EUFORGEN

FOCUS ON FOREST GENETIC DIVERSITY

THEME 5

SEED HARVESTING, TREATMENT, STORAGE, AND NURSERY PRACTICES

How management practices can affect or influence genetic diversity of forest reproductive materials

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The European Forest Genetic Resources Programme (EUFORGEN) is an international cooperation programme that promotes the conservation and sustainable use of forest genetic resources in Europe as an integral part of sustainable forest management. Experts from member countries come together within EUFORGEN to exchange information and experience, analyse policies and practice, and develop science-based strategies, tools and methods to improve the management of forest genetic resources. EUFORGEN is hosted by the European Forest Institute and is funded by its member countries.
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SEED HARVESTING, PROCESSING, STORAGE, AND NURSERY PRACTICES

How management practices can affect or influence genetic diversity of forest reproductive materials

Management of forest ecosystems or forest tree populations is, by its very nature, also the management of forest genetic resources and their diversity under given landscape conditions. When establishing new forest plantations, it is crucial to follow basic silvicultural rules for the handling of forest reproductive materials (FRM) to avoid systemic losses of genes, genotypes, or families, and at the same time adaptive potential of populations. Genetic diversity is vital for continued survival of species and ecosystems in the changing environment, making it essential to develop and implement proper seed harvesting, treatment, and nursery production guidelines.

Many factors contribute to the loss of genetic diversity, such as cone, seed, and seedling processing in seed plants and nurseries, particularly those involving the sorting of seed and seedlings.

It is vital to recognize that most silvicultural, technical, and routine practices used when dealing with forest tree populations have the potential to reduce natural genetic variation. When collecting seeds, it is crucial to preserve the species-specific gene pool and prevent genetic erosion in seed lots as compared to the stand of origin. However, knowledge on genetic structure of a given stand is usually lacking.

The EUFORGEN community proposes clear guidelines, based on scientific knowledge of forest tree population genetics, for consideration by those collecting, treating, or storing seeds, as well as during nursery production processes.

01 SELECTING TREE SPECIES AND SEED SOURCES

It is important to include a variety of local tree species and their genetic diversity while approving seed sources. Not all tree species in European forests are included in the EU Council Directive (*1999/105/EC*). While the scope of the directive is not the conservation of genetic diversity, nevertheless this may lead to some ecologically important species being neglected or avoided in *in situ* and *ex situ* genetic conservation, in seed and nursery production, and in forest restoration measurements.

Seed sources in the EU Council Directive *"source identified"* might be preferred in the case of rare or endangered species, especially if there are no basic materials available in "higher categories". Many species (e.g., the wild fruit species from the genera *Pyrus*, *Malus* and *Sorbus*) do not occur in large stands and are often harvested in seed sources.

The category "source identified" is not accepted in some countries, or is only allowed for a few species, especially for those that are minor/scattered and not stand- forming. Though it is possible for just a single or a few trees to be the source of FRM in this category, regular use of seeds from the same seed source representing only one or a couple of trees in the same area may be undesirable from a genetic point of view.

02 SEED COLLECTION FROM STANDS AND SEED SOURCES

When harvesting FRM, it is crucial to preserve the species-specific gene pool and prevent genetic erosion in seed lots as compared to the stand of origin. High genetic variation can ensure better adaptability and stability for forestation. However, in the case of basic material included in the category "source identified", the lack of information about quality traits and genetic composition is a disadvantage in multifunctional forestry where wood quality is important.

The mast years

Seed collection should be carried out during the mast years when almost all or most of the trees participate in reproduction. Avoid collecting small amounts of seeds from a reduced number of neighbouring trees while the accessible number of maternal trees during the mast year is much bigger.

Stand size, number and distribution

The number, size and structure of forest stands used for seed harvesting, as well as the number of maternal trees harvested per stand determines the genetic diversity in the next generation stand. Minimum population size is defined in FRM laws or decrees in many European countries.

The population size for stand-forming tree species depending on the country and species, ranges from 40–70 individuals taking part in the reproductive process, and for other target species from 10–40. Preservation of the gene pool of the maternal stand in a seed crop is relevant, with minimum numbers of parental trees, as mentioned above. Hypothetically, genetic diversity loss is around 1–2.5%, but as the number of breeding individuals in the population is usually less than the number of individuals in the population, genetic diversity loss may be higher.

If collection is especially dedicated to ex situ genetic conservation purposes, the minimum number of fruiting maternal trees might even reach 100.

Ideally seeds should be collected from maternal trees distributed randomly in a stand to respect its genetic structure and to collect seeds from unrelated individuals. Seeds should be collected from all maternal trees in equal proportions.

Some experimental and simulation studies suggest that a minimum of 25–50 maternal trees may be enough to preserve genetic diversity of the source stand. Such collection may lead to the capture of most of the forest tree populations' genetic diversity, ensuring seed collection from trees carrying rare alleles. If this number is not reached in a given harvest year, it is recommended to mix seeds with those from other genetically similar populations, or with a seed harvest from a year attaining a larger number of individuals. However, the proportion of crop trees changes from year to year and, consequently, the genetic composition of seed lots from the same stand differs annually. Moreover, the degree to which the gene pool of a maternal seed stand is reproduced in the

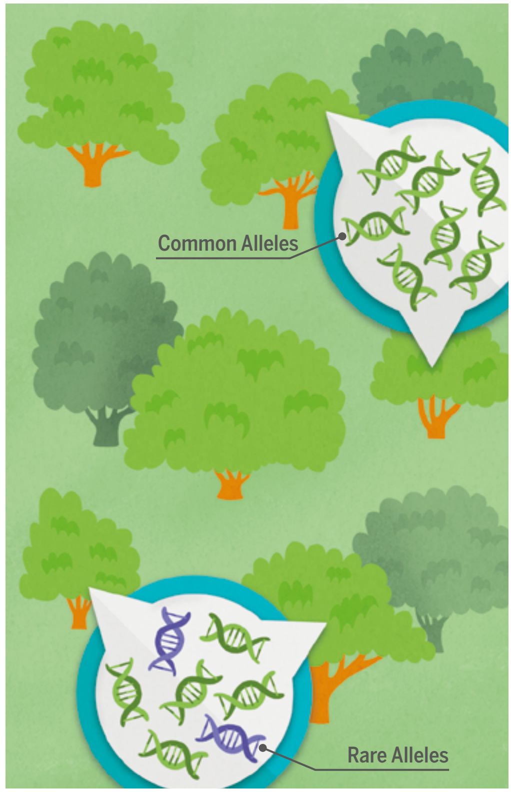

Fig. 1: A minimum of 25-50 maternal trees may be enough to preserve genetic diversity of the source stand, leading to the capture of most of forest tree populations' genetic diversity, ensuring seed collection from trees carrying rare alleles.

collected seed lot depends on mating patterns, which in turn are determined by many factors (e.g., spatial genetic structure, stand density, clonality, pre-zygotic incompatibility and flowering phenology).

Scattered or rare tree species

In the case of scattered or rare tree species, mixing seeds collected from single trees or groups of trees from the whole region of provenance might be beneficial for securing genetic diversity. Nevertheless, knowledge of the existence of clonal structure and spatial genetic structure must be considered to avoid excessive sampling of the same or closely related individuals, e.g., *Prunus avium*.

It is usual to mix seed lots from sources whose genetic value is unknown or for those which are geographically separated meaning that gene flow is limited or does not exist at all. Knowledge on the degree of parental balance is sometimes needed to proceed to proper collection of seeds (e.g., genus *Populus*) as well as the degree of hybridization with other, usually non-native species (e.g., *Larix kaempferi*, species of the genera *Sorbus*, *Abies*, *Populus* etc.), or within native species e.g. species of the genus Quercus where the levels of interspecific gene flow is high, and seeds are difficult to distinguish.

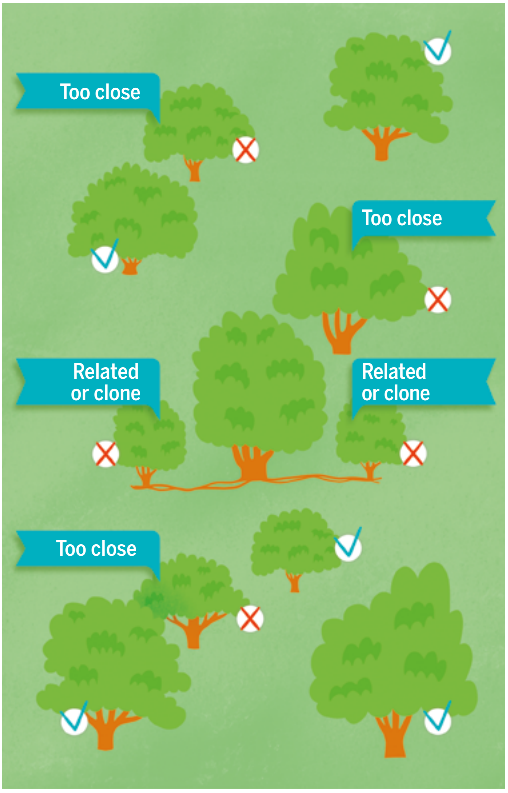

Fig. 2: Forest stand of a scattered tree species with clones and related trees.

Seed collection techniques

Different techniques can be used depending on tree species, kind of seeds (cones, fruits), tree height and crown shape, etc. In principle, the technique used is unlikely to have any detrimental effect on the genetic variation of the progeny.

Note: finding stand with the sufficient number of individuals may be difficult in the case of stand-forming species which do not create large stands (e.g. genera *Larix*), or for species which have been under huge biotic disturbances within the last decades (e.g. genera *Ulmus* or *Fraxinus*).

Nevertheless, labour-intensive and costly collection practices risk disregarding collection rules defined by legislation or guidelines, in particular when collection monitoring by official bodies or competent authorities is not stringent enough. In some countries, official attention is focused on the main commercial species, while rare tree species are monitored less scrupulously. Consequently, fewer heavily fruiting trees might be felled and harvested than is prescribed (only one in extreme cases), if the crop covers the local needs, a highly unrecommended practice.

When to harvest

Appropriate harvest timing is highly relevant for genetic diversity. Harvesting seed in a narrow time frame may result in unintentional selection and may reduce genetic variation. Harvesting seed towards the beginning or end of seed maturity may also favour a certain subset of genotypes and result in genetic shifts in the collected crop. In oaks, the collection date may affect seed dryness, whether the black rot fungus *Ciboria batchiana* has already infested them, and whether germination has started, all influencing susceptibility for thermotherapy as well as the possibility to store these seeds over more than one winter. Beech nuts collected too late and over too long a time period may become infested with pests, or the natural stratification process in the forest may already start, which may have severe implications for seeds' viability in further treatment processes.

03 SEED COLLECTION FROM SEED ORCHARDS

Clonal seed orchards established from vegetative copies of phenotypically-selected or -tested plus trees generally contain many fewer genotypes than those from seed stands. However, studies have shown that genetic diversity in seed orchard crops was similar, sometimes higher, than in natural stands, and that genetic distances to source populations were small. Of course, this depends on the number of unrelated genotypes used in a seed orchard. This is rarely known by forest managers, practitioners or environmentalists, however, who often believe that the use of seeds from seed orchards must necessarily lead to a reduction in the genetic variability of newly planted forests.

Diversity in seed orchard crops depends on effective population size (that is to say, number of breeding individuals in the population), which is affected by the number of genotypes in the seed orchard, seed orchard design, fecundity and pollination from the outside. The way in which seed orchards are designed and managed should ensure maximum genetic diversity in the seed crops produced. Well-designed and managed

Fig. 3: With the right management, studies have shown that genetic diversity in seed orchard crops can be similar, sometimes higher, than in natural stands.

orchards facilitate seed collection, helping to ensure the full genetic range is collected at each harvest by 'visiting' each tree.

Regulation

Beyond the general wording of the EU Council Directive 1999/105/EC, there are no absolute legal restrictions in Europe regarding the number of clones to be harvested or seed lot size per clone. Typically, the whole seed crop is expected to be harvested. However, such restrictions have been imposed in some national regulations. In several European countries, there are simple regulations aimed at maintaining some basic levels of gene diversity in seed orchard crops. For this, cone or seed collection in seed orchards should only be allowed when more than 50 % of clones flower.

If the number of effective clones is less than 15, a coordination committee either prohibits seed dealers or seed orchard managers from collecting the cones or obliges them to mix the crop with a previous seed lot. The option of setting fixed volumes of cones to be collected from each genotype has proved unfeasible, because cone collection control would be too time-consuming, but is still recommended.

Hybridization

For hybridization seed orchards, monitoring flower development and estimating flower abundance and the flowering overlap of the two parent species' genotypes (and possible pollen contamination) is recommended. It would be wise to avoid collecting the cones if there is not a good chance of obtaining a large proportion of hybrid seeds.

Seed maturity

The level of seed maturation affects germinability, especially germination energy, and viability of the seed in storage. Immature seeds are also more susceptible to pathogenic or saprophytic fungi. The physiological maturity of seeds may also affect their germination response to different environmental conditions and change their need and response to dormancy-breaking treatments. For these reasons, high seed quality depends on the collection of seeds only upon maturity; the use of appropriate after-ripening techniques may be needed for some species.

Different genotypes, however, vary in the timing of seed maturation. Finding genetic differences may be more pronounced in extreme populations of the species distribution range, where full maturation does not always occur.

Inappropriate collection times for all clones or individual trees may result in an uncontrolled reduction in effective population size, as immature seeds lose their viability in storage and perform poorly during seedling production compared to seeds from early maturing genotypes. This leads to an unintentional reduction of the crop's genetic diversity.

New plantations from seed orchard crops

While creating new forest plantations with crops originated from seed orchards, it is important to ensure against future natural regeneration, to eliminate the risk of further erosion of diversity in the next generation. Labelling the seed source used to establish a forest plantation in forest information systems is recommended.

04 COLLECTION OF SEEDS FROM PLUS TREES

Few seed collection restrictions are applied to plus trees used as parents of families produced by open pollination in the original stands. One restriction is the number of plus trees that can be harvested; for example, in some European countries, a minimum of 10 plus trees must be harvested and the seed lots are subsequently mixed. This does not automatically imply a balance between families, but climbers usually choose trees with abundant crops; consequently, per-tree seed-lot sizes are mostly quite similar.

If the distribution of trees is to follow the requirements of the EU Council Directive 1999/105/EC, they must originate from the same seed zone (region of provenance) and altitudinal zone; this does not necessarily imply origin from the same population. The risks for the performance of the offspring itself are unlikely to be greater than in the case of other basic materials. Genetic diversity in such mixed crops may even be higher than in a seed-stand crop, as the paternal parent set is much broader.

05 SEED PROCESSING (EXTRACTION, CLEANING, AND SORTING)

Fig. 4: Illustrated flow chart showing the seed preparation process.

Extraction, cleaning, and sorting are usually part of an integrated process to prepare the seed for the market. International standards, and OECD and EU regulations, give standards for ensuring traceability and for the physical and biological attributes in marketable seed lots.

Seed family selection

During seed processing, as well as during seedling production, genetic diversity can only be lost, not gained. It is important that genetic diversity does not unintentionally decrease during the processing stages, from the original collected seed lot to obtaining pure commercial seed. However, seed processing may knowingly lead to a decrease in genetic diversity by reducing the presence of some families in the seed lot. Such intentional influence on genetic diversity should be in line with regulations. Seeds of different families may differ in morphological and physiological characteristics and some families may be subject to selective pressures, which means they may be discarded from the final seed lot. Seed handling does not inevitably lead to lossof genetic diversity, but it is important for the seed practitioner to ensure they use methods that reduce these losses.

Preparing the seed lots

- Seeds must be cleaned of debris and inert material, and the number of dead or damaged seeds needs to be kept to a minimum.
- The technical process must be appropriate for the character of the seed, so as not to reduce the viability of the seeds.
- To preserve genetic diversity, the different parts obtained at the end of a sorting, grading or fractioning process must be mixed.

Seed size: The smallest or largest viable seeds are sometimes removed from seed lots and may be discarded or used separately, because homogeneous sized seeds are often preferred in seedling production to ensure the effective functioning of sowing machines. As seed size may affect germination rate and the size of seedlings, small and large seed are sometimes considered troublesome in nursery production and are thus excluded from the further production process.

Mixing: To ensure that a seed lot is well-mixed, it is often necessary to sort and to separate seeds using different grids of varying sizes. However, to ensure healthy and vital seeds of different sizes are achieved after the grading and fractioning process, seeds should finally be mixed to avoid the loss of the sample's genetic diversity. This is vital if the seed lot is going to be used for both in *in situ* and *ex situ* conservation purposes.

Seed grading and calibration: In some cases, seeds are graded/calibrated for standardization or containerized seeding, at the request of nursery staff, which could reduce genetic diversity.

It is important to highlight the difficulty in mixing seeds of some big-sized species, such as those from oaks, beech and chestnut. Due to homogenization, the marketed seed lot may include a smaller number of genotypes compared to the collected number. Moreover, other nursery activities, such as plant grading or culling, could further reduce diversity.

Homogenous seed sub-lots: From a commercial point of view, seed companies may not only favour removing the smallest and biggest seeds, as described above, but also choose to sort seed from a single seed lot into weight or size fractions. This may optimize economic gain when marketing the seed by offering very homogenous seed sub-lots based on size or weight. The resulting seed lots would have differing properties and vigour, and potentially reduced genetic diversity or a different genetic profile, but they would still be identified with the same Master Certificate Number. It is questionable if such seed fractioning would be in line with the EU Council Directive 1999/105/EC.

Increasing genetic diversity of seed lots: Mixing seeds from two or more seed stands within a region of provenance, or those collected from the same basic material in different years, could be the easiest way to increase the genetic diversity of these marketed seeds. The genetic structure of seeds (genetic factors influencing seed quality and characteristics) is one of the most important components affecting the seed storage process. This is also valid for seed maturity, with mature seeds faring better in storage than immature ones.

06 STORAGE AND STRATIFICATION

Fig. 5: Stratification is one of the most effective methods to break seed dormancy for certain species, allowing seeds to germinate more rapidly and in higher ratios, and maintaining a higher portion of the genetic diversity.

All storage factors (moisture, temperature, duration, and seed maturity) affect genetic diversity of a seed lot by maintaining or reducing seed viability.

Seed germination capacity

Preserving seed germination capacity in the storage process is important for preserving genetic diversity. A variation in viability may eliminate seeds of low relative viability. This is important in the case of long-term storage, or even short-term storage for recalcitrant seeds. Treatments to break dormancy may also induce such irregular seed survival.
A loss of viability in storage will decrease the seedling number and could therefore narrow genetic diversity of the seed lot.

Chemical treatment

Chemical treatment of seeds prior to storage can be performed either to prevent the development of pests and diseases during storage, or to influence

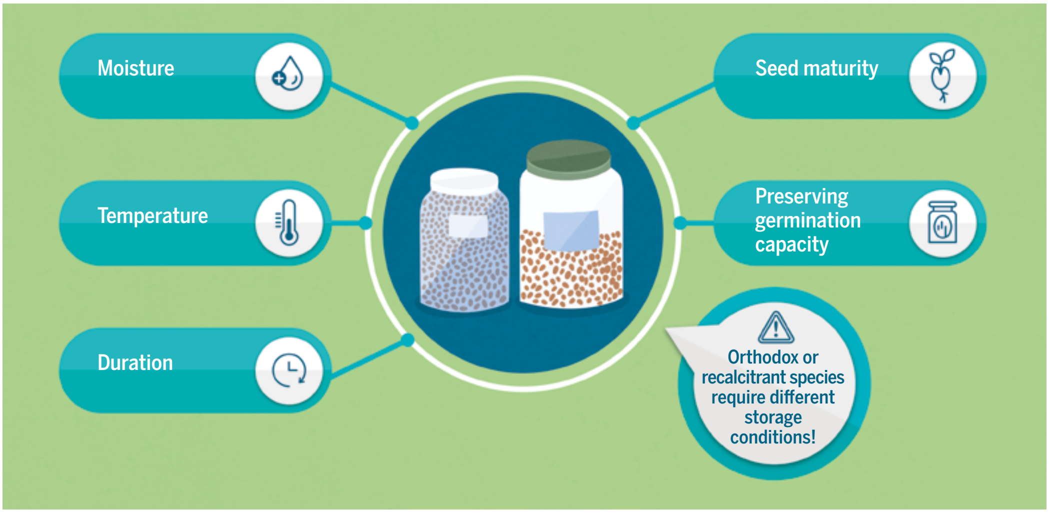

Fig. 6: Storage factors that affect genetic diversity.

their dormancy. Disease prevention or control treatments with chemicals vary, depending on whether the seeds belong to an orthodox or recalcitrant group of seeds. Applying chemicals can prevent germination during storage or neutralize seed dormancy and stimulate germination. Some chemicals used to improve germination (e.g., inorganic salts, fertilizers, growth stimulators and herbicides) can act dually depending on the dosage: low concentrations can stimulate, and high dosages can inhibit germination. Despite the chemical treatment applied, it is important to ensure that genetic diversity is maintained within each seed lot (e.g. some genotypes can be sensitive to chemicals).

Storage conditions

Storage procedures differ among tree species depending on whether they are orthodox or recalcitrant. For taxa with recalcitrant seeds, which do not withstand drying during seed storage, the procurement and handling must be organized differently to species with orthodox seeds tolerant to drying.

Recalcitrant species such as oaks, chestnuts, hazels, and sycamore: Moisture levels must be maintained above the threshold for vitality. Scientific studies indicate moisture levels of 40 - 48 % for oaks and 24 % for sycamore. However, such high-water content favours development of seed diseases and enhances respiration of seeds which can affect their vitality. Therefore, storing recalcitrant seeds needs two constraining conditions: maintaining a relatively high level of seed humidity, whilst minimizing metabolic processes. These opposing conditions can be fulfilled by storing seeds at low temperatures (around 0°C) together with sufficient ventilation.

Orthodox species: Most of the orthodox seeds can be stored at low temperatures and hermetically sealed at a low percentage of humidity for many years.

Stratification

For many temperate tree species, stratification is one of the most effective methods to break tree seed dormancy, which allows seeds to germinate more rapidly and in higher ratios. Response to stratification is under strong genetic control. Although germination capacity may not change, germination speed clearly increases after stratification, while damaged or weak seeds could easily deteriorate during stratification. There is evidence that stratification may help to ensure that the genetic diversity of the original seed lot is not reduced in the germination phase. In some cases, pre-chilling may enhance germination capacity in families, but cannot eliminate family-level variation.

07 SEED QUALITY TESTING

In germination tests, it is important to obtain homogenous and reliable results, which reflect the genetic diversity of the seed lot in question. Germination tests should be conducted using pure samples obtained through purity tests. Samples should be prepared with randomly selected seeds from completely mixed test pieces, otherwise the germination percentage and energy of the seed lots may be wrongly estimated due to variation in germination capacity between tree families and individuals. Seed lot selection based on germination test results may only narrow the genetic diversity of FRM.

08 NURSERY PRACTICES

Nursery production methods may have an impact on genetic diversity of seedlings lot. Irrespective of the production method—bare root or containerized—genetic diversity is maintained or lost in the various nursery production phases and affected by the level of skill with which growth measures are applied.

Choice of seed

The seedling production chain, originating either from seeds or vegetatively, may narrow the genetic diversity of the FRM finally reaching regeneration or afforestation sites. The effects of nursery practices on seedling survival and genetic diversity of cultivated forests may be noticed after out-planting, as production practices

may, for example, affect the growth rhythm of seedlings.
In addition, the desire for both uniform germination and seedling morphology in practical seedling mass production encourages limited genetic diversity. Seed lot price and germinability are often the key attributes affecting choice of seed lots for seedling production, irrespective of production methodology (bare root versus containerized seedling production) and tree species. Also, legislation and regulations on provenance selection in forest regeneration drive the choices of seed lots in nurseries: seedlings with suitable origins for a given area must be produced. The choice of seed lot for nursery production has genetic implications. High germination capacity originating from genetic factors will boost the chance of superior families participating more effectively in seedling crop, while problems in germinability originating from processing conditions could lead to elimination of those superior families. Selection of a seedling in containerized seedling lots, where multiple seeds germinate in a single cell, leads to the unintentional reduction of genetic diversity in comparison to the original seed lot.

Temperature effects on germination

Genetic diversity can be reduced in a seed lot as fixed temperatures in greenhouses or tunnels can be optimal for the germination of seeds from certain families only. Effects on other families include slow germination, or even secondary dormancy which will prohibit germination until the dormancy is broken, despite otherwise favourable conditions. These genotypic differences in germination response can also result in differences in seedling emergence and development in a seedling crop.

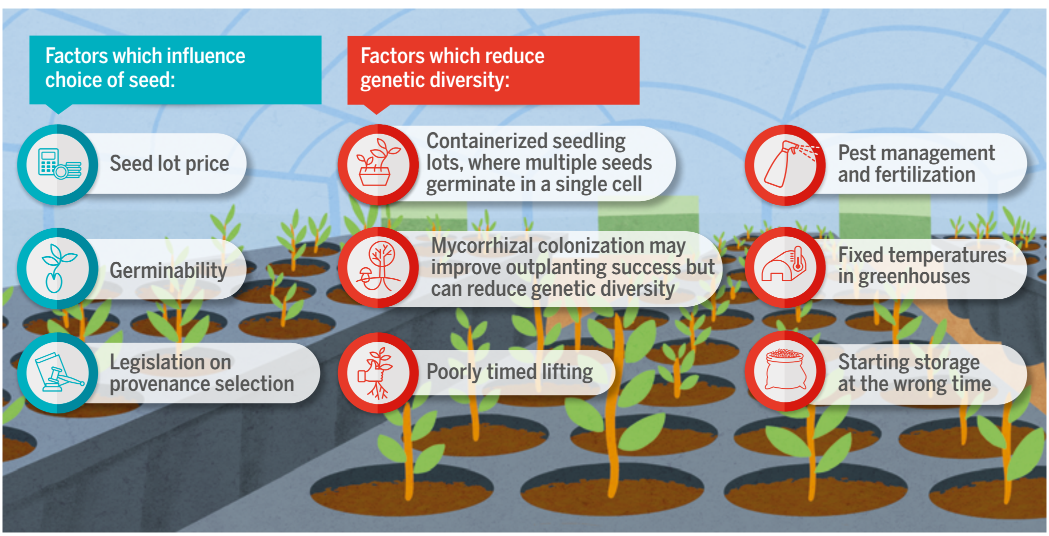

Fig. 7: The choice of seed lot for nursery production has genetic implications.

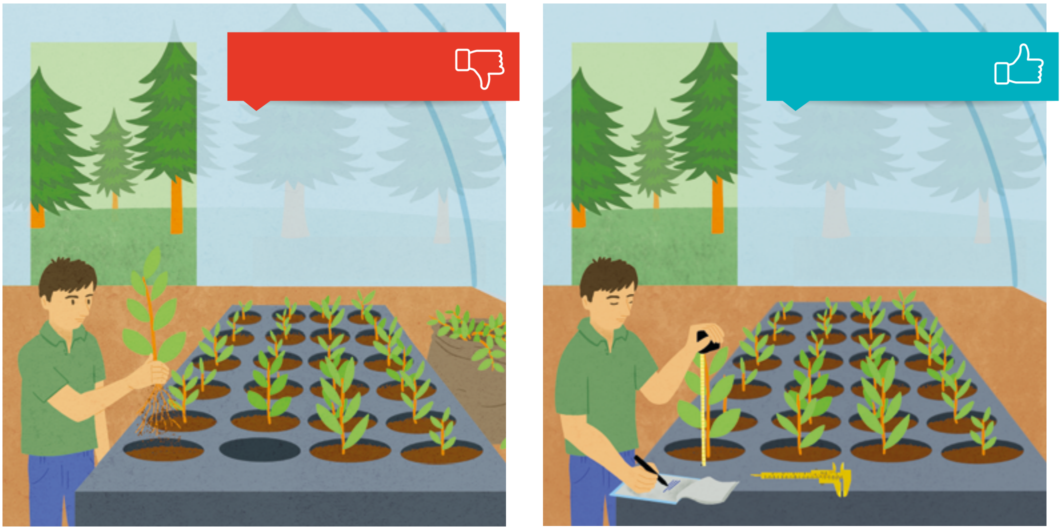

Fig. 8: Grading or culling practices based on size (seedling height, stem diameter, etc.) are likely to influence the genetic composition of the seedling lot.

Such directional selection may cause unintentional underrepresentation of some families in seedling crops.

Pest management and fertilization

Similar reduction of genetic diversity may happen when implementing pest management and fertilization, as different clones and provenances may vary in their susceptibility to plant diseases and other pests during seedling production or use of fertilizers.

Nursery techniques

Due to undercutting and transplantations, increasingly common treatments in nursery production in Europe, seedlings develop a shorter taproot and more intensively developed lateral and hair root system, and are expected to have higher survival rates after out-planting. Under optimal conditions, these treatments typically result in a 5-20 % loss of seedlings but selection on the seedlings' gene pool can be asymmetric, especially if conditions during the treatments are sub-optimal. Both nursery techniques are often optimized for profitability which might also have genetically negative selection effects on seedling lots.

Many nurseries try to sell 1-year-old seedlings, thereby selecting the best growing seedlings and leaving the 'waste products' to grow one to two seasons more in the nursery. Environmental selection pressure on seedlings which are finally out planted in the field is also of great importance. Local conditions of forestation sites might be a strong selection factor in the seedlings' gene pool; therefore, survival rate is a crucial factor in artificial regenerations.

Fungal symbiosis

Each tree species forms a symbiosis with specific fungal species, but host trees may alter their symbiotic partners as they age. Mycorrhizal mycelia enable uptake and re-translocation of water and nutrients to and among plants and receive and re-translocate plant photo-assimilates within their common mycelial networks. Compared with non-inoculated plants, seedlings of some species inoculated by mycorrhizal fungi in the nursery have been shown to be more resistant to water stress, frost, and damage by deer, as well as being less susceptible to *Heterobasidion* root rot and colonized more quickly by native fungi, thus adapting more quickly to the planting area.

Different species and strains of fungi have been shown to be useful in different ways when in symbiosis with different species, populations, or clones of forest trees, influencing the performance in growth, size, and phenology of seedlings in the nursery. The level of mycorrhizal colonization in the seedlings—affected by the host genotype composition—may alter the morphology and other attributes of seedling lots and subsequently the grading of the seedlings in culling, as well as success in establishing them after out-planting. Finally, this may lead to the reduction of genetic diversity of the seedling lot.

Grading and culling

Grading or culling practices based on size (seedling height, stem diameter, etc.) are likely to influence the genetic composition of the seedling lot: culling small—but otherwise healthy—seedlings can cause unintentional reduction in genetic diversity.

Storage

Certain measures taken prior to storage—in addition to the actual storage conditions—may ensure viability during storage and increase out-planting success. The timing of lifting of the seedlings before storage, their stress treatment, dipping the roots of in commercial hydrophilic gel, or the timing of seedling storage are crucial for storage success; more importantly they may alter the genetic composition of a seedling lot. Growth cessation and frost hardiness development is under genetic control and thus starting storage at the wrong time, especially freezer storage, may negatively affect certain genotypes. In bare root nurseries, poorly timed lifting, which precedes storage, may also affect seedling survival and impact genetic structure in seedling lots.

FIND OUT MORE!

These guidelines and recommendations have been synthesized from the EUFORGEN publication:

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