

III.

Innovative approaches in forest restoration

Genetic Quality of Forest Reproductive Materials in Land Restoration Programmes

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Introduction

The choice of plant reproductive materials for restoration programmes include seeds and fruits, whole plants and parts of plants to be used as cuttings for vegetative propagation. The choices made are – or should be – based not only on external aspects but also on genetic characteristics. External quality could be easily evaluated by the user, but genetic quality depends on factors such as genetic diversity, selection criteria for different traits, and a number of biophysical components where the material was collected, none of which are directly observable. The user, therefore, has to rely on the information provided by the collector, supplier, marketing company, or some control authority in charge of the application of the regulation on marketing of the plant reproductive material. The EU scheme on marketing of forest reproductive material is applied to 47 species (or genera) in

TABLE 1. Species under regulation by the Directive 1999/105/CE on marketing of forest reproductive material.

<i>Abies alba</i> Mill.	<i>Larix decidua</i> Mill.	<i>Prunus avium</i> L.
<i>Abies cephalonica</i> Loud.	<i>Larix x eurolepis</i> Henry	<i>Populus</i> spp.
<i>Abies grandis</i> Lindl.	<i>Larix kaempferi</i> Carr.	<i>Pseudotsuga menziesii</i> Franco
<i>Abies pinsapo</i> Boiss.	<i>Larix sibirica</i> Ledeb.	<i>Quercus cerris</i> L.
<i>Acer platanoides</i> L.	<i>Picea abies</i> Karst.	<i>Quercus ilex</i> L.
<i>Acer pseudoplatanus</i> L.	<i>Picea sitchensis</i> Carr.	<i>Quercus petraea</i> Liebl.
<i>Alnus glutinosa</i> Gaertn.	<i>Pinus brutia</i> Ten.	<i>Quercus pubescens</i> Willd.
<i>Alnus incana</i> Moench.	<i>Pinus canariensis</i> C.Smith	<i>Quercus robur</i> L.
<i>Betula pendula</i> Roth	<i>Pinus cembra</i> L.	<i>Quercus rubra</i> L.
<i>Betula pubescens</i> Ehrh.	<i>Pinus contorta</i> Loud.	<i>Quercus suber</i> L.
<i>Carpinus betulus</i> L.	<i>Pinus halepensis</i> Mill.	<i>Robinia pseudoacacia</i> L.
<i>Castanea sativa</i> Mill.	<i>Pinus leucodermis</i> Antoine	<i>Tilia cordata</i> Mill.
<i>Cedrus atlantica</i> Carr.	<i>Pinus nigra</i> Arnold	<i>Tilia platyphyllos</i> Scop.
<i>Cedrus libani</i> A.Richard	<i>Pinus pinaster</i> Ait.	
<i>Fagus sylvatica</i> L.	<i>Pinus pinea</i> L.	
<i>Fraxinus angustifolia</i> Vahl.	<i>Pinus radiata</i> D. Don	
<i>Fraxinus excelsior</i> L.	<i>Pinus sylvestris</i> L.	

all the European countries (Table 1) when used for forestry purposes, but each country can regulate additional species in their territory (e.g., Spain has added 20 Mediterranean species). There are many other species, which could not be under regulation, but the principles of this scheme are valid and can be considered when selecting propagation material for restoration programmes. Therefore, in those cases, it would be desirable to precisely define the type and characteristics of the basic material from which the reproductive material should be collected.

The basic principles on which the regulation is based can be summarised as follows:

- a) The existence of diversity at different levels (species, populations, and individuals).
The genetic diversity among species is easily recognised, but differences among populations are in some cases neglected even they are large for many important traits (Fig. 1). Langlet (1971) presented an historical overview on the differentiation among populations in forest trees, and this genetic variation can be influenced by different life-history traits of the species under consideration (Hamrick 1992). The genetic differences among individuals are easily recognised in many forest species, and especially in those of commercial interest with different breeding programmes (*Populus*, *Salix*, *Castanea*, *Juglans*, *Pinus*, *Picea*, among others).
- b) The importance of some characteristics of the basic material in the future performance of the plantations, specially the origin, the diversity and the selection processes to which populations have been submitted.
- c) The difficulty in assessing such characteristics, and the necessity of an efficient control system at the European level. This control system covers the entire production chain, from seeds to plants, in order to avoid fraud in the commercialization process.



Provenance: *Coca*, Spain



Tamjout, Morocco



Leiria, Portugal

FIGURE 1. Differences among three maritime pine (*Pinus pinaster* Aiton) populations under common garden experiments (in a provenance test conducted in Cabañeros, Ciudad Real).

The genetic quality of the reproductive material should, therefore, ideally be based on sound knowledge of the genetic basis of the processes of selection and characterization of the plant materials under consideration, as well as by evaluation of the material under common garden conditions. This of course is not always possible.

Genetic basis of breeding

The use of species with advanced breeding programmes (i.e., those with several breeding generations) in restoration is usually of limited importance, but we have to understand the principles of selection for the choice of the best reproductive material.

The genetic basis of breeding has been described in different papers (e.g., Zobel and Talbert 1988, Alía et al. 2005). The breeders depend on the existence of phenotypic variability among the individuals, the degree of genetic control (heritability) of the traits of interest, the selection (based on phenotypic or genetic evaluation) of some individuals from the population with desirable properties, and possible crosses among those individuals to advance in breeding (Fig. 2).

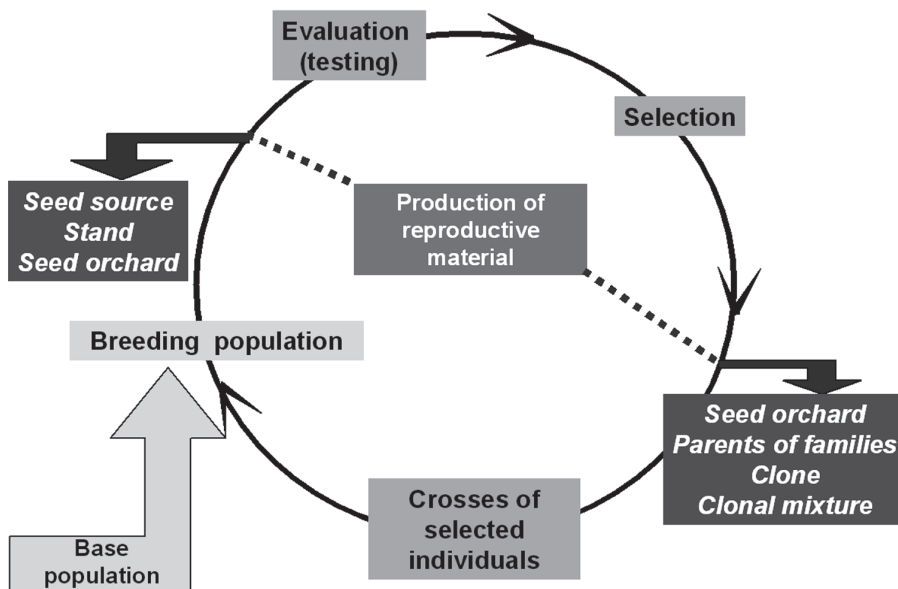


FIGURE 2. Different stages in a breeding cycle.

To be effective, breeding has to be focused on traits of importance, with high heritability (Table 2) and low correlation with undesirable traits. For tree taxa of high economic or social importance, e.g., *Populus*, *Juglans*, *Castanea*, *Prunus*, *Ulmus*, *Pinus*, *Picea*, the most important traits are those related to growth, drought or frost tolerance, and wood properties.

Origin and Regions of provenance

One of the central concepts for the use of forest reproductive material is the *origin* of the material (see Box 1 for definitions, and Table 3). The origin determines many important characteristics related to the future performance of the plants (e.g., traits related to adaptation to climate, traits related to adaptation to biotic or non biotic factors, growth, survival), as a result of the evolutionary factors that shape the genetic structure of the populations in the forest species. Many studies have demonstrated a high level of

TABLE 2. Heritability values for different traits (modified from Alía et al. 2005).

Trait	Species	Heritability
Drought tolerance	<i>Pinus pinaster</i>	High
	<i>Castanea sativa</i>	High
Phenology	<i>P. x euramericana</i>	High
	<i>Populus alba</i>	Moderate
	<i>Populus deltoides</i>	High
	<i>Populus deltoides</i>	Moderate
	<i>Castanea sativa</i>	High
Height	<i>Pinus sylvestris</i>	Low
	<i>Pinus halepensis</i>	Moderate
	<i>Pinus pinaster</i>	Low
	<i>Castanea sativa</i>	Moderate
	<i>P. x euramericana</i>	Moderate
Diameter	<i>Pinus sylvestris</i>	Low
	<i>Pinus nigra</i>	Low
	<i>Pinus pinaster</i>	Low
Wood density	<i>Pinus sylvestris</i>	High
	<i>Pinus pinaster</i>	High
	<i>P. x euramericana</i>	High
Form	<i>P. x euramericana</i>	High
	<i>Populus alba</i>	Very Low
	<i>Pinus pinaster</i>	Moderate
Branching	<i>P. x euramericana</i>	High
	<i>Populus alba</i>	Moderate

Very Low: 0-0.1; Low: 0.1-0.35; Moderate: 0.35-0.6; High: 0.6-0.9; Very High: 0.9-1.0

differentiation among populations for traits under selection (such as bud set, growth initiation and cessation, frost tolerance, and drought tolerance) (e.g., Van Andel, 1998). However, the characters related to migration, isolation, genetic drift (i.e. not under selection) present a variable level of differentiation.

Box 1. Definitions (Source: Directive 199/105/CE on marketing of forest reproductive material):

(a) **Autochthonous stand or seed source:** An autochthonous stand or seed source is one which has been continuously regenerated by natural regeneration. The stand or seed source may be regenerated artificially from reproductive material collected in the same stand or seed source or autochthonous stands or seed sources within the close proximity;

(b) **Indigenous stand or seed source:** An indigenous stand or seed source is an autochthonous stand or seed source, or a stand or seed source raised artificially from seed, the origin of which is situated in the same region of provenance.

(c) **Origin:** For an autochthonous stand or seed source, the origin is the place in which the trees are growing. For a non-autochthonous stand or seed source, the origin is the place from which the seed or plants were originally introduced. The origin of a stand or seed source may be unknown.

(d) **Provenance:** The place in which any stand of trees is growing.

(e) **Region of Provenance:** For a species or sub-species, the region of provenance is the area or group of areas subject to sufficiently uniform ecological conditions in which stands or seed sources showing similar phenotypic or genetic characters are found, taking into account altitudinal boundaries.

TABLE 3. Origin and provenance of the basic material, and the forest reproductive material obtained.

Origin	Provenance	
Basic Material	Basic Material	Reproductive material
Site A	Site A	Autochthonous (origin = provenance)
Site B	Site A	Non Autochthonous (origin ≠ provenance)
		Known origin (origin =B)
?	Site A	Non Autochthonous (origin ≠ provenance)
		Unknown origin

It is necessary to delineate regions of provenance for each species, i.e. zones with similar ecological characteristics (Box 1). These regions are the base of the marketing for source-identified and selected forest reproductive materials (see description of the categories below). Two methods (agglomerative and divisive) have been followed to establish the regions of provenance in Europe (Fig. 3), and they are available for the different European countries. The description is available in different monographs or webpages from the different designated authorities in each country (e.g., CEMAGREF 2003 for France; Martín et al. 1998, García del Barrio et al. 2001, 2004 for Spain).

- a) *Divisive method*: the territory is divided into disjoint ecologically homogeneous regions, taking into account climatic, geographical, and soil traits related to the performance of the species under consideration. This method has been applied in different European countries (see Gordon 1992, CEMAGREF 2003, García del Barrio et al. 2001, 2004). This method has the main advantage of defining the same regions for all the species under consideration, but it does not take into account some possible special characteristics of the species (e.g., patterns of genetic variation, distribution patterns).
- b) *Agglomerative method*. The stands of a species with similar phenotypic, genetic or ecological characteristics are grouped to form a region of provenance. Therefore, each species has different regions of provenance, but they describe more precisely the pattern on known variation of the species. This method can be used for species with precise information on phenotypic, genetic or ecological variation.

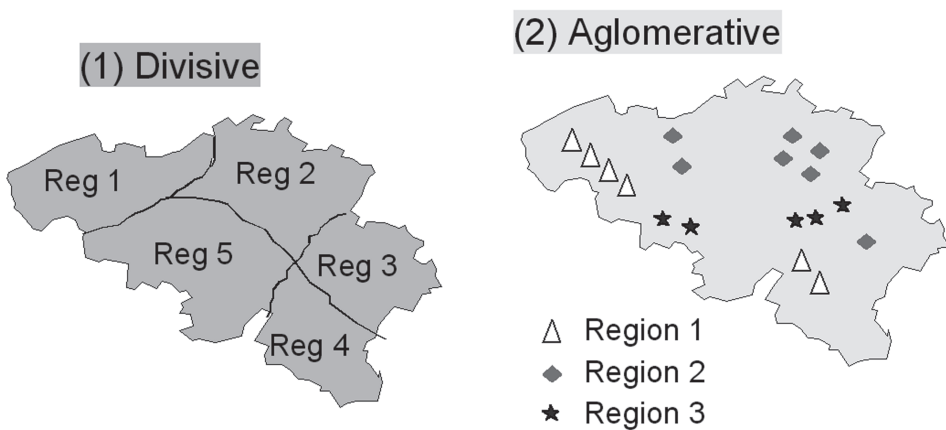


FIGURE 3. Methods applied for the delineation of regions of provenance (CEMAGREF 2003, Alía et al. 2005).

The main characteristics of a system of regions of provenance are the following:

- *The region of provenance determines the geographical limits from which reproductive material can be mixed for commercialization.* Both source-identified and selected reproductive materials have to be collected in seed sources or stands from one region of provenance. They can not be mixed with material from other regions.
- *The region of provenance simplify the marketing of forest reproductive material by identifying zones in which seed or fruits have been collected.* For a species as *Pinus sylvestris* there are 17 regions of provenance in Spain, and more than 400 seed sources or stands. Therefore, it is easier for the user to recognise the 17 regions of provenance instead each of the seed sources or stands.
- *The region of provenance simplifies the seed transfer rules in national forestation programmes.* Usually, seed transfer rules are based on information on a limited number of materials evaluated in a limited set of ecological conditions. Therefore, it is much easier to define the rules for each region instead of rules for specific seed sources or stands.
- *The region of provenance can be used for planning breeding or conservation activities.* A region, or several regions, can be combined to constitute a breeding zone in a breeding or conservation programme.
- *The practical importance for marketing is different for each region, and therefore they are not used similarly in restoration programmes.* Some regions are susceptible to be used in a broad spectrum of ecological situations, whereas some others are restricted to a local used.

Taking into considerations those different aspects, regions of provenance are the first step in the selection of the material for improvement and restoration programmes, and a deep knowledge on the ecological, phenotypic or genetic characteristics of the different regions is essential for a correct choice of the material to be used in each case. After identifying the most suitable region of provenance we have to decide on other characteristics of the material as type of basic material and on the category of the forest reproductive material.

Characteristics of basic and forest reproductive materials

Forest reproductive materials have to be collected in specific types of basic material (if the species is under regulation), in order to guarantee some genetic properties, and those basic materials have to be included in the National Registers of approved basic material for the production of forest reproductive material. The national registers are available from the different national authorities.

The EU certification scheme distinguishes six types of basic materials: seed sources, stands, seed orchards, parents of families, clones and clonal mixture (see Box 2), This is similar to other control schemes for the international trade as OECD (Organisation for Economic Co-operation and Development) and AOSCA (Association of Seed Certifying Agencies).

Box 2. Types of basic materials. (Source: Directive 1999/105/CE on marketing of forest reproductive material, adapted from Nanson 2001).

(a) **Seed source:** Seed is collected within a zone of collection called a seed source. This zone is not necessarily delineated, nor clearly identified. On the contrary, the Region of Provenance where it lies has to be clearly delineated and identified in a National Register (maps).

(b) **Stand:** It is a well delineated population of trees possessing sufficient uniformity, and referenced in a National Register.

(c) **Seed orchard:** It is a plantation of selected clones or families which is isolated and managed to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant and easily harvested crops of seed. There are two main types of Seed Orchards: i) Clonal Seed Orchards, ii) Family Seedling Seed Orchards. These last are in fact progeny tests with small plots, the trees of which are later submitted to genetic selective thinning.

(d) **Parents of families:** They are defined groups of trees (clones) producing open pollinated or controlled pollinated families. These families are afterwards mixed for production. Most often, this mixture of families is vegetatively bulk propagated (e.g.: cuttings of *Picea sitchensis* in Great Britain).

(e) **Clone:** It is a group of individuals (*ramets*) derived originally from a same single individual (*ortet*) by vegetative propagation, for example by cuttings or micropropagation. Individuals of the same clone have the same genotype, unless somatic mutation or error.

(f) **Clonal Mixture:** It is a mixture in defined proportions of initially identified clones. Usually, the ramets of these clones are mixed, bulked and so delivered for afforestation. The clonal identity of the individual ramets is therefore generally lost at the forest stage and often already at the vegetative propagation stage. In current scientific language, clonal mixtures are usually denominated as “multiclonal varieties” or “polyclonal varieties”.

The four categories of forest reproductive material depend on the selection method and the evaluation processes of the basic material (Nanson 2001, Alía et al. 2005). They have to be labelled with different colour when commercialized.

- **Source Identified (yellow label).** The reproductive material is derived from seed sources or stands from one single region of provenance. Basic materials have thus not been submitted to any selection and are only identified by the region of provenance.

- *Selected (green label)*. The basic material has undergone a phenotypic selection at the population level. It is the case of selected stands that are phenotypically superior to stands of the same region of provenance. Presently, the basic material related to this category is still representing the major part in the world, often more than 90% of basic materials in national catalogues.
- *Qualified (pink label)*. Components (trees, clones) of relevant basic materials must have undergone a phenotypic selection at the individual level. Seed orchards are the most common material in this category.
- *Tested (blue label)*. Forest Reproductive Materials produced by relevant basic materials must be found genetically superior, by comparative testing or by an estimate of the superiority of the reproductive material calculated from the genetic evaluation of the components of the basic material.

The EU certification scheme defines the types of basic material accepted for producing forest reproductive material from the different categories (Table 4). Those different reproductive materials differ in terms of genetic diversity (higher for seed sources or stands, lower for clones), genetic gain (higher for clones of clonal mixtures, lower for seed sources), and for the degree of phenotypic and genetic evaluation of the materials (higher for tested material, lower for identified materials). However, the certification scheme does not mean a scale, in which the “value” of the reproductive materials increases from source-identified to tested materials. The source-identified and selected materials are more useful than tested materials for many restoration programmes as they have a determined origin, no or slight selection and high levels of genetic diversity.

TABLE 4. Categories under which reproductive material from the different types of basic material may be marketed (EU Directive 199/105/CE).

Type of basic material	Category of the forest reproductive material			
	Source Identified	Selected	Qualified	Tested
Seed source				
Stand				
Seed orchard				
Parents of families				
Clone				
Clonal Mixture				

Use (*transfer rules*) of forest reproductive material

When deciding the best reproductive material to use in a given restoration programme it is necessary to choose among a list of materials (seeds or plants) available from different providers. These will differ in their basic offerings in term material, region of provenance and categories. It is necessary to take into consideration different factors:

- *Which is the deployment zone?* The main ecological characteristics of the area where the material will be established determine some of the properties of the material to use.
- *Which is the best procurement seed zone (region of provenance)?* To select the origin of the forest reproductive material (for source-identified and selected reproductive materials) it is possible to use information on the ecological, phenotypic and genetic information of the basic material from the different regions of provenance. However, for qualified or tested material, the origin is not so important, as the material can be the result of advanced selection programmes where the pedigree of the material is much more important to decide on its properties. Some additional principles, mainly related to the conservation of forest genetic resources, have to be considered in order to avoid endangering valuable local resources by introducing exotic material. Even when the regulation on forest reproductive material is not aimed at conservation purposes, this aspect should be considered as a general principle in all restoration programmes.
- *Do we need an increment in the mean value of some important traits? (i.e., an increment in wood production or survival).* In commercial plantations, in general, the goal is to maximize production for some characteristics. Therefore, qualified or tested materials are preferred because they have been selected for those characteristics and have been tested under ecological conditions similar to those of the plantation sites. However, when the objectives are related to ecological restoration, other features such as adaptation to some special conditions, or the use of specifically local adapted material may be the top priority. In absence of such materials, selected materials could be more suitable in such conditions.
- *Which level of genetic diversity do we need?* The genetic diversity of the material to be used is important, especially if we are interested in plantations at the long term, and where the natural regeneration can have an important role in the future. The different types of basic material can yield material with different levels of diversity, i.e., with low or very low levels for parents of families, clones or clonal mixtures.

From all these factors, we shall now focus on the relationship among deployment and procurement zones (Buijtenen 1992), as the other factors depend mainly on the specific objectives of the restoration programme. Deployment and procurement zones (region of provenance) can be the same (if using local sources) or different (if using exotic material because of some interesting traits or absence of a desirable local source).

To establish the transfer rules, there are some general patterns established as a result of many transfer experiments (see Zobel and Talbert 1988, as an example):

- Local seed sources are usually locally adapted (Kawecki and Ebert 2004).
- Local seed sources are not usually the most productive (Namkoong 1969).
- We have to take into considerations the seasonality in rainfall and temperature (or other important climatic factors), and not only the mean values.
- It is not desirable to move material from a coastal climate to a continental one, or vice versa.

However, these recommendations are too general, and it is useful to combine information on ecological similarities among deployment and procurement zones, and from concrete transfer experiments. In general, there are site-site transfer rules (among planting sites and seed sources or stands), site-region transfer rules (among plantation sites and regions of provenance) or region-region rules (among deployment regions and regions of provenance). These types of rules are summarized in Table 5.

TABLE 5. Types of transfer rules of forest reproductive material.

Use of forest	Procurement of forest reproductive material	
Reproductive material	<i>Seed source, Stand or Tree</i>	<i>Region of provenance</i>
<i>Plantation site</i>	Site - Site	Site - Region
<i>Deployment Region</i>	-	Region – Region

Depending on the category of the reproductive material, different kinds of information can be used.

Source-identified and selected material. Usually there is ecological information at the region of provenance level, and some information on the performance of a limited number of provenances in a limited number of sites. We have to rely on the phenotypic characterization of the basic material and on the ecological similarities among planting sites and regions of provenance. The most common approach is to establish some relationship among regions of provenance and planting regions for the most important species (García del Barrio et al. 2001, CEMAGREF 2003). An example of these transfer rules is shown in Table 6. When information on the performance in provenance tests is available, it is possible to use this information to establish some predictive models (e.g., Hamann et al. 2000, Westfall 1992, Parker and Lesser 2004).

Qualified material. Usually there is information on the phenotypic characteristics of the individuals, the ecological characteristics of the sites where the materials were selected, and some information (derived from the empirical experience) on the performance of the materials. This material is not linked to a region of provenance, and we have to establish the similarity between the planting site and the site from where the material was selected.

Tested material. In this case, there is information on the performance of the material on different experimental sites, including the importance of the genotype-environment interaction. It is known the region of likely adaptation within the country in which the test was carried out and the characteristics which might limit its usefulness. These characteristics can help to determine the transfer guidelines of such material. This information is available for some of the species (e.g., Padró 1992, CEMAGREF 2003).

TABLE 6. Example of transfer rules for different species in Spain (Alía et al. 1999, García del Barrio et al. 2004). For each deployment zone, the codes of the most suitable regions of provenance and the level of recommendation, from low (light green) to very high (dark green), are included.

Deployment Zone	Procurement zone (Region of Provenance)					
	<i>E. sylvatica</i>	<i>P. halepensis</i>	<i>P. nigra</i>	<i>P. pinaster</i>	<i>P. pinea</i>	<i>P. sylvestris</i>
1				1a		10
2	1			1b		10
3	2-4			1a		10
4	1-2-5		7			10
5	3-5		2-7	2-9		1-8-10
6	7-8			1a		2
7	5-6-8	4	3	3-9		2-3-4
8	9-11-12		2-3			3-4-5-6-7
9	10-12-13-14	3-4	1-2-3-4-5		7	3-4-7
10		1-2		6-9-C	6-7	
11		3-6	3-4-5	6-B	6-7	4-7-16
12		3-5-6-14				
13		5-9-14	7	10		14-15
14		4-6-9	5-7	9		4-8
15	16-17		3-10	9		8
16		9-14	5-7-10	8-9	1	8-10
17		9-14		2-8	1	
18				1a-4-6		10
19		14	8-9	6	2	10-11
20	18	14	8-9	6-7	2	9-10
21		5-7-9	7	11-12		12-14
22		7	7	12-13		12
...						

Conclusions

The genetic quality of the reproductive materials used in restoration programmes determines some of the future characteristics of the plantations, as variables related to adaptation, genetic diversity and growth have a strong genetic determinism.

The EU scheme on marketing of forest reproductive material is applied to 54 forest species, but the principles of such scheme are valid for other species relevant in restoration programmes. In those cases, it would be desirable to precisely define the type and characteristics of the basic material from which the reproductive material must be collected.

Therefore, when choosing reproductive material, we should take into consideration different factors as the characteristics of the basic material from which the material was collected, the category of the reproductive material, the region of provenance and origin of the material, and the information on the seed transfer rules available for the species or materials under consideration. Most of these factors are related to the future adaptability of the tree plantations, and many studies are now focused to provide information on such characteristics. The managers in charge of planning a restoration program must analyse the different materials available (species, regions, of provenance, type of basic material, category of the reproductive material) in order to select the most adapted to the planting conditions. It is necessary to take into consideration that this material can also affect the genetic diversity of the present and future forests.

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