materials beyond seeds from

phenotypically selected stands, which have been delimited in the 1990s (Gordo et al., 1999). Even a hypothetical edaphic adaptation to singular soils conditions of some endemic populations, e.g. on gypsum-rich marl, has resulted to be without genetic base when evaluated in reciprocal common garden trials (Gordo et al., 2007).

On the other hand, the same as in most agronomic wooden species selected for fruit or nut production, the clonal propagation by grafting is ultimately based on cultivar selection, that is preferred to breeding-by-crossing, given the time scale needed for the latter, including the delay by the long juvenile state of progenies before reaching maturity for their evaluation, as well as the presumably heterozygous and quantitative genetic structure for traits under selection.

Therefore, the potential of genome-based techniques that would allow faster and cost-efficient breeding, is still to be shown in this species. As a first step, genotyping within the B4EST project aims at exploring applicability of, for instance, genomic selection based on possible QTLs or SNP profiles linked to valuable genotypes. Nevertheless, the lack of continuity and funding for breeding initiatives and field trials has been a setback producing several but disperse phenotypic data series for growth and cone production, whose systematic rescue and documentation has been part of B4EST activities for this species, in order to study allow for genotype-phenotype correlations.

3.1.2 Mass propagation

Although there have been first experiences of applying techniques for somatic embryogenesis or organogenesis to this species (Valdés et al., 2001; Alonso et al., 2006), the only mass propagation method used at operative scale is still classical tip-bud grafting, normally in nursery or on saplings in situ (Guadaño & Mutke, 2016).

3.1.3 Orchard management

Focussing on stone pine as novel nut crop or as tree element of agroforestry systems, there is still a need for establishing optimized orchard management techniques (planting grid, fertilisation, pruning, etc.), before offering a attractive business model for standard forest owners (Loewe & Delard, 2019, Teixidó et al., 2020). In Portugal and Spain, this development is tackled by research institutes and technological centres in collaboration with the sector through the aforementioned operational groups and projects.

3.2 Environmental and socio-economic factors

3.2.1 Environmental changes

The ongoing climate change, as well as other environmental or biotic challenges (pests, diseases), already impact stone pine growth and cone yield in its current, drought-prone growing areas, which are near the rearing edge of the Mediterranean biome, and future climate projections are worsening this panorama (Pardos et al., 2015; de-Dios et al., 2018). Nevertheless, stress is better tolerated by stone pine than by the often sympatric maritime pine that in several regions is already suffering decline or decay processes due to droughts and biotic agents (Prieto-Recio et al., 2015; Menéndez-Gutiérrez

et al., 2018; Gea-Izquierdo et al, 2019). Shifts in composition of seminatural mixed Mediterranean forest are taking place, observed when assessing natural regeneration success of different species (Vergarechea et al., 2019).

On the other hand, stone pine for cone production has been proposed as possible alternative for plantations forestry when, and where, pure maritime pine plantations are declining due to climate or biotic stresses, in Portugal and NW Spain namely the prevalence of the pine wood nematode, *Bursaphelenchus xylophilus*, which is a fatal pest for this pine, but not for stone pine (Menéndez-Gutiérrez et al., 2018). Nevertheless, expansion of planting area of stone pine to the north, both along the northern coast of the Iberian peninsula and into SW France, essentially in an assisted migration approach, will need further field data before setting up operative novel deployment strategies, for instance by adjusting transfer functions or norms of reaction with data from common garden trials out of the current Mediterranean distribution area of stone pine, viz. the Reinfforce arboreta currently under evaluation (Correia et al., 2018a). Moreover, the performance of the species under current and forecasted climates must be studied beyond simple stem growth. Especially both the resistance to extreme weather events like frost or heavy snowfall, to which the candelabra crown of *Pinus pinea* is quite sensitive, and of course the potential cone productivity must be assessed under a range of climate conditions, avoiding direct extrapolation of cone production models based on allometries with growth that were developed for Mediterranean climate zones. Ultimately, stone pine will be planted only if expected yields allow for profitability (Teixidó et al., 2020).

3.2.2 Societal demands

Beyond provisioning of cones/pine nuts as its main marketable forest product, stone pine as forest or plantation tree offers other provisioning (timber, biomass, mushrooms), regulating (soil and water protection) and cultural (landscape, recreation) ecosystem services. But there are clear trade-offs between ecosystems services offered by, for instance, semi-natural Mediterranean mixed forests with presence of stone pine or by agronomically managed orchard plantations for cone production planted in regular grids controlling ground vegetation. Even when the latter is a clear bio-based business opportunity for rural development, the transition between forestry, agroforestry and horticulture can be gradual fluid and might depend on sites conditions, for instance if erosion risk in excessive slopes were limiting feasibility of tilling.

Therefore, discussion among decision makers, both forest owners and politicians, is still necessary about private interests and profitability, public values, public expectations and regulations for forest management that will shape introduction and deployment of stone pine and of its improved forest reproductive materials in forests or farmland in the coming decades, especially in regions out of its current growing area.

4 Conclusions

The use of open-grown stone pine orchards, the same as chestnut groves or almond plantations elsewhere, can contribute in northern Portugal, NW Spain and southern France to diversification of rural landscapes, at one hand introducing tree elements in otherwise “empty” open farmland, but on the other hand allowing for finer-scaled, more wildfire-resilient structures than the currently often

overarching continuous maritime pine or eucalyptus plantations. At the same time, they will broaden the portfolio of wood and high-valued non-wood forest products, which are regional resource base for a bioeconomy (Martínez de Arano et al., 2021). For this purpose, the best available reproductive materials must be delivered, which compels a network of ex-situ comparative trials for evaluating different selected base materials (clones) of stone pine in putative expansion areas north of its current distribution area.

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SPECIES CASE STUDIES

Norway spruce

Authors: Arne Steffenrem & Andreas Helmersson

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Norway spruce is a fundamental species in most forest ecosystems in Northern Europe. The species is also one of the most commercially important conifers in Northern Europe. The straight stems of high stiffness relative to weight is the most suitable material for structural use, and the long and lean fibers are valuable for many pulp-based products. In addition to the industrial importance, Norway spruce plays a major role as carbon storage in forest and forest products.

1 Breeding activities

1.1 Current breeding programs in Europe

A thorough review of the European efforts made in breeding Norway spruce are given by Jansson, et al. (2013). In the Nordic countries, the tree breeding programs for Norway spruce started in the 1940s when clearcutting followed by planted regenerations became the prevailing silvicultural methods in this region (Myking, et al. 2016). Since then, there have been established breeding programs in many European countries, initiated with the selection of plus-trees and establishment of 1st generation seed orchards categorized as “qualified”.

In the Nordic countries, the breeders started progeny testing of the plus-trees which, after two-three decades, have yielded breeding values for backward selection so that many regions now can benefit from seed orchards with the category “tested” (1.5 generation). The breeding programs in the three Nordic countries are about to finalize the first round of progeny testing and a second round is well in progress. In Lithuania, the second cycle is finalized, and breeding arboretums with the selections are being established.

1.2 Breeding objectives

The breeding objective has traditionally been to improve volume production and the stem quality by reducing the frequencies of stem defects caused by double leaders and spike knots (Karlsson and Rosvall 1993, Skogfrøverket 2017, Jansson, et al. 2017). Underlying these objectives are necessarily climatic adaptation, health and vitality. The breeding objectives are now diverging more among the breeding zones since the effect of ongoing climate change pose different stresses and opportunities. The experience have also shown that the growing conditions in some regions are becoming so favorable that more attention has to be paid to wood quality traits such as wood density (Skogfrøverket 2017), and also bud flush and frost risk in spring (Skrøppa and Steffenrem 2021, Liziniewicz and Berlin 2019).

The adaptation of spruce, underlying all breeding activities, follows large scale clinal trends with latitude and elevation (Dæhlen, et al. 1995, Skrøppa and Steffenrem 2019, Skrøppa and Steffenrem 2020) mediated by chilling requirements during dormancy, temperature, photoperiod and the interaction between these factors. The trees are not resistant to frost during the elongation of shoots but tolerate temperatures as low as minus 45 – 50 degrees under full hardiness during winter (Strimbeck, et al. 2015).

In general, growth is a breeding goal with a heritability often around 0.15-0.30 in the forest field situation (Kroon, et al. 2011, Hallingback, et al. 2010). When spruce is grown in dense stands, the size and number of branches and the resulting knots in wood is rarely a crucial factor for the structural performance of wood. However, in more open grown stands, knot dimensions in lumber might exceed the critical values for strength (Høibø 1991). The number and diameter of branches show a heritability

of around 0.30 and 0.15, respectively, and the size of branches are positively correlated with growth (Hallingback, et al. 2010, Steffenrem 2008). Although, the major proportion of spruce stems are sufficiently straight for the most important commercial products, such as construction wood there is genetic variation in stem straightness with a heritability around 0.20 - 0.30 (Hallingback, et al. 2010, Steffenrem 2008) and breeders exclude the worst families after scoring progeny tests. Straightness of stems are also reduced in stands where spike knots occur frequently. Both the knot itself and the crook occurring in the stem reduce strength of the construction wood. Spike knots in the lower part of the stem most often occur in relation to frost. But it seems as it also relates to the occurrence of lammas shoots, a second flush after the end of the growing season that is developing a lower hardiness for the winter (Skrøppa and Steffenrem 2017, Katrevics, et al. 2018). Lammas shoots occur most often at fertile soils and the trait might increase height growth performance by 10-14 % (Neimane, et al. 2015). The phenomena show a heritability of 0.15 – 0.40 depending on the situation (Skrøppa and Steffenrem 2017).

The density of the wood, with heritability as high as 0.50 – 0.60 (Steffenrem, et al. 2016, Hannrup, et al. 2004, Hylen 1997) is important both for construction purposes and pulp yield. The negative genetic correlation with height is weak compared with a stronger negative correlation with diameter increment, and many breeders prefer to perform selection on the basis of height rather than diameter. The grain angle, or spiral grain, is a determinant trait for the dimensional stability during drying of the sawn boards and wood in service (Säll 2002). The heritability is well documented to be around 0.30 (Steffenrem, et al. 2016, Hannrup, et al. 2004, Hallingbäck, et al. 2008) but the trait is not included in operational breeding yet.

Of the pathogens, the prospect of breeding for increased resistance against the root rot pathogens (*Heterobasidion* ssp.) is promising since the pathogen causes substantial loss in the quality of biomass produced in forest. There is genetic variation in the resistance to this pathogen with broad and narrow sense heritabilities around 0.15 – 0.20 (Steffenrem, et al. 2016, Wellendorf and Thomsen 2008, Swedjemark, et al. 2012). There are several other pathogens causing reduced health and vitality to Norway spruce stands, such as needle rust (*Chrysomyxa abietis*) and Pineapple gall adelgid (*Adelges abietis*), but the damages are more temporary and often not weighted much attention in breeding.

1.3 Breeding and deployment strategies

The breeding strategies followed in the Nordic countries are summarized Jansson, et al. (2017) and the main breeding system is presented in Danell (1993) and Karlsson and Rosvall (1993). A major part of the breeding programs started with selection of phenotypically superior plus-trees, those exhibiting good growth and stem quality, from natural stands. Selections were also made from provenance trials and commercial plantations with known background. Scions from the plus-trees were collected and grafted in 1st generation seed orchards and breeding arboretums. Progeny tests were established from open or controlled pollinated mating and established as short or long-term experiments on agricultural land or in the forest field. This initial testing has resulted in breeding values for the parents used to decide on their deployment in 1.5g seed orchards and as parents for subsequent breeding generations. The parents had then been kept as grafted clones in seed orchards or breeding arboretums awaiting the testing.

From a large base population of tested plus-tree (founders), each Nordic country aim to select a long-term breeding meta-population of 900 - 1200 individuals each (Jansson, et al. 2013, Karlsson and

Rosvall 1993, Skogfrøverket 2017). This meta-population is divided into smaller sub-populations, size of 50 individuals in Sweden and Norway and 160 in Finland, that are the subject to long-term operational breeding activities. In Sweden and Finland, these sub-populations will be distributed along photoperiodic gradients facilitating assisted migration for adaptation to climatic changes (Danell 1993). A similar structure is chosen in Norway (Skogfrøverket 2017), but here the breeders aim to preserve somewhat more of the geographic origin in the population structure since the climatic gradients are steep with a considerable influence from the oceanic climate in the west. The reasoning is related to other adaptive traits than those following the large-scale gradients.

Within the sub-populations, controlled mating is performed to propagate subsequent generations. The prevailing strategy is to do new crosses between the tested selections to assure that subsequent generations are based on superiority both on the maternal and paternal side. After a round of pre-evaluation of the seedlings, cuttings are produced and the "clonal-replicated progenies" are planted in long-term field progeny tests (Jansson, et al. 2013, Karlsson and Rosvall 1993). The selection is then made within these full-sib families, based on clonal means that yields a more precise forward selection. This strategy has been evaluated and found to be the most efficient way of moving fast forward for this species (Mullin and Lee 2013, Stejskal, et al. 2022). Organizational and economic differences between regions have resulted in various ways of deploying the strategies along the way. An example are some breeding populations established in Norway that are based on selection from 30–40-year-old experiments with open pollinated families. Without having access to the parents, the breeders have made forward selection of the 2. generation without having the possibility to validate the breeding value of the father. In other cases, genotyping has been used to validate the father and relatedness to keep track of potential inbreeding in the population.

Pre-screening for clonal replicated progeny testing

A large number of progenies from each full-sib family is planted in a dense pre-screening trial in a nursery. After 2-5 years, the most healthy and vigorous 20 - 40 plants of each family are selected as donors for cuttings. These donor clones are now being replicated in sufficient numbers to establish multi-site long-term field experiments with at least four ramets of each clone at each site. These field experiments facilitate selection of the best clones effectively within each family. Testing the same genotypes across multiple environments should also facilitate evaluation of phenotypic plasticity and the stability of the clones (Skrøppa and Steffenrem 2021, Karlsson and Högberg 1998).

1.4 Breeding organizations

The breeding organizations in Finland, Sweden and Norway are Luke (Natural resources institute Finland), Skogforsk and the Norwegian forest seed center (Stiftelsen det norske Skogfrøverk), respectively. In Finland and Sweden, the breeding organizations are responsible for the breeding programs that support the seed orchards owners with improved materials. In Norway, the Norwegian forest seed center manages everything related to seed procurement, seed orchards and breeding activities.

1.5 Achievements

1.5.1 Seed orchard production of seed

For Norway spruce, the most effective way for mass propagation of the genetic gain achieved in breeding programs is to produce seed in seed orchards (Almqvist and Wennström 2020).

In Sweden there are in total 490 ha of seed orchards, 325 of these older than 10 years and currently productive (Almqvist and Wennström 2020). Even if 165 ha becomes productive within the next decade, there is expected to be a shortage of seed for most regions with a total deficit of 400 – 700 kg year-1. This is equivalent to 20-40 % of the total seed consumption in Sweden. The genetic gain will vary from approximately 10 % for the older 1g seed orchards to 25 % for the 1.5 g orchards established from 2004 and onwards. The latter category contributes to 57 % of the total productive seed orchard area within the next decade. A "Fourth round" of seed orchards are now being established containing 2g clones that have been tested in clonal replicated progeny tests that increase precision of selection and a proposed gain of 32 % in volume production (Högberg and Uggla 2021).

In Finland there are currently 200 ha of 1g and 250 ha of 1.5g orchards. The 1.5g orchards are still young and not productive. The strategy is to cover 100 % of the seed demand for nurseries in southern Finland with 1.5g SO seed, while the plan for northern Finland is 50 %. The target is 1400 kg of 1.5g seed year-1. By 2030, the first 2g SOs will be established from the ongoing 2g generation clonal trials established from 2011.

Also, in Norway the strategy is to cover all supply of seed for the annual regeneration of 50 million seedlings from seed orchards. The first-generation clonal seed orchards established in the 1960s, 70s and 80s are now being replaced by 1.5g seed orchards after the first round of progeny testing. There are presently 110 ha of clonal seed orchards that are productive, or will become productive in the near future, and work is underway to establish 90 ha more until 2040 (Myre, et al. 2021). There is an increasing proportion of 2g selections in the newer orchards, but a difference from Sweden, these have been selected from old progeny tests without clonal replication and lower precision in the selections. F1 selections from the clonal replication program will be available sometimes between 2030 – 2040.

Across all countries, seed and cone pathogens cause considerable and unpredictable damage to seed crops and are frequently the main reason for regional deficit of improved seed (Almqvist and Wennström 2020, Myre, et al. 2021, Kaitera and Karhu 2021).

1.5.2 Breeding centers for advanced generation breeding

The breeding center is an important infrastructure facilitating effective breeding activities. One important function is to facilitate safe SHE environments for the breeders to effectively produce new families from controlled crosses. Another important function is to promote flowering in the breeding arboretums here kept as large potted grafts. The flowering is promoted by moving the grafts into a greenhouse for a period during June to obtain the needed temperatures, in combination with a hormonal treatment with gibberellin A(4/7).

meristems into reproductive buds for flowering the following year (Johnsen, et al. 1994, Almqvist 2007, Högberg and Eriksson 1994, Almqvist 2003). Another benefit of this process is that relatively young and small grafts can flower, which makes the controlled mating easier without climbing tall trees in the field. For producing the clonal replicated progeny trials, the breeding centers often have a function both for the initial pre-screening of mother-plants and rooting of cuttings. The centers often host

short-term trials, and sometimes even older trials and breeding arboretums. Grafts for new seed orchards are produced in large scale here.

In Sweden, there are breeding centers at Skogforsk research stations in Ekebo and Sävar. In Finland there is one breeding center at the field station of Luke in Loppi, and another research unit in Savonlinna, which is focusing on somatic embryogenesis of Norway spruce. In Norway Skogfrøverket is running their centers in collaboration with Skogplanter Midt-Norge in Overhalla, and Skogplanter Østnorge in Gjøvik and at NIBIOS research station in Ås. The model chosen in Norway is to collaborate with the commercial nurseries at the sites to utilize their competence and labour in peaks of the field season to limit the costs of the centers. Also, in Lithuania, a breeding center with greenhouse facilities to induce breeding are under establishment.

2 Constraints

2.1 Genetic diversity across a wide range of environments

The wide distribution of the species also covers a wide span of environments. The main constraints in breeding of Norway spruce relates to the need for many and large breeding populations to maintain adaptation and genetic diversity across the wide distribution. Norway spruce show a strong clinal adaptation to temperatures and photoperiods along the wide gradient of distribution and importance for industry. This requires a range of populations or sub-lines managed in breeding, such as the distribution of 22 sub-populations distributed across the temperature climate and photoperiods in the Swedish conifer breeding programs (Danell 1993). In similar fashion, the large altitudinal variations in Norway force the breeders to manage three breeding zones in the same region, covering adaptation from sea level and a growing season of 1500 day-degrees (>50C) to 1000 m elevation and 400 day-degrees.

The concern about diversity in Norway spruce relates to two main factors. One is the general environmental concern that the spruce forests are changed into something else making the ecosystem different and not suitable for the genetic diversity present today. It is important to emphasize that the forest production sites are the same sites as those being habitats for biodiversity, recreation, and non-timber values. The other is that the improved forest becomes too low in diversity to adapt and resist climate change and the expected extreme climatically induced events. To account for these potential negative effects, the breeding populations must be sufficiently large across the adaptive gradients (sub-populations) increasing the cost and organizational challenges in breeding. There will be breeding populations covering forest regions where a few hundred thousand to tens of millions of seedlings are produced annually with almost equal efforts since forestry is an important tool in regional policy. In the long run, concern and careful management of diversity will safeguard long-lived breeding programs since they comprise a sufficiently broad genetic base.

2.2 Late fecundity and uneven cone setting

The breeding cycle takes at least 30 years due to the species late age of sexual maturity and the uneven cone setting every 3rd to 5th year (summarized in: Uddenberg, et al. 2013). This is a constraint not only in the seed orchard production of seed. But there is considerable variation between clones, sometimes making it a decade-long process to cross through a subpopulation. There are also a few stubborn individuals not flowering at all even in the breeding centers where flowering can be induced

by temperature treatments and gibberellin injections. Also, in the orchards treatment with gibberellin has been evaluated (Eriksson, et al. 1998), but it is not put into operational practice on a large scale.

Since the breeder experiences challenges of mating sufficiently large proportions of the breeding population simultaneously the progeny tests must be a compilation of families mated in different years. This is not unproblematic since the adaptive traits of Norway spruce varies with the temperature (Johnsen, et al. 2005) in different seed years (Solvin and Steffenrem 2019). The uneven mating progress within the breeding populations adds "mating year" as an extra factor of variance to the progeny testing.

2.3 Late expression of economically important traits

At highly fertile soils, under the best climatic conditions, the rotation for saw timber production of Norway spruce might be as low as 50 years. However, the rotation age within the Nordic countries is more typical 70-80 years, and often more. While growth traits and survival important for volume production is sufficiently expressed as early as at 10-15 years (Skrøppa and Steffenrem 2019, Westin and Sonesson 2005), traits related to structural wood quality (wood density and spiral grain) and resistance to root rot (*Heterobasidion* ssp.) in the field situation are expressed much later (Wellendorf and Thomsen 2008, Hallingback, et al. 2010, Högberg, et al. 2014, Steffenrem, et al. 2016).

2.4 Climate change

The expectations of climate change are that there will be warmer, drier and more extreme episodes of wind and precipitation (Hanssen-Bauer, et al. 2017). The ongoing breeding activities are based on a robust long-term breeding population structured in sub populations that undergo cyclic mating, testing and selection. For most regions, these populations are established, well managed, well suited for evaluating the breeding objectives and sufficiently large to maintain genetic diversity over many generations. However, for other regions, there are needs for supplementing the existing breeding population, or establishing new populations. This latter is particularly relevant when the need for new breeding objectives is identified, and the existing structure of the population or progeny tests does not sample well the variation for these objectives. As we now observe, there are four points of concern for Norway spruce.

Low drought tolerance: In Central Europe and southern parts of the Nordic countries, there was considerable increased mortality and loss in productivity due to drought stress early in the 2000's (Hentschel, et al. 2014) and after the drought year of 2018 (Svein Solberg, pers. Comm.). Among many of the native species, Norway spruce show poor tolerance to extreme drought episodes, particularly above its middle age of 40-50 years. In many regions, breeding programs must improve the selection for higher drought resistance without already having the necessary progeny tests established at drought prone sites. Hence, even if there is a potential for selection for higher drought tolerance, the breeding programs present might not capture the necessary environmental variation in their testing regime.

Frost risk during flushing in spring: The reduced survival, growth and quality related with frost damages of the elongating shoot of the most early flushing individuals are experienced across Northern Europe (Jansson, et al. 2013) and is expected to become an even larger problem with climate change (Langvall 2011). Since the heritability of flushing is very high and the genetic correlation with growth, the frequency of stem defects and lammas shoots are favourable and the most "phenotypic stable and

robust" materials flush moderately late (Skrøppa and Steffenrem 2021, Skrøppa and Steffenrem 2017, Skrøppa and Steffenrem 2016), it should be effective to perform selection for later flushing individuals. However, bud flush and growth cessation are strongly correlated so the selection should be moderate to avoid loss of hardiness and adaptation to late summer frosts and winter hardiness (Skrøppa 2022, in prep.)

Lammas shoots, and the utilization of the prolonged growing season: The prolonged growing season is important to utilize for increased biomass production. However, this adjustment to the growing season must be done without the cost of increasing the risk for frost damages. It is therefore important to know the potential for breeding for a longer growing season, and the correlations with growth (there are several references on this). A proportion of Norway spruce trees growing under favourable conditions tend to prolong their growing season by flushing of lammas shoots during late July and August (Katrevics, et al. 2018, Beda 1988, Søgaard, et al. 2011, Landis 2012). In many regions, this flushing increases the risk of damage to the leader causing spike knots. It is shown that early flushing families are more prone to produce this lammas shoots or related defects (Skrøppa and Steffenrem 2017, Helmersson and Westin 2019) while it is the late flushing provenances that are expressing the same problem (Skrøppa and Steffenrem 2017).

Winter damages to seedlings due to shorter periods with protective snow cover: Since large parts of the boreal region are covered by snow during winter, seedlings under establishment have been well protected against typical winter desiccation or "exhaustion". When the period of snow cover now are decreasing, severe winter mortality often as high as 50 % is reported (Rolandsen 2015). However, there seem to be genetic variation present as many trees survive and stay healthy and observations also suggest presence of provenance variation. Hence, the situation might be improved with increased knowledge of the physiological background that cause variation among trees.

Higher pathogenic pressure: Higher temperatures, prolonged growing season, and reduced protective snow cover during winter does also increase the potential for spread of existing and new pathogens in the forest, and their consequences might increase (Solberg, et al. 2016). One example is the spread of the root rot pathogens *Heterobasidion* ssp. that occur under a longer period of the year. Other examples, such as bark beetles, benefit from the trees being stressed after drought or storms.

2.5 Phenotyping

With the described biotic and abiotic constraints to breeding, there are also a number of traits that are demanding or practically impossible to phenotype with present techniques. Efficiency and accuracy on breeding therefore rely on more work on these methods.

In Norway spruce, there is a complete lack of efficient methods to evaluate resistance to an important trait such as drought. The same regards hardiness against winter desiccation of young seedlings.

Resistance to the root pathogens *Heterobasidion* ssp. is heritable, but the phenotyping methods based on inoculations under bark (Swedjemark, et al. 2012, Skrøppa, et al. 2015, Skrøppa, et al. 2015, Swedjemark, et al. 1997) or in stem (Chen, et al. 2018, Capador-Barreto, et al. 2021, Lind, et al. 2014) or branches of 2-year-old seedlings have several weaknesses as there are considerable GxE reported, possibly due to environmental interactions with the inoculum (Skrøppa, et al. 2015). The trees phenotyped in these studies are also very young compared with the age where the resistance is important in the natural situation.

Wood quality traits are late expressed as they are usually not useful to measure before 20-25 years from seed, and laborious to measure. Wood density can now be well characterized in relative figures with the Resistograph (Fundova, et al. 2018, Fundova, et al. 2019) or accurately measured with high-throughput x-ray techniques (Steffenrem, et al. 2014, Jacquin, et al. 2019) when the traditional pilodyn penetration is considered to be too inaccurate. The main challenge with the more accurate x-ray techniques is that it requires several steps of data analysis that tends to delay the breeding progress. Spiral grain can also be phenotyped in the field (Hannrup, et al. 2004). However, there are more difficulties in the implementation of direct measurements of stiffness and strength as there are weaknesses with the most effective acoustic methods for use in field (Fundova, et al. 2019) since the physicians claim that the distance for sound travel (often 1 m) is too short to obtain the most relevant information.

Even if height and diameter measurements are effectively measured by the manual methods present today, there is no doubt that more effective methods would increase the breeder's possibility to increase the size of the progeny tests for the limited budgets. Further, as new breeding strategies suggest use of plantations across the landscape, there is a need for methods that can phenotype the whole landscape (more about BWB below).

2.6 Data management and utilization

Data management and genetic evaluation increase in complexity when breeding programs span over several generations, and new genotyping and phenotyping techniques are implemented. In several regions there are a considerable bottleneck in the utilization of the data present and many breeders experience lack of time and knowledge to utilize these effectively, and even correctly.

3 Perspectives for future breeding

3.1 Technological factors

3.1.1 Genomics

The Norway spruce genome has been sequenced (Nystedt, et al. 2013), which is of fundamental importance for our further development of tools in breeding. Further, a 50k SNP array has been developed by Bernhardsson, et al. (2021) for high-throughput genotyping with the purpose of genomic prediction (GP) of breeding values and pedigree analyses. One of the great benefits with the SNP arrays is that they give objective / concrete scores for the allelic variants at the loci, increasing the repeatability in use. However, the SNP array is biased for variable regions of the genome and probably not suited for population genetic analyses. In a comparative study Chen, et al. (2018) found significant gain in GP within a population with full-sib structure when time of breeding cycle was considered.

The combination of efficient DNA sampling, storage and extraction, standardized genotyping platforms, results as standardized relationship matrices and genomic breeding values is about to realize the potentials of genotyping as an operational tool in breeding. Further, there are now biobanks that are specialized in simplifying the workflow from tissue samples to extracted DNA for further analysis and storage. It is important that the breeders, that not necessarily have the competence within genomics, can use these tools to reconstruct pedigree for populations, estimate genomic breeding values, joined with the phenotypic values and environmental factors at the planting site, include all this information in the breeding value estimation often made in Asreml.

3.1.2 Phenotyping

Unmanned aerial systems (UAS), or drone collection of massive amounts of data, make up a promising set of tools comprising high resolution aerial photography for multiple specters such near infrared (NIR) and red edge (RE). Lidar laser scanning is also in the toolbox. Hence, UAS can be used for mapping of seed orchards, breeding arboretums and progeny tests, phenotyping of field trials, and evaluation of cone setting.

Height measurement is maybe the most obvious use of UAS in breeding, particularly after canopy closure in stands that are taller than 10-12 m. Here the treetops are difficult to see from ground during conventional measurements. 3D photogrammetry based on the visible RGB has been used to process 3D models of the canopy height (CHM) with promising results for Norway spruce progeny trials. When the CHM is subtracted from the digital terrain model (DTM) individual tree heights to the uppermost whorl can under ideal circumstances be estimated with an accuracy of ± 14 cm (2.8% of tree height) for intermediate and dominating trees (Solvin, et al. 2020) and ± 60 cm (12%) for all trees including suppressed trees. Higher accuracy for estimation of all trees, including automatic tree detection, was found by (Guerra-Hernandez, et al. 2016) while a larger error was found by (Liziniewicz, et al. 2020). With the image processing systems used, it has not been possible to obtain a reliable estimate of the tree height including the last apical leader (Solvin, et al. 2020). Another limitation is that trees heavily suppressed by their neighbours are “disappearing” in the canopy among the lower branches of more dominating trees and their heights becomes overestimated due to the interpolation algorithms in the system making the 3D model. Further, compression of image files into e.g. JPEG might cause reduction in colour depth and loss of detail and accuracy increasing the measurement error (Liziniewicz, et al. 2020). This error seems, however, to be unbiased. Another source of error is the detail of the DTM available. When this is established from national laser scanning made after canopy closure, the surface will be artificially evened out due to interpolations and the errors might be considerable in rugged terrain. Last, but not least, misidentification of trees is a considerable issue as they might be out of their expected grid when planted (Steffenrem, et al. 2014), or they might be tilting. Many of these errors, such as misidentification of individuals or erroneous tree heights for suppressed trees might not be critical for many breeding purposes. But it is important to be aware of the possible bias and search for tools to screen for records that are likely to relate to a larger error.

Lidar scanning from drones is now available to an affordable price. The lidar scanning produces a point-cloud of physically measured distances from the scanner to the surface. The processing needed to obtain a high-quality CHM is minimal. With a sufficiently dense cloud also a high quality DTM can be generated from the same scan. When measuring 10 – 20 m tall trees, we found the standard error to be around 30 cm (2 %). However, this error is likely shared by the lidar data and the manually measured data. With lidar, several of the issues described under the RGB-method are solved. However, accurate positioning of the trees is still fundamental. Tilting or heavily suppressed trees are still difficult to obtain reliable heights for.

As proxies for adaptation, Solvin et al. (2020) tried to estimate the annual growth initiation and growth cessation from the leader elongation of individual trees by creating CHM for six different flying dates through a growing season. Correlations between manual measurements and UAS estimated data were poor since the leader itself was difficult to capture based on these data and the measurement error of ± 14 cm is considerable when elongation of the leader is under investigation.

The drones can also be used to collect twigs, DNA samples, scions and cones as a sawing system is available for the larger drones.

The development of UAS for breeding purposes is still under development. Important experiences made so far is that coverage of RTK-corrected GPS is simplifying all procedures. In rugged terrain, it is important to establish the digital terrain model (DTM) before canopy closure, preferably already at site establishment. It is also important to collect accurate positions of individual trees at an early stage as this will simplify the extraction of data from the CHM at later stages. This can be done with an RTK-corrected GPS or the high-resolution orthophoto right after establishment.

3.1.3 Breeding techniques

As an alternative to the conventional breeding strategies, El-Kassaby and Lstibůrek (2009) proposed a concept they called Breeding-without-breeding (BWB) for forward selection of candidates to breeding populations and seed orchards. The concept utilizes existing plantations established from natural mating in the parental populations (seed orchard or breeding arboretum) rather than the laborious system of controlled mating and progeny tests. The main advantage is the reduced time of initiating the breeding program. The stands are phenotyped by conventional methods and then genotyped to obtain the pedigree of the phenotyped trees for breeding value estimation. The most effective implementation of the methods seems to be that, based on the initial phenotyping, a top candidate population of e.g. 600 individuals and a random sample of the rest of the population of 600-1200 are genotyped to obtain the pedigree matrix (Lstiburek, et al. 2015). This matrix will comprise full-sibs and half-sibs. Hence, the new ad-hoc progeny tests comprise 1200 individuals, where 600 are candidates for forward selection. If the target population should hold an effective size of 20, the gain from this strategy would vary from 80 – 95 % of the gain from a conventional full-sib breeding strategy. The strategy are also presented as a concept for forwarding the breeding population in an advanced generation breeding scenario, making use of large plantations, not systematic in any way, planted across the landscape to sample the most relevant environmental variance for reaching the breeding objectives (Lstibůrek, et al. 2017). The following studies have addressed the issues with minimizing costs (Lstiburek, et al. 2012), positive assortative mating (Lstiburek, et al. 2005) and pollen contamination (Lstiburek, et al. 2012).

The plasticity present in the species can be even more utilized. It has been shown that the adaptive performance of Norway spruce is influenced by the reproductive environment influences the adaptive performance of the progenies (summarized in Johnsen, et al. 2009). The experience is that higher temperatures during zygotic embryogenesis and seed maturation (Johnsen, et al. 2005, Johnsen, et al. 2005), alter the phenology so that the progenies are adapted to an extended growing season (Solvin and Steffenrem 2019), without any known genetic selection on the way (Kvaalen and Johnsen 2008). Also, other conifers seem to have similar regulation, e.g. Scots pine (Dormling and Johnsen 1992), Lodgepole pine (Wei, et al. 2001) and the Picea glauca x engelmannii complex (Webber, et al. 2005). This plasticity, often connected with epigenetic regulation of gene expressions, can be important to utilize to promote rapid adaptation by the means of localisation of seed orchards in the ”future climate”, or modification of the temperature during somatic embryogenesis. It is also important to understand these mechanisms since this rapid adaptation will have importance for the need of assisted migration.

3.1.4 Genetic evaluation methods

Tools for genetic evaluation span from the most fundamental BLU-prediction of breeding values for individual traits, to integrated analysis across the breeding population and multiple traits, to decision support tools. Skogforsk in Sweden has implemented their breeding programs in the DATAPLAN / TREEPLAN / SEEDPLAN system (Kerr, et al. 2001) while Finland and Norway have built their own database systems with a structure of tools for analyses on top.

Breeding value estimation is most often done with mixed linear modelling systems in software packages like R, SAS, Asreml and Asreml-r. A great advance for complex breeding situations is the use of the "animal model" in Asreml (Gilmour, et al. 2009). This makes it easier to estimate breeding values across a series of trials with various mating schemes without the need for complex model systems. The system can also utilize genomic pedigree data, and probably take advantage of the genomic predicted breeding values based on SNP analyses. In addition, autocorrelation models are used to adjust for some of the heterogeneity in growing conditions not captured by the replicates (Dutkowski, et al. 2002, Dutkowski, et al. 2006, Zas 2006, Chen et al. 2018).

Tools for strategic breeding planning, planning and selection based on breeding values and restrictions on diversity, are increasingly important as advanced generations increase complexity of breeding. Tools in use are Opsel (Mullin 2014), Xdesign (Mullin 2017) and SEEDPLAN modules. Use of these tools requires high competence and time of dedication which might be a challenge for some breeding organizations.

The final establishment of the seed orchard can be facilitated with tools that increase the rate of mating between individuals with high specific combining ability and reduce the rate of mating between related individuals in advanced generation seed orchards with relatedness (Chaloupkova, et al. 2016, El-Kassaby, et al. 2014, Lstiburek and El-Kassaby 2010). Similar tools are also available in SEEDPLAN.

3.1.5 Mass propagation

The main mass propagation of improved FRM of Norway spruce is from seed orchard production of seed. However, spruce is possible to propagate vegetatively by cuttings and somatic embryogenesis (SE).

Vegetative propagation facilitates production of elite FRM of uniform quality and with superior growth or other economically important traits suitable for special end-uses, offering a promising alternative to the conventional seed orchards. First attempts toward commercial propagation of rooted cuttings of Norway spruce were taken in Sweden, and on a smaller scale in Finland, already in the late 1970s. These programs were eventually not capable of solving various technical and economic challenges of large-scale cutting production and were short-lived. More recently, the interest has shifted from cuttings to somatic embryogenesis (SE), which potentially offers a very high multiplication rate and could therefore have an enormous impact on forestry. Cost efficiency plays a major role in the commercialization of the method is dependent on cost efficiency and for plant production, requiring the need to automatize critical parts of the SE process. R & D toward Research and development on somatic embryogenesis is currently underway both in Finland (Luke) and Sweden (SweTree). Now, the use of the method is functional in a laboratory scale and in plant production is still limited to small-scale propagations for commercial partners. Vegetative propagation of improved material would

facilitate implementation of new breeding objectives efficiently, such as resistance to root rot (Heterobasidion ssp.). It would also go hand-in-hand with genomic selection.

3.2 Environmental and socio-economic factors

3.2.1 Environmental changes

The main environmental impacts on decisions in breeding are discussed under preceding chapters.

3.2.2 Societal demands

Raw material for bioeconomy: Norway spruce comprises a fundamental resource in past, present, and future bioeconomy since it goes into a diverse range of products from food additives to structural and massive building constructions. There are a large number of pulp and paper products from spruce, but now there is also research on production of proteins for animal and fish fodder.

Due to its wide range of uses, and considerable unknown potential in the future, many end-users connect a certain risk with the highly specific breeding goals that have been suggested for short rotation species, such as reduced lignin content in poplars and southern pines. It might be safer to continue producing the assortments that are produced today in higher volumes and with somewhat fewer defects.

Carbon storage: CO2-sequestration in forest and forest products are important contributors to reduced global warming. Norway has already implemented forestry practices and breeding into the set of tools for increasing the C-storage in forest (Miljødirektoratet 2020) and it is estimated that breeding can contribute with increased storage equivalent to the emissions due to domestic air traffic within 2070 (probably earlier).

The use of the forest resource as carbon-storage adds additional factors to the weighting between traits. Of course, volume will always be important as the main factor for increased C-storage. However, wood quality traits become important as they might be decisive for the durability of the forest products. E.g., a construction wood in a wooden house stores the carbon longer than newspapers or packaging. Further, the stability of the forests becomes increasingly important, since it can be argued that the most significant gain to storage will be achieved if the forest is kept 10-20 years longer than the strict economic rotation time.

Biodiversity: The concern about diversity in Norway spruce relates to two main factors. One is the general environmental concern that the spruce forests are changed into something else making the ecosystem different and not suitable for the genetic diversity present today; the forest production sites are the same sites as those being habitats for the biodiversity, recreation, and non-timber values. The other is that the improved forest becomes too low in diversity to adapt and resist climate change and the expected extreme climatically induced events.

3.2.3 Organizational evolutions

National infrastructure for breeding and seed supply is fundamental for a stable national seed supply, and each nations ability to collaborate about Nordic state of readiness in case of seed deficiency (Skrøppa, et al. 2015).

4 Conclusion

Norway spruce will remain one of the most important contributors to the growth and prospects of forest based bioeconomy in northern Europe. The potential gain from breeding is high, but this is only fully utilized in Sweden, Finland, Norway and Lithuania at the moment. Breeding faces climatic challenges with climate change. Many of these can be solved with increased knowledge both of genetic and physiological character, but also by technology. The possibility of making the breeding program more effective and attractive with the implementation of existing technology is considerable.

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SPECIES CASE STUDIES

Poplar

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1 Breeding activities

1.1 Current breeding programs in Europe

Species belonging to poplar genus include the most intensively cultivated forest trees (Mohrdiek, 1983). According to recent inventory information there were about 900.000 hectares of poplar plantations managed in Europe (IPC, 2016). Poplar genus is usually classified in 6 major sections known as *Populus*, *Tacamahaca*, *Leucoides*, *Turanga*, *Abaso*, and *Aigeiros*, according to morphological traits (Coddington and Levi, 1991), although clustering analyses based on nuclear rDNA and mitochondrial sequences suggest the polyphyletic origin of some sections and a different clustering of poplar species (Hamzeh and Dayanandan, 2004). To date, the most economically relevant poplar species used to sustain commercial hybrid breeding belong to section Aigeiros, which includes black poplar (*Populus nigra*) and eastern cottonwood (*P. deltoides*), and to section *Populus*, which includes quaking aspen (*P. tremuloides*), common aspen (*P. tremula*), and white aspen (*P. alba*) (Coddington and Levi, 1991) (Table 1).

Table 1: List of the most used poplar species for breeding in EU

Species	*Section*	*Note*
P. deltoides	*Aigeiros*	Female parent of *P.* ×*canadensis* Parentof *P.* ×*generosa*
P. nigra	*Aigeiros*	Male parent of *P.* ×*canadensis*
P. alba	*Populus*	Parent of cross hybrids with *P. tremuloides*
P. tremula	*Populus*	Female parent of *P.* ×*canescens* Parent of cross hibrids with *P. tremuloides*
P. tremuloides	*Populus*	Parent of cross hybrids with *P. tremula*
P. trichocarpa	*Tacamahaca*	Parent of *P.* ×*generosa*
P. maximowiczii	*Tacamahaca*	Parent of cross hybrids with *P. deltoides*

Following the introduction of eastern cottonwood in Europe during the late 18th century, the first valuable cultivars (e.g., 'Robusta') were selected within natural hybrids originated from open pollinations between *P. deltoides* and the native *P. nigra* (Stanton et al., 2010). In Italy, Jacometti initiated poplar conventional breeding in 1923 using controlled hybridization of *P. deltoides* and *P. nigra* species to create new clones of *P. ×canadensis* (e.g., 'I-214' and 'I-488') having an improved resistance to spring leaf and shoot blight induced by *Venturia populina* (Gennaro and Giorcelli, 2019).

Over time, large-scale breeding programs were established in the last quarter of the 20th century as the genetic improvement of poplar trees was considered pivotal to satisfy farmer and industry needs (Mohrdiek, 1983). Unlike other perennials, the short time to maturity, the widespread interspecific crossing ability and the fast generation turnover have contributed to reach the remarkable progresses of poplar breeding (Stanton et al., 2010).

To date, breeding programs are currently active only in a few countries such as Finland, Germany, Sweden (mostly to obtain Aspen cultivars to supply pulp industry and the bio-energy chain), France, Belgium, Hungary, and Italy (mainly to select *P. ×generosa* and *P. ×canadensis* cultivars suitable to produce high quality poplar plywood and particle boards).

Poplar improvement programs carried out in Italy are based on the exploitation of heterosis in *P. × canadensis*, which derives from enforced crossing between *P. deltoides* and *P. nigra* (Table 1), in order to pursue the characteristics defined by the desired plant ideotype.

1.2 Breeding objectives

The main objectives of poplar breeding are based on obtaining new clonal varieties selected for fast growth, rooting capabilities, vigour, plant architecture, resistance to biotic and abiotic stresses, wood quality, and adaptation to sites and climate conditions. Recurrent or semi-reciprocal recurrent selection approaches have been used for gradual population improvement and to create elite cultivars for commercialization (Berguson et al., 2017; Neale & Kremer, 2011). The most efficient long-term strategies involve the constitution of base populations, the selection of parents for intra- and inter-specific hybridizations, and the selection within the progenies. On the other hand, short-term strategies can also be applied simultaneously to increase the number of clones released on the market; progeny testing of parents has rarely been attempted for the performance of F_1 families rather than a general combining ability of the parent trees.

Enhanced wood quality is particularly important to satisfy the needs of end-users: low density, white or creamy yellow color, and fiber length in the case of plywood manufacturers and companies.

One of the main objectives of the Italian Council for Agricultural Research and Economics – Research Centre for Forestry and Wood (CREA-FL, Casale Monferrato) breeding programs is *Phloeomyzus passerinii*, the woolly poplar aphid (WPA), resistance as it can cause up to 10% reduction of potential production (Carletti et al., 2016), mainly in European and American countries where the majority of cultivated clones are susceptible (Arru, 1975). For this reason, the development of new poplar clones resistant to WPA is pivotal to contrast the spread of the infestation.

Like for other perennials and annual crops, climate change is changing the environmental conditions of poplar plantations, for which the vulnerability to both biotic and abiotic stresses is increasing. The impact of climate change on poplar breeding objectives is reviewed in section.

1.3 Breeding and deployment strategies

CREA-FL and former organizations merged with CREA-FL have driven poplar breeding programs since 80s' for selecting *P. × canadensis* hybrids using semi-reciprocal recurrent selection on large collections of both *P. deltoides* and *P. nigra* species. These improvement programs implement a systematic methodology in which long-term and short-term approaches are integrated (Bisoffi 1990, 2001) (Fig. 1).

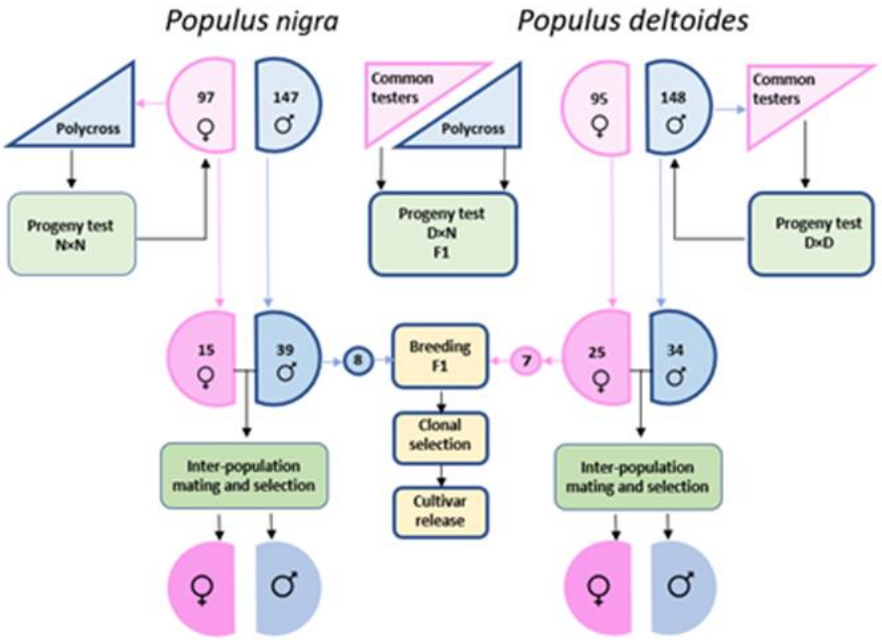

Figure 1: Overview of the poplar breeding programs currently ongoing at CREA-FL. These programs aim to carry out semi-reciprocal recurrent selection of P. deltoides and P. nigra to simultaneously improve base populations and create F1 hybrids. The number of individuals currently included in P. deltoides and P. nigra populations are reported.

Exploiting of heterosis with hybrid breeding usually requires developing heterotic groups including lines that may or may not be genetically related and that can produce heterosis upon crossing. In the context of poplar breeding, heterotic groups coincide with the different sets of parental lines belonging to different species and make F_1 inter-specific hybrids the most effective strategy to maximize heterosis.

In CREA-FL breeding programs, the long-term breeding objectives aim at improving both *P. deltoides* and *P. nigra* populations with pedigree selection and progeny tests to create elite parental populations (**Fig. 1**). This approach is outlined as follows:

1. Assembling of base populations of *P. deltoides* and *P. nigra*, currently including 242 and 245 individuals, respectively.

2. Carrying out intra- and inter-specific enforced crossings to evaluate specific combinatorial aptitude.

3. Selection of the best genotypes and constitution of elite populations of *P. deltoides* (currently including 59 individuals), and *P. nigra* (composed by 54 individuals).

Differently, short-term operations aim to select new commercial hybrids based on progeny tests combining the ability of *P. nigra* and *P. deltoides* clones to satisfy market needs. At CREA-FL this strategy is implemented with enforced interspecific crossings with the best parental lines, which currently include 7 clones of *P. deltoides* and 8 clones of *P. nigra* previously identified based on their specific combining ability.

Parental lines of *P. deltoides* and *P. nigra* included in the elite populations are usually further clustered into sub-populations according to their characteristics and to the traits to be improved (e.g., growth rate, rooting ability, resistance to WPA for *P. deltoides;* branchiness, resistance to *Marssonina, Venturia, Melampsora spp.,* and WPA for *P. nigra*). Nonetheless, the phenotypic evaluations for the traits considered in these breeding programs are time-consuming and the entire process for the release of commercial clones can take up to 20 years (**Fig. 2**).

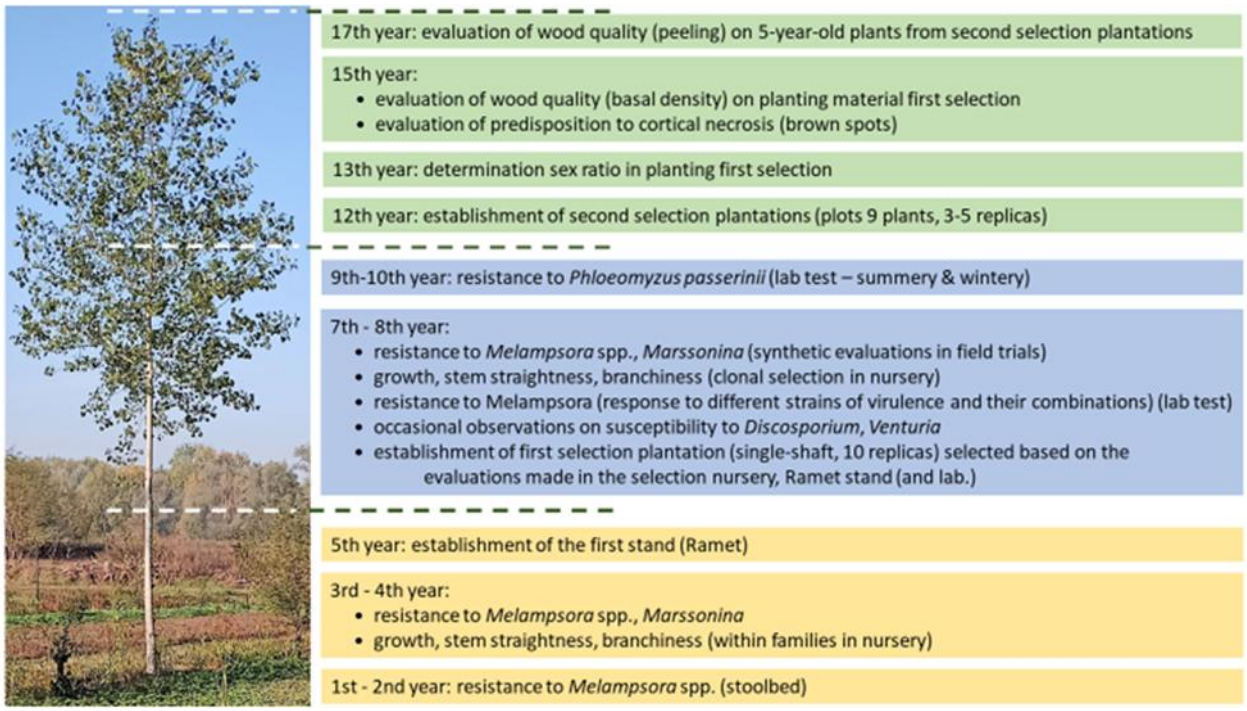

Figure 2: Outline of the selection process adopted at CREA-FL.

Using the aforementioned breeding schemes, thousands of poplar clones have been phenotyped at CREA-FL during the last 25 years.

1.4 Achievements

During the last cycles of poplar breeding, CREA-FL has created and registered several commercial hybrid clones of poplar, most of which are reported in **Table 2**.

Table 2: List of poplar clones released by CREA-FL during the last breeding cycles.

Name	Species	Section	Sex	Reference to breeder's right
'Aleramo'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (38492)
'Baldo'	P. *deltoides* W. Bartram ex Marshall	Aigeiros	Male	Community Plant Variety Right (n. 25211)
'Diva'	P. ×*canadensis* Moench	Aigeiros	Female	Community Plant Variety Right (n. 38690, awarded 2012)
'Imola'	*P.* ×*canadensis* Moench	Aigeiros	Female	Terminated
'Moleto'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 40714, awarded 2013)
'Mombello'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 40715, awarded 2013)
'Moncalvo'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 40713, awarded 2013)
'Oglio'	*P. deltoides* W. Bartram ex Marshall	Aigeiros	Male	Community Plant Variety Right (n. 29841, awarded 2009)
'Orion'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 25212, awarded 2007)
'Soligo	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 13691, awarded 2001)
'Taro'	*P.* ×*canadensis* Moench x P. ×generosa Henry	Aigeiros × Tacamahaca	Male	Community Plant Variety Right (n. 13692, awarded 2001)
'Tucano'	*P.* ×*canadensis* Moench	Aigeiros	Male	Community Plant Variety Right (n. 38493, awarded 2012)

Currently, CREA-FL is responsible to maintain the purity of these genetic materials (**Table 2**), while the distribution to farmers is delegated to private nurseries, which commercialize these poplar clones in different European countries.

2 Constraints and opportunities

There are multiple sources of constraints that hamper poplar breeding, some of which are obviously tied to the biology of poplar and are in common with other perennials, while others are poplar-specific or have a socio-economic base (**Table 3**).

Table 3: List of constraints hampering the genetic improvement of poplar.

Constraint type	Description	Effects
Biological	Late expression of some relevant traits (e.g., wood quality traits) Late development of reproductive structures	Prolonging breeding and selection cycles New selection possibilities for earlier evaluations of wood quality traits.
Climate change	Accurate predictions of the effect of climate change on poplar traits and the emergence of new poplar diseases are lacking	Breeding and selection for poplar clones that might be useless in changing environment.
Economical	Long breeding cycle and stumpage prices	Return on investment for selecting new clones might be uncertain and unpredictable
Societal	The intensive cultivation models, adopted in the past in specialized monoclonal plantations, are often criticized by environmental associations more favorable to natural forests	Restrictions on the cultivation of hybrid clones in sensitive areas and along rivers Increased interest in sustainable management of plantations and polyclonality with resistant genotypes

One of the most important constraints that hamper poplar breeding is the late expression of some important traits (e.g., wood quality traits and resistance to certain biotic stresses) that are usually targeted in poplar breeding programs. In poplar, this phenomenon is the main cause that slows clone selection, prolonging breeding cycles and, consequently, the duration of the entire program. For instance, phenotyping for basal density and peeling, which are two important traits tied to wood quality, is usually carried out at the 15^{th} and 17^{th} year of the selection process (**Fig. 2**). New selection

possibilities based on predictive models for the juvenile-mature correlation for wood quality have recently been developed and represent powerful tools to speed up the entire process.

Although hermaphroditism has been observed across several sections, poplar species are generally dioecious, with male and female reproductive structures present in different plants. The development of reproductive structures usually occurs in 7–10-year-old trees, depending on the species and environmental interactions, and along with the late expression of some key target traits, contributes to slowing down breeding activities. Particularly, the long-term breeding objectives rely on the development of sub-populations (heterotic pools) of *P. nigra* and *P. deltoides* parental lines using reciprocal recurrent selection, which requires to inter-cross selected parents recurrently. As for other trees, the late sexual maturity is one of the main constraints that slow down the genetic improvement of poplar.

As a consequence of the long breeding cycle of poplar, there is the need for long term financial support to guarantee breeding operations. In Italy, only governmental organizations (e.g., CREA-FL) have carried out significant breeding activities for poplar (**Table 2**), which is almost entirely supported with public fundings. One of the reasons that might explain the lack of interest of private organizations for poplar genetic improvement is that a typical duration of hybrid breeding program can extend until 17-20 years (**Figure 2**) and during this long period, market needs and conditions might change (e.g., the stumpage prices) and new clones might not guarantee the expected return on investment, making breeding for poplar clones a high risk business.

The financial returns from hybrid poplar plantations is one of the main factors that enhance the request of improved traits and clones. Analyses carried out in Italy have shown that the range of possible financial returns from hybrid poplar plantations varies depending on investment costs and stumpage prices applied to the amount of harvested timber. These results show that at high stumpage prices poplar plantations can be profitable even in the case of high management costs, while low stumpage prices can make poplar plantations unprofitable (Pra and Pettenella, 2019). Consequently, the trend of stumpage prices determines breeding objectives and the traits to improve.

Climate change is modifying the bioclimatic parameters in which poplar plantations are established. While there are plenty of simulation models that offer reasonable predictions of the future environments across Europe and Mediterranean areas, for poplar it is still unclear how climate change in relevant environments might foster the emergence of new pests and diseases or the insurgence of abiotic stresses. Current evidence points out that climate change underlies the expansion of the geographical ranges of disease of forest species towards the higher latitudes and the emergence of diseases in new territories where susceptible plantations are located (Brodde et al., 2019; Burdon and Zhan, 2020). Understanding the relationship between changing environmental conditions and the spread of poplar pests and pathogens is pivotal to define breeding objectives and the traits to target for new territories where susceptible plantations are located. This is a huge constraint for poplar hybrid as there is the concrete risks to create new clones with improved traits that might be not useful because of the lacking of the appropriate resistances. Breeding poplar for climate change requires to accurately predict which diseases and pests will shift and appear in new environments. Accordingly, the activity carried out within the WP1 of the B4EST project allowed an in-depth analysis of the emerging problems concerning the impact of climate change on the spread of poplar pests and diseases in Europe, as crop simulation analyses carried out in other word areas (e.g., New Zealand)

point out an increase of the average degree of susceptibility to *Melampsora larici-populina* for the next decades (2031–2070) (Mcivor and Jones, 2015).

3 Perspectives for future breeding

The future sustainability of poplar plantations depends on the development of new clones that should fulfill three key requirements:

1) high rooting capacity to ensure good survival, growth and adaptability to limiting water conditions;

2) yield improvement and persistence under biotic and abiotic stresses, and climate change;

3) correct plant architecture and high technological characteristics and quality of wood for end users.

3.1 Technological factors

To deliver the aforementioned objectives and sustain poplar genetic improvement, the implementation of new technologies and methodologies in poplar breeding is needed to make the selection process more efficient. Some of the emerging methodologies and technologies that have the potential to accelerate poplar breeding are reported in **Table 3**.

Table 3: List of potential technologies and methodologies that might improve the efficiency of poplar breeding.

Technology	Application in hybrid poplar breeding	Pros	Cons
Molecular breeding techniques (marker assisted selection)	Selection of parental lines; Selection of hybrid clones	Early selection of individuals	Requiring the previous knowledge of markers associated with the traits of interest
Genomic prediction	Recurrent reciprocal genomic selection	Accelerating the creation of heterotic groups of parental species Reducing costly progeny tests	Requiring genome wide molecular markers and know-how to run models

Genomic prediction	Hybrid clone selection	Accelerating the selection of hybrid clones	Requiring genome wide molecular markers and know-how to run models
Precise phenotyping	Phenotyping in nurseries	Accelerating phenotyping operations	Requiring high CAPEX and tools for managing and carrying out automated image analysis
Unmanned Aerial Vehicle-Based Phenotyping	Phenotyping in field trials	Accelerating phenotyping operations	Requiring high CAPEX and tools for managing and carrying out automated image analysis
Hormone stimulation	Accelerating the development of reproductive structure and reducing breeding cycle	Accelerating breeding cycles	
Grafting	Accelerating the development of reproductive structure and reducing breeding cycle	Accelerating breeding cycles	

3.1.1 Molecular breeding and genomic prediction

Marker assisted selection (MAS) requires to have molecular markers associated with different traits to be used for the analysis of genetic diversity in populations and for the assisted selection of both parental lines and progeny in the first years of the selection process, a period in which the intensity of selection is more drastic and poorly targeted. The deployment of MAS enables the selection at seed or seedling stages, reducing time and costs and enhancing the efficiency and sturdiness of breeding (McClure et al., 2014; Allwright and Taylor, 2016). Even if the bottleneck of the long juvenile phases of perennial crops are still needed to reach sexual maturity, an early selection allows to identify only the individuals carrying the desired alleles associated to specific traits, thus requiring reduced spaces and costs for field trials (Migicovsky and Myles, 2017).

Genomic prediction (GP) aims to regress genome-wide Single Nucleotide Polymorphisms (SNPs) or other types of DNA markers on phenotypes to simultaneously predict their effects (Meuwissen et al., 2001), which, in turn, are used to estimate genomic breeding values (GEBVs) of individuals for which only genotypic information is available. Potentially, GP can speed up breeding cycles, increase the accuracy of selection, and enhance the realized genetic gain per unit time. In actual breeding schemes, GP is used to rank individuals based on their GEBVs without using phenotypic information. GP applications for the improvement of tree species are still in its infancy, nevertheless the lesson learned from other crops points out that this methodology might be a game changer for accelerating tree breeding as it allows increasing the genetic gain per unit time and improving accuracy. As for other hybrid crops, GP can be implemented in poplar breeding to predict the performance of unknown interspecific hybrids using only molecular markers or for improving heterotic populations of parents (*P. deltoides* and *P. nigra*) using recurrent reciprocal genomic selection, shortening the selection cycles.

3.1.2 Early flowering as tool to speed up poplar breeding

As mentioned above, one of the main constraints in poplar breeding is the long juvenile phase. Poplar trees take several years before flowering, which delays crossings and results in decades-long breeding programs. The opportunities to tackle this problem are essentially limited to the adoption of biotechnological strategies. These strategies, however, require efficient genetic transformation protocols adapted to the specific genotypes used and are largely dependent on political decisions and public acceptance. Transgenic poplars overexpressing different FLOWERING LOCUS T (FT) paralogues (FT1, FT2) display shortened juvenile phases that allow flowering within one year (Böhlenius et al., 2006; Hsu et al., 2006). FT proteins move through the phloem to reach shoot apical meristems and promote the transition to the reproductive phase. However, attempts to accelerate flowering in scions grafted on rootstocks that overexpress FT have been unsuccessful (Zhang et al., 2010).

Considering that the FT transgene is hemizygous and dominant, it is theoretically possible to introduce it in a breeding genotype to accelerate crossings for several cycles, and then to segregate it away in the last cross whose progeny would be the only one to undergo to a long juvenile phase. Thus, the final genotype obtained in the process would not be transgenic (McGarry et al., 2017). However, this strategy would still be dependent on the regulations that limit the possibility of growing transgenic plants in the field for experimental purposes. Furthermore, open-field trials with transgenic trees at flowering stage should be preceded by the development of effective strategies for genetic containment.

A new breeding strategy targeting poplar genes for inducing early flowering might significantly reduce the breeding cycles of this species. Transgenic trees start to flower at an age of up to one year, and trees bred and released from this breeding strategy contain no foreign genes. Crossings could be performed using one year old early-flowering poplars. Molecular analyses of the F_1 plants revealed the transgene-free status in 49% of the progeny (Hoenicka et al., 2014). Thus, the practical application of early flowering transgenic polar for cross breeding has been shown for the first time worldwide. For apple, early flowering plants should be used for the production of disease resistant apple plants. For the first time, it was feasible to control the flowering dates and not to change the growth habit of the tree. As a result, a progeny could be produced from crosses between early flowering transgenic apple plants and the wild variety *Malus robusta*, being resistant to fire blight. The seedlings are currently being investigated in respect to resistance properties (Kellerhals et al., 2017).

3.1.3 Remote sensing

In recent years, remote sensing techniques applied in forestry have found mounting interest, allowing the possibility to extract information for forest planning and sustainable management such as the structure, composition, volume, or growth of forests and plantations. Precision Forestry becomes important to facilitate the implementation and integration of information and communication technologies as essential elements of innovation in the national forestry and wood production system. The use of remote sensing technologies by forestry technicians, owners and managers of the forest heritage and wood plantations allows the development of operational tools also suitable for non-specialist use, for:

1. carrying out complex analyses of forest and territorial data of interest in a guided, simple and economic way

2. supporting planning and design decisions of forestry operations according to standardized and reproducible conceptual paths

3. promoting the integration between technicians, operators in the sector, policy makers and the social community, while guaranteeing the transparency of forestry asset management operations, on the basis of shared, standardized and highly accessible information platforms (e.g., Web-Gis and App for everyday mobile terminals)

The use of precision forestry can provide reliable and non-destructive measurements of the volume of trees and contribute to accelerate phenotyping. One of the remote sensing under test for poplar phenotyping is the terrestrial laser scanning (TLS), which is a reliable method and non-destructive method for measuring volume allometries in poplar plantations and holds great potential to enhance conventional tree inventory and monitoring. These non-destructive, high precision and low-cost measurements can be applied to both tree crown volume and tree position.

3.1.4 Robotic and automation

The remote sensing applicable to wood plantations is similar to the site-specific ones that in precision agriculture are focused on variable rate approaches (VRT) to allow the optimization of the quantity of agronomic inputs (fertilizers, herbicides, irrigation water) as a function of the spatial variability of crops. In the context of wood plantations, geo spatialized information (GNSS and GIS) is also used to facilitate the optimization of planting activities.

The issue of product traceability is closely linked to the mobilization of wood resources that can be extracted from woods and forest plantations, as required by EU Regulation - Timber Regulation - n. 995/2010 (Due Diligence system). The transmission of data relating to the flow of timber taken, both in terms of quantity and geographical location will contribute to significantly improve the control and protection policy of resources.

The latest technological innovations have seen remote sensing realized using drones. This technique has a range of benefits such as reduced costs, flexibility in time and space, high accuracy data, and the advantage of no human risks. Parameters such as canopy cover, number of trees, volume estimation, vitality or composition of stands are important parameters in forest planning and sustainable forest management.

Plant phenotyping can operate at different levels of resolution and dimensionality, from molecular to the whole plant, and, in different environments, from controlled to field conditions. Although each level focuses on certain traits, the ultimate goal is to acquire and integrate this knowledge in poplar breeding to produce higher performing clones. In this regard, the use of plant phenotyping methods as part of breeding programs has become a powerful research tool for breeders to generate more adaptable cultivars to different challenging environmental scenarios (Lobos et al., 2017).

3.2 Environmental and socio-economic factors

3.2.1 Environmental challenges

Poplar breeding has been traditionally conducted aiming at improved biomass yield and wood quality traits for specific utilizations. However, associated with the ongoing climatic change, the challenges posed by drought, heat waves, and extreme weather events, including floods and windstorms, are predicted to increase in importance in the coming years. Thus, it will be also important to focus on traits related to resilience and tolerance to abiotic stresses.

Water shortage is the most limiting factor for many crops around the world. It causes severe damages to the plant cells by osmotic stress and excessive reactive oxygen species (ROS) accumulation with consequent protein and membrane denaturation. It also limits growth by reducing carbon fixation and by inducing loss of turgor in the cells with consequent reduced expansion. In trees, drought poses additional threats associated with their aboveground architecture and long-life cycle. These include embolism formation, which can result in branch or even tree death, and carbon starvation due to depletion of carbohydrate reserves that results in physiological imbalance and limited growth not only during the stress event but also in subsequent years.

Heat stress is predicted to increase due to increased frequency of heat waves and causes yield losses by directly damaging cellular components and processes including nutrient uptake and photosynthesis. Heat and drought stresses are closely related because often occur simultaneously and aggravate each other: during drought conditions leaf temperature can increase drastically due to stomata closure and consequent reduced transpiration cooling; on the other hand, it has been recently shown that an increase in leaf temperature in poplar induces stomatal opening, leading to increased water loss by transpiration (Urban et al., 2017).

Environmental challenges have been addressed in pluriannual selection programs by setting field trials in multiple locations characterized by different pedoclimatic conditions. However, there is nowadays the need to elaborate more focused breeding strategies that specifically consider stress-related traits and their genetic basis. Once established, these traits should be phenotyped from the early stages of the selection process along with precise measurements of climatic and soil water content data.

The need for specific methods for the evaluation of abiotic stress tolerance traits in breeding programs is highlighted by several studies that have shown that poplar clones characterized by high productivity are generally the less tolerant to these stresses, and vice versa (Monclus et al., 2006; Attia et al., 2015; Viger et al., 2016). These studies provide useful insights on the trade-offs between yield and stress-tolerance, but also suffer from limitations due to the fact that in most cases measures have been conducted in controlled conditions (greenhouse or growth chamber), using relatively young shoots instead of full-grown trees, and for a limited time, rarely exceeding one growing season. However,

these studies also highlight a high degree of genetic variability among poplar clones which could be further explored by re-assessing, in different environments, clones that are already established for their productivity and are grown in specific areas.

A major bottleneck for the integration of abiotic stress resilience traits in selection programs is the availability of practical and effective methods for phenotyping. Traits related to drought tolerance that can be measured without too much effort and are thus potentially suitable for large scale screening include plant biomass productivity (tree diameter and height), leaf size, stomatal density and size, and leaf temperature (which is related to stomatal opening and transpiration). Stomatal density traits have been recently investigated for the ability to predict growth performance in drought conditions (Himes et al., 2021). Other traits require the set-up of experimental protocols but still seem measurable as, for instance, the speed of stomatal movement in response to a drought condition. More problematic traits, for which the development of dedicated methods would be especially needed, are those involving root morphological characteristics which are difficult to measure and often display low heritability. Research efforts directed to identify root traits that can be measured in early growth stages have recently made some progress. For instance, the ratio of cumulated superfine root length per unit of leaf area has been identified as a promising trait to predict growth performance under limiting water conditions (Meyer et al., 2021).

The large body of information provided by functional studies on herbaceous model plants can be used as useful guides for the identification of key traits for abiotic stress responses in poplar, as the major signalling pathways involved appear to be conserved. Recently, genome-wide association studies have led to the identification of gene polymorphisms that are associated with stress response traits, including stomatal density, in *P. trichocarpa* (Chhetri et al., 2019). The identification of similar polymorphisms in breeding populations may pave the way to significantly speed up their introgression into commercial clones by crossing or by biotechnological methods such as cis-genesis or genome editing. However, it is important to stress that additional factors associated with the perennial habit and the long-life cycle can play significant roles and can only be assessed in pluriannual trials. These include stress memory effects associated with DNA methylation changes that can potentially modify the response to recurrent stress events in the same growing season as well as in subsequent years (Georgii et al., 2019; Sow et al., 2021). Phenotyping for wood quality traits should also be performed in different environments and stress conditions. Contrasting changes in wood composition following a drought stress treatment have been reported, including a reduction in cell wall polysaccharides in *P. trichocarpa* and, unexpectedly, an increase in saccharification potential in *P. nigra* (Wildhagen et al., 2018; Hori et al., 2020).

3.2.2 Societal demands

At European and particularly Italian levels, standards for forest certification schemes (FSC® and PEFC) and sustainable cultivation (“Linee di indirizzo per la pioppicoltura sostenibile”) have been defined for poplar plantations to satisfy the requests of eco-friendly raw material.

Users of poplar wood and final consumers can decide to choose finished products deriving from certified sustainable plantations managed with low environmental impact practices and genetic materials resistant to biotic and abiotic stresses.

To achieve these objectives, poplar breeding should be able to develop new FRM tolerant to new pests and diseases that will be predominant as a consequence of climate change.

The possibilities of innovation of poplar breeding programs on the basis of applications developed also within the various WPs of the B4EST project will be an opportunity for attention and satisfaction for both stakeholders of the supply chain and civil society.

Furthermore, the new European directives drastically limit the use of phytosanitary products, making poplar productions based on sensitive clones difficult for the containment of insects, and other biotic adversities.

A more shared and homogeneous approach in regulations at national level is also desirable in view of the new European Rural development plan. For this reason, it could be useful to support the certification schemes of sustainable management (FSC and PEFC) and increase the commercial availability of poplar clones with greater environmental sustainability as well as promoting polycyclic plantations able to provide an anticipated and differentiated income over time.

3.2.3 Organizational evolutions

Currently, one of the critical issues in the wood sector is the scarcity and fragmentation of updated and reliable information and statistical data. Among the future prospects of adapting wood production in accordance with market needs, the creation of cooperation projects and the realization of supply chain agreements involving nurserymen, poplar growers, and industrialists becomes important, as well as the availability of adequate communication tools and disclosure. Currently, the FSC and PEFC production regulations provide for the use of a percentage per hectare of improved clones to obtain certification, which could be enhanced. To date, in Italy the information regarding the available certificated nursery material on the market is obtained from the official registration bodies, but of this material it is not known how much is planted in Italy and how much is exported.

The implementation of FRM and information to producers of the potential of these new genotypes, as well as information on the annual productions of poplar nursery materials from FRM, supported by inventory monitoring of poplar cultivation, can be real indicators of the availability of certified raw materials in the medium term, to allow greater investment planning capacity for the various actors involved. It could be useful to create a network that can be consulted by the various stakeholders in which information can be obtained on where to procure nursery material for growers, as well as the final wood product for industries (Coaloa et al., 2019). By this way, the poplar supply chain managed in an eco-sustainable way with the use of FRMs, can contribute to consolidate in Italy one of the most dynamic sectors of the green economy.

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SPECIES CASE STUDIES

Scots pine

Authors: Matti Haapanen & Torgny Persson

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1 Breeding activities

1.1 Current breeding programs in Europe

Genetic improvement activities of Scots pine (Pinus sylvestris L.) have been underway in Europe for decades, starting in many countries between 1930 and 1980. The magnitude of the breeding work has reflected the economic resources and the importance of Scots pine in the forestry of each country. Information on the present status of Scots pine breeding in various countries is scant. Many programs do not seem to have lived past the first stages of breeding, which have typically involved phenotypic selection of seed stands and plus trees and establishment of first-generation seed orchards (Krakau et al. 2013).

The most advanced active breeding programs seem to reside in three countries of northern Europe: Sweden, Finland, and Latvia. All these programs have completed the mass selection of several thousands of plus trees from natural stands, determined their genetic merit by progeny testing, established tested-category (1.5-generation) seed orchards and advanced further to produce F1 materials for use in advanced-generation (second cycle) breeding through controlled crossing of the best progeny-tested plus trees.

Breeding programs of somewhat lower intensity seem to be underway at least in Estonia, Lithuania, Poland, Czech Republic, Spain and France. In some countries, such as UK (Scotland) and Norway, there are plans to restart Scots pine breeding after a long inactive period. More information on the status of Scots pine breeding in various countries may be found in the summaries by Krakau et al. (2013), Ruotsalainen and Persson (2013), and Stener (2015).

1.2 Breeding objectives and selected traits

The long commercial rotation of Scots pine forces breeding objectives to be rather generally defined so that they maintain their relevancy under prevailing conditions as well as various future scenarios. Strict goals based on production functions and specific end-products would probably turn out to be futile over time since industrial processes and needs are not likely to remain unchanged over long periods of time.

The overall objective of Scots pine breeding is to increase wood production and the economic value of end products (Rosvall 2011, Ruotsalainen & Persson 2013). Scots pine wood is commonly used for sawn timber which emphasizes the importance of good stem quality (knot size, absence of defects). Because of the ongoing environmental changes, stable performance over a range of sites and climates as well as horizontal resistance against various pathogens and pests is becoming more important goals (Rosvall 2011, Krakau et al. 2013). Adaptability to poor sandy sites is a specific goal of Scots pine breeding in France (Mullin et al. 2011).

The most common traits assessed in Scots pine trials include height and diameter growth, traits associated with branchiness (branch size and angle, the occurrence of ramicorns and forking), stem straightness, symptoms of diseases, autumn frost hardiness, overall vigor, and survival rate. Good autumn frost hardiness (which is sometimes assessed using freezing tests) is important in the harsh climate of northern Sweden and Finland where low survival rate of planted stands is often the primary reason for suboptimal per unit area volume growth. Wood properties such as wood density and stiffness have not been directly selected, but as they appear unfavorably correlated to some selected

traits such as diameter and volume growth (Hong et al 2014), there is a need to monitor them and ensure that breeding for growth will not affect wood quality negatively in the long run.

1.3 Breeding and deployment strategies

To maintain sufficient diversity for long-term breeding, the metapopulations of Scots pine consist of about 1000 unrelated individuals both in the Finnish and Swedish program. The breeding materials are divided into multiple independent subpopulations targeting a certain climatic condition. The multiple population structure is based on the rather narrow adaptation of Scots pine to temperature and photoperiod on the northern range of its distribution.

The most common breeding strategy is based on recurrent selection, where plus trees from natural stands are evaluated in progeny trials and the results are used to backward-select the best performing parents to breeding and multiplication populations (seed orchards). The next-generation offspring (the recruitment population) is reproduced through controlled crosses between plus trees based on a positively assortative mating design. From this point on, several alternative breeding schemes to proceed forward exist. The length of the breeding cycle (generation interval) varies from 21 to 36 years depending on the breeding scheme (Rosvall 2011). The various options were reviewed by Mullin & Lee (2013, p. 12–17). Several software tools are today available that can help the tree breeder handling relatedness among selected next generation candidates. The tools maximize the genetic value of a selected population, while at the same time imposing a constraint on acceptable loss of genetic diversity.

The Swedish Scots pine breeding is based on a combination of different strategies, rather than a single strategy (Rosvall 2011). The Finnish strategy aims at selecting an expanded set of 2nd generation candidates from full-sib families growing in F1 trials. The selections will be grafted in clone banks and pair-crossed to produce a 3rd-generation recruitment population. This mostly corresponds to the “Field forward with expanded BP” breeding scheme as described by Mullin & Lee (2013). Independently on the chosen scheme, the forward selections from the recruitment population are based on BLUP breeding values which exploit all the information available on the potential candidates and their relatives through a pedigree file.

The advent of the genomics era has not yet manifested as major changes in the breeding strategies of ongoing Scots pine programs. It seems that genomic tools could be adopted faster to new programs than to the more advanced ones. For instance, the commencing Scots pine program in Norway plans to skip the initial phase of plus tree selection in natural stands and instead perform the first selections in commercial planted stands consisting of improved materials. This approach, utilizing a concept called “Breeding without breeding” by El-Kassaby and Lstiburek (2009), treats plantations as ad-hoc progeny trials and makes use of informative DNA markers to fingerprint and reconstruct the pedigree of the phenotypically forward-selected candidates.

Seed orchards established with grafted propagules of phenotypically selected (1st generation) or progeny-tested (1.5-generation) plus trees (typically 25 to 35 clones in an orchard) have been the main method of mass propagation in Scots pine. In the summary of an international questionnaire (not covering all European countries) Sweden, Finland, Germany, Poland, Czech Republic, and Lithuania were listed as countries having more than one hundred hectares of Scots pine seed orchards in 2013. Seedling seed orchards have been established only in Poland (Krakau et al. 2013).

1.4 Achievements

Jansson et al. (2017) published a review of the economic impact of tree breeding programs in Scandinavia and Finland. Published estimates of genetic gains for Scots pine were available from two countries, Sweden and Finland. Genetic gains in unit area volume growth for seedlots from seed orchards comprising phenotypic plus trees fall between 10 and 12%. 1.5-generation seed orchard seedlots were reported to yield up to 25% more wood per hectare than unimproved seed sources. Genetically improved reproductive materials are widely used in both countries. As of 2020, 91% and 98% of the nursery seedlings in Finland and Sweden, respectively, were raised from seed orchard seed. Statistics of the use of improved seed sources in reforestation seem to be lacking for other countries where Scots pine has been a subject of breeding. The Scots pine programs in both Sweden and Finland are progressing toward deploying 2nd generation candidates in seed production.

2 Constraints

2.1 Late sexual maturity

Regeneration of breeding materials through controlled crossing or open pollination requires that trees have undergone the physiological transfer from the juvenile stage to the mature reproductive stage. In open grown stands of the boreal climate, the flowering of Scots pine begins at the age of 8 to 20 years (Sarvas 1964). Even though some trees show exceptionally early (precocious) flowering, most Scots pine trees do not attain the flowering competence before age 10. On the 60th latitude, the first female flowers occur at the age of 10 to 15 years. In more northern regions (64th latitude) female flowering starts at the age of 13 to 18 years. The onset of male flowering occurs 3 to 5 years after female flowering (Curt Almqvist, pers. comm. 16.5.2019).

Tree size seems to be an important component of the flowering competence. Hence, the best strategy for obtaining rapid sexual maturation in many tree species is to grow the seedlings as rapidly as possible to a certain minimum size and then apply some flower-inducing treatment which is appropriate for the species (Hackett 1985). Sufficient size is also important for the tree to produce viable seed (Chalupka & Cecich 1997). On the boreal region, greenhouse conditions, where controlled excess/deficit in light, temperature or water during the period of flower bud initiation may be used for flowering stimulation, are practically essential in achieving substantial reductions in the timing of the first flowering.

Both pine and spruce species have been successfully stimulated to flower by exogenous treatments of the gibberellin hormone. The best applications have included simultaneous stimulation by GA4/7 and other stress treatments (water and temperature). The result from the stimulation is sensitive to the proper timing.

Speeding up breeding progress by selecting very young materials (using either genomic or phenotypic information) is likely to be very difficult in Scots pine due to the long period it will take to raise new offspring from the selected candidates. The outcome of flowering stimulation treatments varies by the genotype, but in general it is very difficult to force early flowering in a large proportion of juvenile breeding materials. As some trees are slower than others at reaching flowering competence, early selection is likely to favor early flowering genotypes. This may have unexpected consequences on the other traits through the system of genetic correlations.

Most effective breeding strategies accept the late flowering as a biological fact. In a conventional breeding scheme, the recruitment population is raised in field trials for phenotypic assessment and selection which are scheduled to occur around the time of the first flowering. When the juvenile stage is fit to the length of field testing, controlled crossing work in the grafts of the selected candidates can be started without any unnecessary delay. Another reason to avoid selection at early ages is the need to obtain scions from the selected candidates for crossing archives and seed orchards. Obtaining adequate numbers of scions from the single selected genotype is usually a challenge even when those trees are 15-20 years old when selected.

2.2 Vegetative propagation ability

Genetic evaluation of candidates by progeny testing is an elementary part of any tree breeding scheme. Progeny trials comprising clonally replicated candidates offer significant advantages to trials established with seedling progenies of the candidates. Information on multiple clonal replicates of the candidates improves the accuracy of genetic evaluation. Cloning also allows the same genotype to be directly evaluated in multiple different environments, which is generally considered important as breeding programs want to improve the robustness of the breeding materials to climate change.

The poor rooting ability has constrained the use of clonal material in Scots pine progeny testing. Although shoots from young Scots pine seedlings (1–2-year old) have been successfully rooted as cuttings, the shoot production and especially sufficient rooting response seem to vary greatly by the genotype (in a manner quite typical to vegetative propagation). The current protocols do not seem to be robust enough when applied to a large number of genotypes and thus, many candidates are lost in the process. A recent Nordic collaborative project (Högberg et al. 2011) found its results generally low and erratic and admitted that it was not successful in developing improved and reliable protocols for Scots pine cutting propagation. Despite these difficulties, clone testing is used to some extent in the Swedish Scots pine breeding program.

2.3 Deployment of genetic gains through mass propagation

Grafted seed orchards have proved to be efficient in producing masses of improved seed for Scots pine reforestation. After Scots pine seed orchards reach the productive stage, which usually takes about 10 years since the establishment, they are estimated to remain productive at least 40 to 50 years, which is a benefit for the seed producer. However, the long production time can also constrain the deployment of best materials from breeding programs if commercial seed producers choose not to replace their aging seed orchards with new ones as soon as new superior genotypes from breeding become available.

3 Perspectives for future breeding

3.1 Technological factors

3.1.1 Genomic applications

DNA markers of various types have been used in Scots pine breeding for decades for identifying labeling and grafting errors and ensuring that correct genotypes are deployed in controlled crosses and seed orchards. It is likely that genotypic fingerprinting will remain as one of the most important applications of DNA markers in the future.

Advances in high-throughput sequencing of the DNA have quickly expanded the range of potential breeding applications. Thus far, the progress in forest trees has been mostly hampered by the lack of cheap and rapid high-throughput genotyping platforms. The advent of whole-genome single nucleotide polymorphism chips for forest trees, such as the new 50K SNP genotyping array developed for Scots pine within the B4EST project, represent important technological steps toward operational breeding applications. The new PiSy50K array was suggested to be useful for four primary breeding and research purposes: 1) Genomic selection, 2) Pedigree reconstruction, 3) Genome-wide association studies and 4) Genetic mapping (Kastally et al. 2021).

In genomic selection (GS), genomic estimated breeding values (GEBV) of candidates are predicted as the sum of the effects of individual marker alleles across the genome, based on the assumed linkage disequilibrium of the thousands of markers with genes affecting the trait of interest. GS allows superior individuals to be identified early in life. Benefiting from this requires, however, that the selected candidates reach reproductive maturity soon after the prediction of GEBV. As previously stated, the late sexual maturity is a major biological constraint for Scots pine. Whereas this implementation of DNA markers has revolutionized dairy cattle breeding, similar benefits through faster generation intervals do not appear likely in Scots pine.

Another implementation of GS in Scots pine breeding could be pre-selection for traits that will express at a late age, such as wood density, heartwood content etc. Limiting phenotypic evaluation to the most promising genotypes would potentially increase selection intensity and reduce phenotyping costs. Selection intensity is positively dependent on the size of the population from which the selections are drawn. However, the relationship is not linear. Thus, to increase selection intensity substantially, recruitment materials would have to be greatly larger compared to conventional breeding schemes. Reproducing such materials is hampered by the generally slow rate of reproduction and small seed yields of Scots pine. Higher selection intensity would require longer periods spent on crossing, which would eventually slow the breeding cycle. Utilising DNA markers this way would require a careful economic analysis that would consider the costs of genotyping, crossing work, field trials and phenotyping together with the time factor.

One potential use of DNA markers in Scots pine breeding would be to use them to improve the accuracy of breeding value predictions. In this application, marker information is used to calculate the realized relationships which equal the proportion of the genome that is IBD between two individuals. Breeding values (so called G-BLUPs) can then be predicted directly by incorporating the matrix of relationships among individuals into conventional mixed model analyses. An extension of GLUP, the so called single-step analysis simultaneously considers genotyped and non-genotyped trees and combines the additive genetic relationship matrix of the non-genotyped individuals with the genomic relationship matrix of the genotyped individuals into a joint (H) matrix (Cappa et al. 2019).

Accurate information on genomic relatedness seems to offer the most applications for future Scots pine breeding. Known genomic relationships could be used, for instance, to bridge unconnected series for a multi-environment genetic evaluation and to estimate the degree of co-ancestry and genetic diversity in breeding materials. Pedigree reconstruction by using DNA markers opens opportunities for implementing less costly breeding strategies, such as polymix breeding which replaces controlled crosses with polycross and following parentage analysis (Isik 2014). Such applications could be

particularly attractive in countries which have limited resources to implement conventional breeding schemes.

In general, DNA markers may increase genetic gain from breeding by shortening the breeding cycle (early selection), by enabling higher selection intensity (more candidates evaluated) or higher selection accuracy (through better information on Mendelian segregation). For Scots pine, the last application is the easiest one to adopt to operational breeding whereas the first application seems the least likely. In the future, we may seed more applications that benefit from phenotypic trait-marker associations. Rosvall (2011) raises an interesting concept of DNA-based kits developed for rapid assessment flowering competence, diseases resistance and winter hardening, for instance.

3.1.2 Phenotyping

Genetic evaluation in Scots pine is usually done based on phenotypic data from 10 to 20 -year-old progeny trials, collected by assessor groups of two persons. Multiple traits are screened, such as height and diameter growth, several branch traits, stem sinuosity, the frequency of ramicorns and the occurrence and severity of damages etc. Acquiring the phenotyping information for genetic evaluation is usually expensive and time-consuming due extensive field tests. The cost and errors of manually performed assessment are the main motivation to adopt more efficient phenotyping platforms.

Laser scanning by an unmanned aerial vehicle, a terrestrial scanner, or a mobile scanner seems to be the most promising technology to gather large amounts of phenotypic field data quickly and relatively inexpensively. Airborne scanning is already widely used in forestry investigations for estimation of traits such as tree height, stem volume, tree crown volume and tree species (Chen et al. 2017, Pont et al 2021). The airborne technology seems best suited to obtaining data from tree heights and canopy metrics. The use of this technology in breeding trials seems limited because the laser has insufficient penetration through tree canopies. It could still be useful for collecting tree growth data from older trials which are harder to assess manually because the trees have grown large and canopy closure has occurred.

Terrestrial scanning seems to be better suited to producing the kind of detailed information on various tree attributes (especially stem quality traits) that are usually assessed in Scots pine progeny trials. More importantly, the new technology is not limited to the traits recorded in conventional field measurements, but potentially able to provide more versatile information on the trees. For the full utilization of the potential of TLS, efficient algorithms based on sufficient reference data need to be developed.

3.1.3 Breeding techniques

Completing controlled crosses on the selected individuals is often the most tedious phase of Scots pine breeding, calling for techniques for fast and robust flowering acceleration. The main methods used include top-grafting onto sexually mature inter-stocks, gibberellin treatments and various damage-causing treatments applied to grafts of the selected trees. Sufficient flowering may be increasingly more difficult to achieve in advanced-generation candidates selected from field trials at teen-ages, calling for more efforts on research and development. Because of the genotypic variation in flowering ability, accelerated breeding usually means accepting, at least to some degree, that the mating combinations will be determined by flowering maturity rather than the breeding values of trees.

3.1.4 Mass propagation

The improved forest reproductive materials of Scots pine are exclusively produced in seed orchards established with grafts of selected trees. The method has proved to work well for Scots pine and no major changes in the main method of mass propagation are to be expected. Although vegetative mass propagation through somatic embryogenesis (propagating Scots pine by cuttings is difficult) has been demonstrated to work in Scots pine, the current protocols are not sufficient for large-scale propagation of neither mature tested trees or untested genotypes (seed embryos). Whereas the technical obstacles to the mass propagation of Scots pine using SE could be solvable, it is difficult to foresee a scenario where costly SE seedlings would have a competitive edge against sexually reproduced improved seeds and seedlings used in operational forest regeneration. It seems likely that SE will be used for specific research purposes and grafted seed orchards will remain as the predominant option of mass propagation.

3.2 Environmental and socio-economic factors

3.2.1 Environmental changes

The ongoing climate change may have a generally positive effect on the wood production of Scots pine under boreal conditions (Bergh et al. 2010). This potential is best realized by utilizing forest regeneration materials with adequate performance both in the current (survival) and the future climate (growth). A tool to assist landowners to choose the best of the currently available reproductive materials in Sweden and Finland has already been introduced for Scots pine (Berlin et al. 2019).

The general response of current breeding programs to environmental changes is to improve the overall stability and vigor of breeding materials by testing them in multiple test environments. One part of this strategy is to test candidates in warmer than average environments.

Scots pine has low nutrient demands and it naturally (supported by mycorrhization) grows on relatively nutrient-poor, dry sites. Thus, it is likely to have a small problem with sufficient drought tolerance in the boreal region. However, it might be a problem in more southern environments.

The predicted warming is likely to be associated with changes in the biotic environment of the Scots pine trees and influence them in ways that will be more difficult to predict than the changes in the abiotic environment. Whereas breeding can improve the overall vitality of trees, such improvements are gradual and too slow to be of any significance if, e.g., outbursts of new insects or pathogens attacked Scots pine stands in new favorable conditions. Such damages must be primarily dealt with pre-emptive silvicultural measures. The emergence of new type of damages might force commercial rotations to be shortened in the future. Improved materials of Scots pine play an important role in such scenarios by enabling faster growth per unit of time.

It seems probable that the potential adverse effects of global warming on Scots pine are most efficiently mitigated by a combination of breeding efforts aiming at good overall tolerance of biotic and abiotic stresses, assisted migration utilizing scenarios on the speed of warming and ensuring that the mass propagation populations are constantly recycled with the best breeding materials available at the time.

3.2.2 Societal demands

Public concerns on the effects of commercial harvests on biodiversity and carbon sequestration of forests are intensifying in many European countries. Whereas none of these concerns are specific to Scots pine or breeding, the negative views may provide harm to Scots pine breeding and restrict the deployment of improved FRM especially if the current artificial regeneration practices used in member countries became restricted or regulated through domestic or EU legislation.

Apart from the negative public image of genetically transformed biological materials, there are not strong common perceptions about genetically improved materials. Misunderstandings often seem to stem from the poor lack of knowledge regarding basic concepts and methods of breeding and qualities of improved FRM. Communication of the benefits of improved FRM in terms of genetic diversity, increased carbon sequestration, increased land expectation values, and improved tolerance of future views will be increasingly important in the future.

3.2.3 Organizational evolutions

Current breeding programs of Scots pine in Europe are organized separately within each country, and usually led by public research institutions on behalf of state and other funders. Most programs have historically relied on public funding. There are no signs of ongoing programs with different strategies, histories, breeding materials and funding sources being merged into a bigger joint program. Such a fusion would be complex to accomplish, and it would offer few obvious benefits. Well-established programs are already cooperating and there are many examples of, e.g., exchange of breeding materials. Most of the ongoing breeding programs have already undergone organizational restructuring at some stages of their history. These are difficult to predict since political decisions and factors other than the needs of breeding often play the predominant role in such evolutions.

Realizing genetic gains from breeding programs to operational plantations requires long-term breeding plans, technical resources. Because of the long-term commitment needed, the government typically plays a major role in funding Scots pine breeding programs. The whole chain also involves seed orchard management, nursery propagation and marketing of improved materials.

One potential novelty with desirable effects for many breeding programs of Scots pine and other species would be the emergence of a common genotyping service for forest trees. If the mass genotyping phase could be outsourced, it would be helpful for breeding institutions and could speed up the incorporation of genomic methods in operational breeding.

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