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11

Multiple Approach for Plant Biodiversity Conservation in Restoring Forests

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1. Introduction

The current extinction crisis requires dramatic action to save the Earth' s biological diversity. In the mid-1980's the word "biodiversity" was coined to catch the essence of research into the variety and richness of life on Earth, that is, the variety of life expressed at many levels (Wilson & Peter, 1986). These levels include the genetic diversity within species as well as the array of genera, families, and still higher taxonomic levels that, taken together, comprise communities of organisms within particular habitats and physical conditions that form entire ecosystems. It is widely demonstrated that more species contribute to a greater ecosystemic stability. Moreover, individuals, populations and ecosystems are tightly linked and interact to maintain landscapes, large socio-economic systems and man's health. As a consequence, biodiversity maintenance is fundamental for the planet life, and should be carried out with "passive" conservation measures implemented with "active" procedures using the most recent progress in technique and policy. In this context, reforestation programmes have to be considered as dynamic actions devoted to the biodiversity conservation toward the recovery and/or the enlargement of such areas essential for coenosis' evolution. This concept of reforestation is relatively new and still has difficulty to be established.

Most conservation biologists recognize that although we can not save everything, we should at least ensure that all ecosystem and habitat types are represented within regional conservation strategies that have been applied at a number of geographical scales, from single watersheds to entire continents (Hummel, 1989; Eriksson et al., 1993; Caldecott et al., 1994; Krever et al., 1994; Noss & Cooperrider, 1994; BSP et al., 1995; Dinerstein et al., 1995; UNEP, 1995; Ricketts et al., 1999; Abell et al., 2000).

Forests are the single most important repositories of terrestrial biological diversity. They provide a wide range of products and services to people throughout the world. Forest trees and other woody plants help support many other organisms, and have developed complex mechanisms to maintain high levels of genetic diversity. This genetic variation, both inter- and intraspecific, serves a number of fundamentally important purposes. It allows trees and shrubs to react to changes in the environment, including those brought about by pests, diseases and climatic change. It provides the building blocks for future evolution, selection and human use in breeding for a wide range of sites and uses. And, at different levels, it supports the aesthetic, ethical and spiritual values of humans. Forest management for

productive and protective purposes can and should be rendered compatible with conservation through sound planning and coordination of activities at different geographical levels. Accordingly, the conservation of these resources should be seen as an attempt to preserve groups of genotypes or populations, and their various combinations of genes (Gregorius, 1991). Therefore, the aim of forest resource management is to maintain conditions in which the genetic makeup of a species can continue to evolve in response to changes in its environment (Eriksson, 2001). At the same time, management for conservation aims at reducing the rates of genetic erosion (FAO et al., 2004).

Different conservation strategies and practices have been developed. *In situ* ('in place') conservation implies the continuing maintenance of a population within the environment where it originally evolved, and to which we assume it is adapted (Frankel, 1976); this type of conservation is most frequently applied to wild populations regenerated naturally in protected areas or managed forests, but can include artificial regeneration whenever planting or sowing is carried out, without directional selection, in the same area where the seed was collected. *In situ* conservation in general has the advantage of conserving the function of an ecosystem rather than just species. This means that *in situ* programmes for conservation of selected target species often result in valuable conservation of a number of associated animal and plant species (Thomson et al., 2001). *Ex situ* ('out of place') conservation measures are mainly concerned with sampling and maintaining as much of the genetic variation as possible that resides within and among populations of selected target species. *Ex situ* conservation requires substantial levels of human intervention, in the form either of simple seed collections, storage and field plantings or of more intensive plant breeding and improvement approaches. Unlike breeders of agricultural crops, forest tree breeders cannot rapidly produce new varieties, nor can they quickly breed for new variations among populations. Therefore, the existing genetic diversity among populations is important and fundamental to the conservation of forest genetic resources, particularly as it may relate to maintaining genetic diversity in viable populations in the long term. This also suggests that special attention must be given to conserving intraspecific genetic variation in peripheral or isolated populations, as they could possess higher levels of characteristics such as drought resistance, tolerance to various soil conditions (Stern & Roche, 1974), or features that will help to protect them from future climate change (Muller-Starck & Schubert, 2001). The important features of an *ex situ* conservation programme for any particular species are: to be an important backup measure should other *in situ* conservation means be unworkable or unavailable, to ensure that a wide range of the diversity (phenotypic and genotypic) available in a species is conserved, and to manage the regeneration of the species outside its original natural range (provenance) in a more controlled way (which is likely to further develop the population(s) for use or conservation) (Amaral & Yanchuk, 2004).

Recently, a European funded project, EUFGIS (European Information System on Forest genetic Resources) established a web-based information system to serve as a documentation platform for national forest genetic resources inventories and to support practical implementation of gene conservation and sustainable forest management in Europe (further information at http://www.eufgis.org). The main purpose was to assess pan-European minimum requirements and data standards for the dynamic gene conservation units of forest trees, i.e. selected areas which emphasizes the maintenance of evolutionary processes within tree populations to safeguard their potential for continuous adaptation.

Those tools are adopted in programmes devote to afford issues such as the conversion of forest land to other uses. Increasing pressure from human populations who aspire to higher standards of living, without balancing the sustainability of resource utilization underpinning such developments, raises concerns in this regard. It is inevitable that changes of land use will occur in the future, but such changes should be planned to help ensure that the complementary goals of conservation and development are achieved. In recent times there has been a growing awareness in this topic, and the research community is looking for moving away from the dominant focus on deforestation and resources' conservation to examine the patterns and the processes associated with reforesting landscapes (Rudel, 2005). Developing a more comprehensive understanding of the factors that can help to promote reforestation is therefore critical, if we are to increase useful policy interventions to arrest or reverse deforestation, and encourage forest regrowth. Yet, it is important to recognize that forests are embedded within larger-level ecological, socio-economic and political settings, which have the capacity to significantly influence outcomes. Thus, discussions of context (biophysical, geographic, ecological, socio-economic and institutional) are essential to the development of our understanding of this area of study (Nagendra & Southworth, 2009). This implies awareness of the availability of efficient tools to comply with traditional management strategies, as well as action plans and guide lines at large scale. Under these circumstances the Council of the European Union promoted a legislative tool in 1999 that recognized social, economic, environmental, ecological and cultural functions of forests. Both the restocking of these forests and new afforestation require a sustainable forest management in relation to the Forestry Strategy for the European Union, that include the use of reproductive material which is genetically and phenotypically suited to the site and of high quality (European Council, 1999). In this context, the definition and delimitation of Regions of Provenance have been proposed as fundamental to select reproductive material and to approve basic material with highest possible standards.

However, an improvement of genetic knowledge about forest plants is surely required to accomplish the requirements of the Directive, and should also contribute to better define what inter- and intra-specific biodiversity is. One of the latest standardized molecular approach is DNA Barcoding (Hebert et al., 2003) that identifies living organisms by joining specific sequences of DNA and electronic information retrieval. Biodiversity characterization and improvements in genetic knowledge would be two of the main benefits of the widespread application of Barcoding, in terms of speed, low cost, reliability, and improved resolution power. Besides taxonomy, a powerful research complement for molecular ecology, diversity studies and population genetics is clearly to be expected. DNA Barcoding may lead to many useful applications in forestry sciences, such as community ecology (to describe plant-animal interactions and vegetation dynamics/changes), biodiversity surveys (aimed at habitat and species protection), silviculture (to assess forest regeneration), and nursery activities and market regulation (to establish wood, secondary products and germplasm certification). Conversely, it must be emphasized that some species-rich tree genera may prove very difficult to barcode, especially those in which species circumscription is affected by complicated taxonomies, biogeographies and/or reproductive biology.

Since plant biodiversity is strictly related to natural restoration and rehabilitation of ecosystem functions, with respect to its health, integrity, and sustainability, all the tools mentioned above are linked to the reforestation techniques proposed by scientists and

experts from decades. New insights both in theoretical and in practical actions have been developed as innovative methods to foresters and ecological specialists. Among these, the Miyawaki method based on the vegetation-ecological theories, seems to be a reliable approach that include the principles of self-organized criticality and cooperation theories in forest ecosystems, also fulfilling the objectives anticipated by the Regions of Provenance.
The tools mentioned above face the same issues, and try to get practical solutions for conserving and/or restoring forests. However, they approach plant biodiversity conservation in a piecemeal fashion with projects and management plans focusing narrowly on one or a small range of techniques that coincide with the responsibilities, philosophies, and capabilities of the people working in a given setting.
In this chapter we would like to propose a multiple approach as a potentially powerful system for facing the challenge of conserving, but mainly expanding forests over long time horizons. A critical review on the methods mentioned above toward an holistic point of view will be discussed. We believe that multidisciplinary would be the way to follow out, and our effort regarded the setting up of a linkage between the mentioned strategies and practices. In the next paragraphs a deeper description of Regions of Provenance, DNA Barcoding, and reforestation using Miyawaki method will be presented, focusing on recent achieved results, improvements and proposals. Some study cases in Italy will be also shown, in order to shed some light on the criteria for detecting best actions in the Mediterranean Basin. Finally, we will attempt to logically order these tools in an improved and well-organized "toolbox".

2. Regions of Provenance in Europe

The relationship between genetic variability and adaptability for a species is particularly important if we refer to forest plants, because they are characterized by long life cycle and consequently more exposed to environmental changes. Looking at biodiversity within a single species, very important are those populations with specific adaptations that could characterize local ecotypes. When populations are geographically separated and genetic flux is interrupted, differentiation processes can lead to speciation.
In many countries, the uncontrolled use of germplasm of unknown origin favoured serious phenomena of genetic erosion and pollution, in particular after the implementation of the Regulation EEC 2080/92 which encouraged the reforestation of agricultural land. In Italy, for instance, the Rural Development Programme 2000-2006 promoted reforestation on huge surfaces, and many land owners joined the program. The lack of enough autochthonous propagation material to supply the demand, leaded the operators to use plant material from several ecologically different geographical areas; as a result , many reforestation plans failed because of diverse pedoclimatic requirements of the adopted material, and with the rising up of infestations by new parasites.
For these reasons, the use of high quality propagation material, phenotipically and genetically appropriate to the plantation area is fundamental. Such principle, previously introduced in two European Directives (EEC 404/66 and EEC 161/71) was finally integrated in the Directive EC 105/99 about the marketing of forest reproductive material. Moreover, the Directive establishes that the basic material for reforestation has to be harvested from selected stands, and underlines the importance of delimiting Regions of Provenance, defined as "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 where appropriate". It is also specified that forest reproductive material (seeds, cones, fruits, parts of plants, planting stocks) is classified in the following categories: Source-identified, Selected, Qualified and Tested. About the source-identified and the selected materials, they belong to seed sources, stands, seed orchards, parents of family, clones or clonal mixtures located in a single Region of Provenance. Selected materials include also the phenotypic ones, identified at population level, and fulfilling the requirements of origin, isolation, population dimension, age and development, homogeneity, phytosanitary status, quali-quantitative production, status and morphology. Indeed, the Directive highlights the need for each Member State to define the Regions of Provenance for a correct use of reproductive material, in order to ensure forest biodiversity conservation with specific regards to the nursery activities. However, for reforestation practices, the Directive's contents does not suggest the use of basic material in accordance with the Regions of Provenance.

In the last twenty years, many European countries developed management systems based on ecoregions, adopting national measures accordingly to the Communitarian legislation. Anyway, the delimitation of the Regions of Provenance is very elaborate, since it requires the definition of the actual relationships between the ecological features of an area, the ecophysiological characteristics of each species, the peculiar propagation dynamics (pollination, dissemination, diffusion methods) of the species, and the intra-specific genetic diversity at both the individual and the population levels.

2.1 Common criteria for the definition of the Regions of Provenance

The subdivision of the distribution range for a species in spatially and genetically homogeneous regions complies the hypothesis of an intra-specific differentiation according with the environmental selection effects. This argument is valid only if populations have enough genetic variability to face, in terms of adaptability, and possibly to mild the environmental changes that may occur within a certain physical area. Some reproductive isolation derived from the genetic differentiation is a necessary prerequisite for allowing adaptability processes at a local scale. Therefore, the delimitation of the Regions of Provenance plays a key role in identifying those basic materials from which harvesting forest reproductive materials. Despite the environmental and genetic homogeneities are essential requirements to define different provenances, a weak point is detectable: the genetic composition of a population, i.e. the main indicator of adaptability derived from the evolutive processes, is commonly assessed throughout the analysis of the phenotypic performance, while a description through the use of molecular markers would be more appropriate. The adaptability at local environmental conditions, together with a genetic peculiarity, are essential features to reveal the autochthony of a population, possibly witnessed by historical documents. Recommendations by national and regional measures underline the importance of autochthonous resources for environmental restoration, starting from considerations about species' adaptability. Referring to forest populations, "autochthony" indicates the continuous occurrence of a species, in terms of genealogy, in a defined site since the last post-glacial migration. However, adaptability, as a peculiar feature of autochthonous populations, raises further considerations about the surface size where populations occur, as well as the time they passed under the same environmental conditions. For these reasons, the meaning of adaptability has been redefined several times, but always focusing on the spatial and temporal continuity in constant environment settings. Such quantitative characterization allows to consider the autochthony of a

population as a phenomenon in terms of degree rather than presence/absence. This suggests the need for defining the population extension, the size and structure, and the assessment of a continuous occurrence through the time in a specific area. At the same time, it is important to specify meta-populations' structure preserving their reproductive coherence by genetic flux. According to the spatial scale of investigation, the local genealogic continuity could appear relatively low in some stands, because of punctual extinction events or other disturbances. An appropriate spatial scale should be only established after the understanding of the reproductive coherence within the species' distribution range, and the analysis of the homogeneity of the environmental characteristics. Temporal and spatial scales, and the degree of environmental heterogeneity could be indirectly observed in the genetic structure of an autochthonous population, as the consequence of evolutive processes of adaptability. This circumstance derived from the presence of heterogeneity variation within a population; such variation has to be heritable, so the availability of genetic diversity is fundamental.

As mentioned above, it is often hard to check for the main adaptability determinants, as well as to accurately measure the features of autochthony. There is a significant mass of literature about the most commonly applied methods for delimiting Regions of Provenance according to the factors mentioned above (e.g. Geburek & Konrad, 2008; Kleinschmit et al., 2004; Lindgren & Ying, 2000; Krusche & Geburek, 1991; Raymond & Lindgren, 1990). They usually refer to the division of the territory (*divisive method*), if ecological parameters are considered, instead of joining of similar populations (*agglomerative method*) according to common biological features. Three clustering approaches are generally followed:

1. clusters according to homogeneous environmental conditions;
2. clusters according to genetic markers;
3. clusters according to phenotypic response.

The first procedure consists in grouping areas that share congruent ecological conditions. The selected parameters useful to characterize these conditions are supposed to be important for maintaining and expanding the referred species. The complexity of the growth regulation phenomena make difficult the selection of such parameters; however, some artificial delimitation of the Regions of Provenance based on administrative boundaries have been adopted to facilitate the management procedures. A specific problem raises when the reproductive coherence within a Region does not match with the potential effects on the intra-regional genetic differentiation; this difficulty is typical of areas with plantations, where the individuals have significant differences in terms of geographic origin, and no genetic information resulting from the adaptation at the local conditions.

The second clustering procedure gives more emphasis on the genetic variability of every single species, intended as peculiar feature for observing intra-specific differentiation at level of regional areas, populations, individuals, etc., and needed for defining the Regions of Provenance. Unfortunately, the correspondence between adaptability and neutral molecular markers (e.g., isoenzymes, plastid and mitochondrial microsatellites) may not be sufficiently clear to mirror differences between Regions of Provenance, and the more variable nuclear markers (e.g., microsatellites, AFLPs) may prove too difficult and expensive for large scale investigations (Karp et al., 1997; Duminil et al., 2007). On the other hand, adaptive traits could be more efficiently dissected by use of QTLs analyses (Lewontin, 1984; Borevitz and Chory., 2004), but the technical requirements of the method (and the complex biology of trees) has limited the number of available information on a short number of world species.

Finally, the most recent advancements of molecular biology (identification and characterization of ecologically important Candidate Genes, transcriptomics and metagenomics) are highly promising but still hindered by prohibitive costs and difficulties (Pflieger et al., 2001; Eveno et al., 2008; Derory et al., 2010).
The last procedure is based on statistical researches, starting from test areas, about relationships between growth performance and environmental variables, such as stem growth, bud set, flowering time, and geographic coordinates, photoperiod, altitude, etc. These affinities are used to cluster populations according with specified range of values calculated by statistical functions. Resulting clusters are built minimizing the distance between the origin of propagation material and the area to under investigation.
In conclusion, all these criteria focus on reducing the risk connected with the transfer of the material in ecologically heterogeneous areas. Such risks are evaluated in terms of adaptive failure and undesired phenotypic traits (habitus, seed productivity, etc.), but the methods applied are still under discussion and development. Indeed, every useful strategy devoted to minimize the mentioned risks has to be based on a spatial delimitation consistent with the real and/or potential adaptability of a population through the time.
The main goals to define provenances concern the species' range, metapopulation and subpopulation delimitation, the estimation of adaptability, and the assessment of the adaptive effectiveness in terms of evolution. Therefore, the common methods to achieve these goals involve a spatial-genetic clustering of at least a second generation of adult individuals, the heterogeneity analysis of life mechanisms and functions, and the connexion of adaptive phenotypic variability with the genetic one, developing transplant tests with adaptive differentiation study.
In many Communitarian experiences, the definition of Regions of Provenance leaded to delimitate large areas (e.g. oak species in Germany, Scots pine in the Baltic region), because of the results obtained on experimental fields where the adaptive flexibility of several populations were tested at different environmental conditions. In any case, Regions of Provenance have to be directed to the preservation of the natural mechanisms of persistence for a species within its range.

2.2 State of the art

In the European Union, several countries actuated the delimitation of the Regions of Provenance for forest species after the adoption of the Directive EEC 161/71. The progress project reports differ State by State, but practically match the requirements suggested by the Communitarian normative. An important step concerns the mapping of species' occurrence in each country, and the characterization of homogeneous areas using ecological indexes. In many cases, the Regions of Provenance have been delimited summarizing the results obtained by the application of several clustering approaches, and taking also into account regional and/or provincial administrative boundaries; such last conformation is particularly useful for those countries which have commissioned the competence for environmental topics to the local authorities.
In the following table the Member States adopting the Regions of Provenance in accordance with the Directive 105/99 have been listed (thus, Norway has been excluded even if adopting the Directive), providing synthetic information about used parameters and number of target species (Table 1, modified from Alía et al., 2008). This summary table could be subjected of updating, as the Regions of Provenance is a dynamic process still in progress in several countries (e.g. in Italy).

	Countries																		
Parameters	AT	BE	CZ	DE	DK	ES	FI	FR	GB	GR	HU	IT	IR	LT	NL	PL	RO	SE	SI	SK
Geographical information	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
Altitude	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
Climate	X	X		X		X	X	X	X	X	X	X	X	X		X	X	X	X	X
Soil	X	X	X	X	X	X		X		X	X	X	X	X		X	X	X	X	X
Neutral markers		X			X	X		X				X			X		X			X
Field test					X	X		X	X			X	X	X	X	X				
Nursery/phytotron test						X						X		X	X					
Growth				X		X		X				X	X	X		X				
Phenology			X	X				X				X	X	X		X			X	
Resistance to disease/pests			X	X					X											
Vegetation/ phytogeography	X			X		X		X	X	X	X	X							X	
Overall adaptation				X	X	X	X	X	X			X	X	X	X	X	X			
Administrative boundaries		X					X		X	X		X		X				X	X	
N. of target species	24	37	23	50	36	56	14	53	54	10	25	78	28	9	22	10	35	17	11	7

Table 1. Overview of the criteria adopted by the countries in the European Union that have defined the Regions of Provenance and total amount of forest target species. Short abbreviations of country names are given according to the ISO 3166-1-alpha-2 code.

The differences highlighted between countries suggest some critical remarks. Against a common acceptance of the Directive 105/99, there is not a pan-European strategy for the Regions of Provenance; some difficulties could be detected such as the available information, the possibility to exchange, and the different formats of the data. Moreover, large differences regard the size and the methodologies for the delineation, the number of target species and the knowledge about their biological parameters that varies from species to species.
A standardization process devoted to set up a common approach in the delineation of the Regions of Provenance surely will take long time, as it requires both technical and political actions. However, it is possible to suggest improvements for the methods, using additional ecological variables and/or techniques to explore phenological and biological behaviours of forest species not considered yet. A study case is presented in the next paragraph and regards the delineation of Regions of Provenance in the Latium District (Italy).

2.3 New proposals and improvements for delineating Regions of Provenance: The case of Latium

The clustering procedure according to homogeneous environmental conditions could be considered as the easiest approach for defining Regions of Provenance, because of the large databases about chemo-physical parameters that each country has stored since the beginning of the last century. Generally, we have more information about the property of a

territory instead of the living species that occur there. These simple assumptions could justify why the divisive method is often used. Nevertheless, additional variables could be considered for improving the delimitation of the Regions of Provenance, for instance phytoclimatic indexes as the Mitrakos Winter Cold Stress (WCS) or Summer Drought Stress (SDS), and the Emberger coefficient (Mitrakos, 1980; Emberger, 1955). These parameters demand for time-series climatic data, and refer to a data point network of weather stations widespread in an area; but, it is possible to spatially extend them by using numerical and mathematical techniques dealing with the characterization of spatial phenomena, using geostatistic analyses that rely on statistical approaches based on random function theory to model the uncertainty associated with spatial estimation and simulation. Using the geostatistic methods, as implemented in many GIS softwares, it is also possible to go beyond the interpolation problem by considering the studied phenomenon at unknown locations as a set of correlated random variables. In the case of Latium, both Mitrakos indexes and Emberger coefficient have been spatialized using Kriging interpolation from 85 data points recording precipitation and temperature for 15 years at least; topography and continentality have been also considered as supplementary variables extrapolating data from the Digital Elevation Model (DEM) of Italy with 75x75 m grid cells. The resulting outputs have been overlapped to other chemo-physical variables, i.e. mean annual temperature, minimum temperature of the coldest month, maximum temperature of the warmest month, annual precipitation, geomorphology, soil, etc. A summary layer storing all the spatialized variables has been performed and areas with homogeneous ecological features have been finally detected. Moreover, the boundaries of these areas have been buffered to better represent the gradual spatial shifting from an ecological context to another (Figure 1).

According to the main phytoclimatic parameters, as well as the vegetation maps proposed by several authors (Blasi, 1994; Tomaselli, 1973; Pavari, 1916), Latium has been divided in 3 Primary Regions of Provenance and subsequently in 17 Secondary Regions including also the geomorphology and the soil characteristics (Figs. 2, 3). This procedure basically follows the common strategies adopted by the other European countries, but increases the number of variables to be considered for a deeper ecological investigation that includes plant response to climatic conditions.

Since the evaluation of the effects of natural selection and bioclimatic responses across space is at the base of the definition of the Regions of Provenance, a better characterization of basic material should be achieved by combining already showed results with parameters related to species performance (biological responses to ecological factors) and to the altitudinal gradient (as suggested by the Directive 105/99), in order to provide homogeneous material both for afforestation and genetic preservation. In those countries where the knowledge about forest species' biological and genetic features are studied from years, or for peculiar species with great economic impact (e.g. *Populus spp.*, *Castanea sativa* Mill., *Quercus petraea* Liebl., *Picea abies* (L.) Karst., *Pinus sylvestris* L., *Fagus sylvatica* L., *Quercus suber* L., etc.) such improvements have been made; in particular, as showed in Table 1, phenology, growth performance, and neutral markers for genetic characterization are the most used parameters. Nevertheless, it is possible to consider further investigations focusing on bioindicator species' behaviours, and extending the method to all forest species. Dendroecology can contribute to these studies by improving the analysis of tree growth response to environmental gradients, thereby refining the classifications that are based on climate–vegetation interactions. This approach was previously taken on 17 beech forests in central Italy (Latium and Abruzzi) to obtain horizontal and vertical gradients in tree–climate

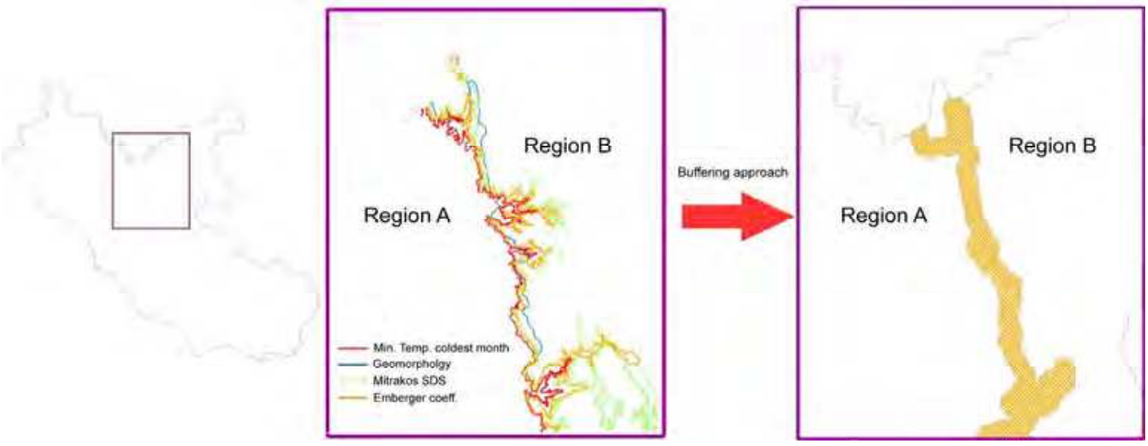


Fig. 1. Example of buffered boundary between two Regions of Provenance in Latium. The buffer zone represents a gradual transition of the ecological characteristics from Region A to B and vice-versa.

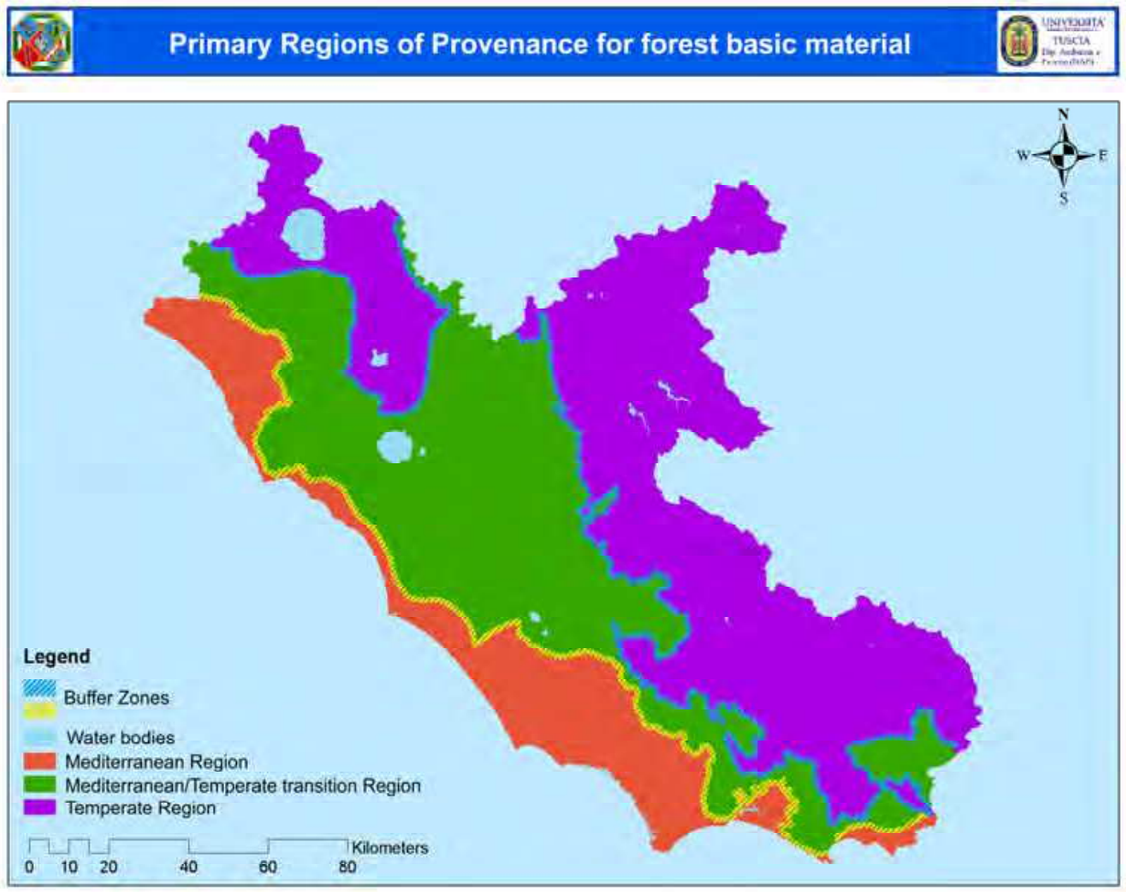


Fig. 2. Delineation of Primary Regions of Provenance for Latium using the improvements cited in the text.

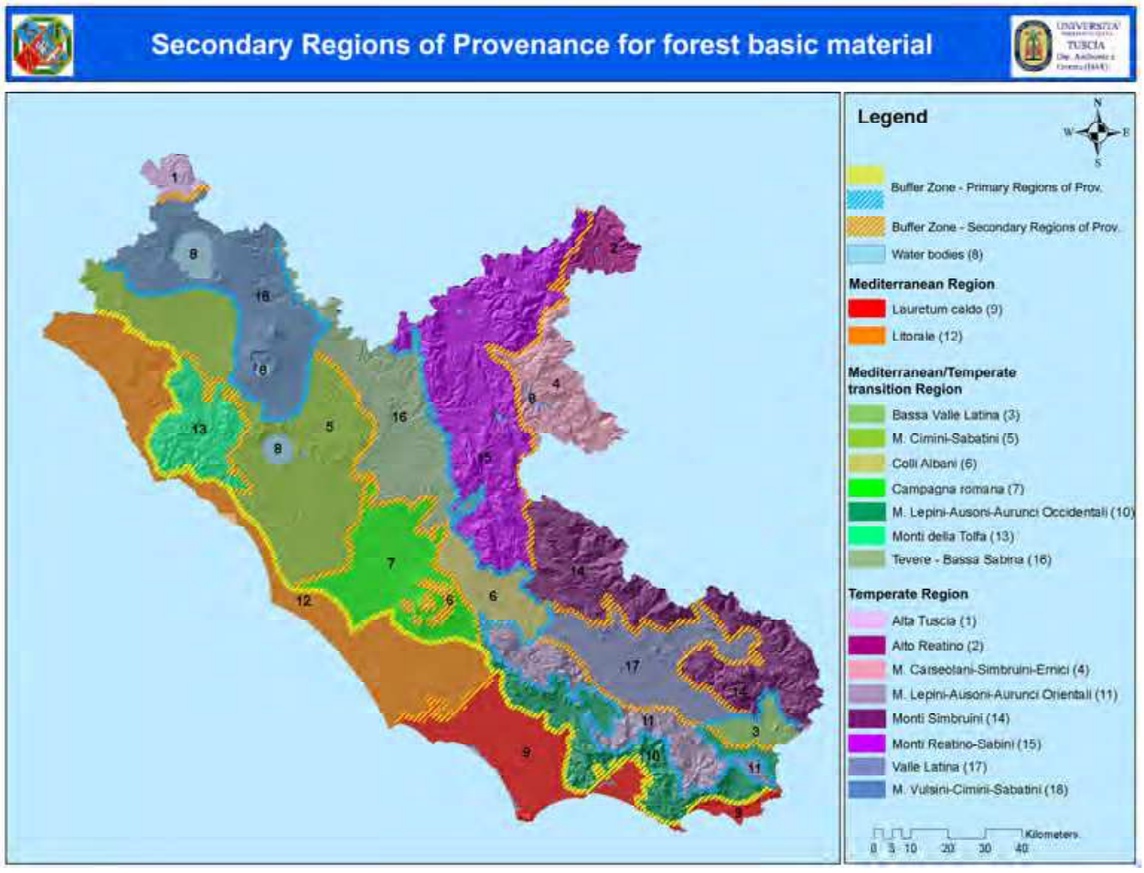


Fig. 3. Map of Secondary Regions of Provenance for Latium derived from phytoclimatic, vegetation and chemo-physical variables.

relationships, thus providing the basis to assess bioclimatic units in terms of the leading dendroclimatic signals (Piovesan et al., 2005). Evidence from tree-ring analysis demonstrated that tree growth is strictly related to elevation, generating distinct beech forest types. In agreement with previous studies (e.g. Biondi, 1992; Biondi & Visani, 1996; Dittmar et al., 2003), distinctive radial growth–climate relationships uncovered in the tree-ring network are organized along altitudinal and latitudinal gradients. Since beech could be considered a good bioindicator, i.e. its dendroecological features are significantly related to elevation, comparisons were extended to all the Latium forest surfaces, including the altitudinal belt where beech is not present in the landscape, i.e. a non-beech belt with bioclimatic traits that generally do not allow the growth of beech. The following results could be considered a starting point for the selection of basic material used in genetic and provenance studies, to accomplish the definition of the Regions of Provenance for Latium previously showed. It is a new approach that checks the agreement between the dendroclimatic classification and the phenological traits analyzed by remote sensing measurements, expressed by the normalized difference vegetation index (NDVI).

The NDVI allows decadal (10 day) monitoring of terrestrial vegetation, at regional to global scales, using the spectral reflectance measurements acquired in the red and near-infrared regions. These spectral reflectances are themselves ratios of the reflected over the incoming radiation in each spectral band. NDVI reflects the chlorophyll and carotenoid content in the

leaves (Tucker & Sellers, 1986), but it is also related to leaf area index (LAI) (Fassnacht et al., 1997) and the fraction of photosynthetically active radiation absorbed by leaves (fPAR) (Veroustraete & Myneni, 1996). The NDVI expresses the greenness of a pixel, and it is a good remote sensing methodology to detect interannual and seasonal changes in forest ecosystems. Using the GIS software an NDVI time series spanning 11 years (1998–2008) was developed. The data have a spatial resolution of 1x1km^2. To detect the area covered by broadleaved forests in Latium, the Corine Land Cover (CLC) database (3.1.1 classes— Broadleaf woods) was used. Only pixels (1x1km^2) with a forest area above 60% were used in the analysis. Raster data were reprojected to the same coordinate system of the subset vector grid map obtained, to overlap the CLC forest surface with satellite images. NDVI mean values were calculated for each selected cell and partitioned using k-mean clustering. Four fixed *a priori* clusters (referred to in the text as NDVI classes) were chosen to test the correspondence with the four bioclimatic zones obtained by the dendroclimatic classification.

The NDVI class assigned to each cell was graphically overlapped with bioclimatic altitudinal belts, showing a good spatial correspondence between results obtained by the dendroecological and the NDVI classification (Figure 4). The relative frequency distribution of NDVI clustered cells per altitudinal belt ranged between 61% and 92% (Figure 5).

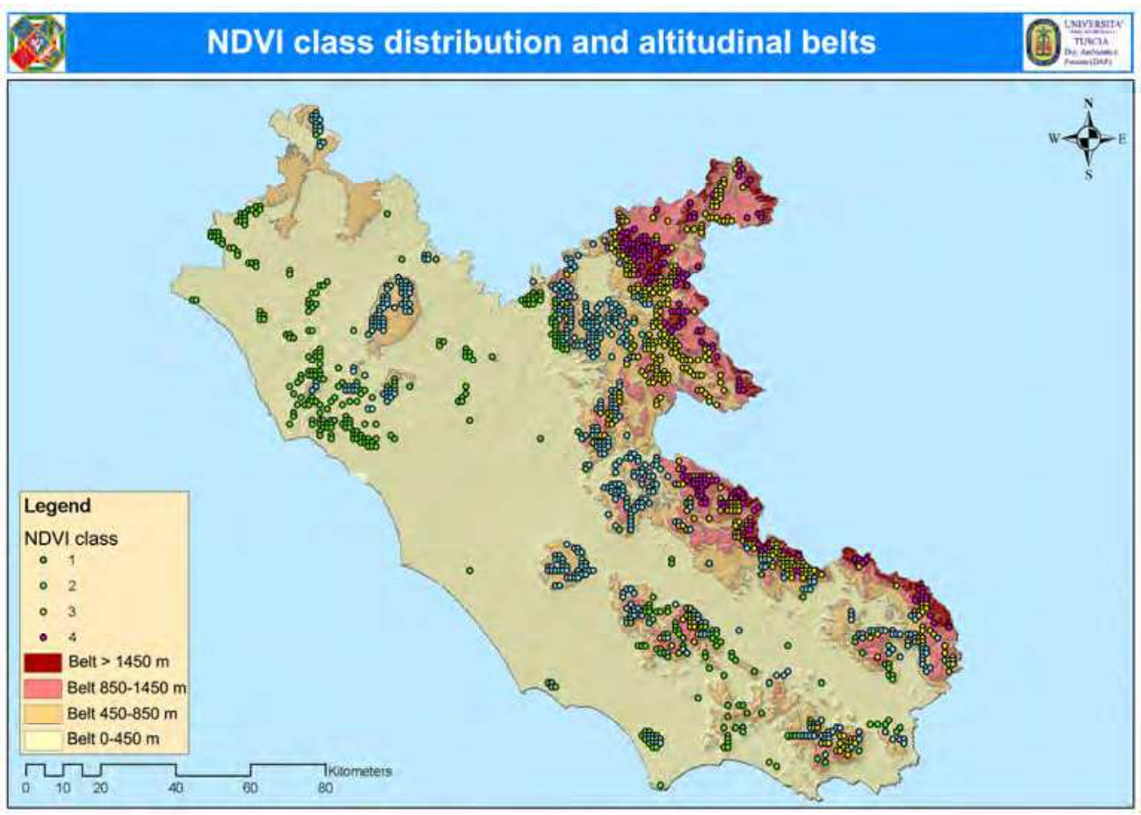
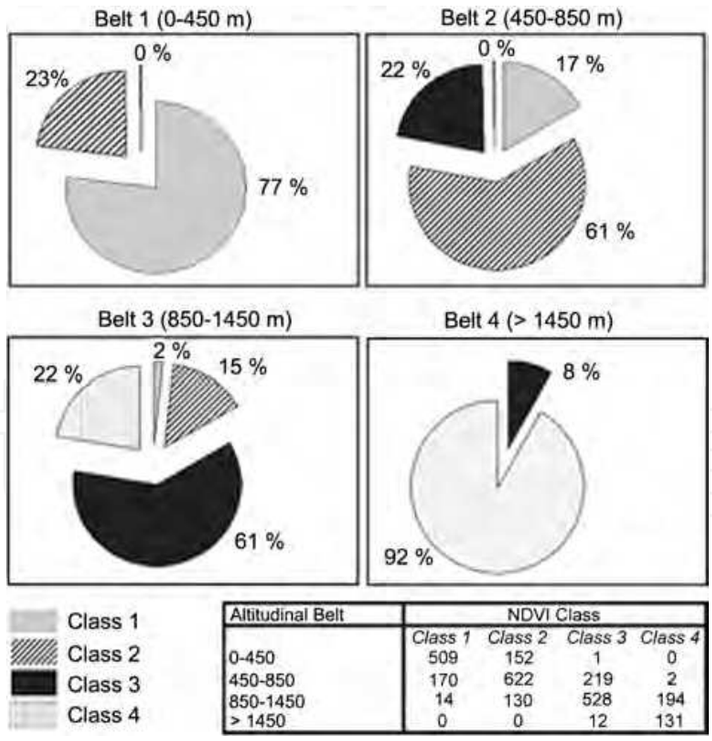


Fig. 4. Map of the spatial overlapping between normalized difference vegetation index (NDVI) classes and altitudinal belts detected using the dendroclimatic approach.

Physical parameters, such as aspect or edaphic conditions, could play a fundamental role where other non-correspondent NDVI class cells were present. This confirmed the general role of elevation as a key factor in controlling both the growth and phenological behaviour of forest stands in central Italy. The NDVI varied greatly among months and NDVI classes, stressing the difference in photosynthetic activities throughout the growing season of distinct forest bioclimatic belts; in particular the growing season length shortened according to increasing elevation (Figure 6). These results assess that it is possible to link tree-ring climate signals to phenology for each altitudinal belt by combining the two methods, adding important clues to the further comprehension and modelling of the bioclimatic organization of these forests. The two methods were mutually validated, and therefore would be useful in defining Regions of Provenance as agglomerative approach. The main benefit is in providing an automated approach at local spatial scale useful to map these regions. The coupled dendroecological application and NDVI can offer a prompt, economic and operative tool to check and manage homogeneous ecological areas, objectively identifying Regions of Provenance according to plant responses. Moreover, this approach could be combined with other biological and genetic parameters, e.g. growth performance, resistance to diseases, DNA markers, for a wider scenario of species' behaviour. At the same time, matching the full dataset of ecological, biological and genetic variables a more completed delineation of Regions of Provenance could be achieved (Alessandrini et al., 2010).



Altitudinal Belt	NDVI Class			
	Class 1	*Class 2*	*Class 3*	*Class 4*
0-450	509	152	1	0
450-850	170	622	219	2
850-1450	14	130	528	194
> 1450	0	0	12	131

Fig. 5. Pie charts of percentage correspondence between assigned normalized difference vegetation index (NDVI) classes and tree-ring altitudinal belts. The panel below the charts shows the number of cells per class and belt.

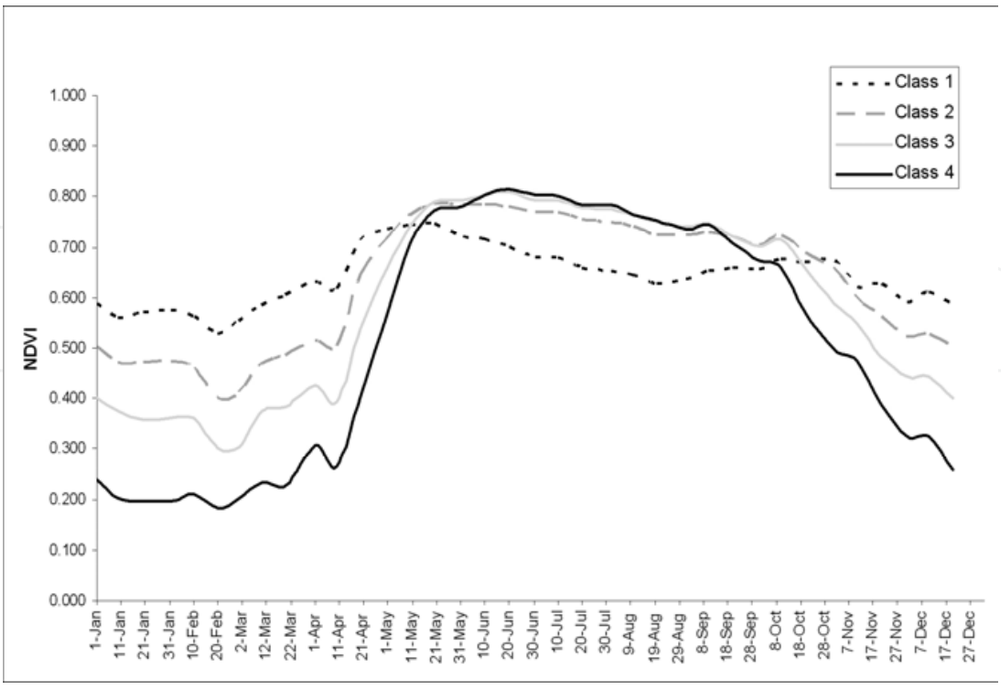


Fig. 6. Annual normalized difference vegetation index (NDVI) profile of the four classes obtained by k-means clustering.

3. DNA barcoding approach: A new challenge for species identification and conservation

DNA barcoding is a standardized molecular approach to label living organisms by joining specific sequences of DNA and electronic information retrieval (Hebert et al., 2003), and it has recently become an increasingly attractive tool for species identification in terms of accuracy, speed, cost and functionality. Ideally, a universal barcode system would be a valuable resource to provide objective and worldwide comparable results, which can be efficiently used in turn to compile biodiversity surveys in local floras (Lahaye et al., 2008; Gonzalez et al., 2009; Kress et al., 2009, 2010). Additionally, the method allows the analysis of poor, fragmented samples at any life stage (Chase et al., 2005) and it can be easily repeated even by non-taxonomist specialists. The primary goals of barcoding are thus species identification of known specimens and discovery of unnoticed species to enhance taxonomy for the benefit of science and society (Kress & Erickson, 2008). The term "DNA barcode" refers to a short DNA sequence-based identification system which may be constructed of one locus or several loci used together as a complementary unit (Kress & Erickson, 2007). Necessary prerequisites of DNA barcodes are ease of application across a broad range of *taxa*, sufficient sequence variation to distinguish between species, and absence of intra- and inter-specific diversity overlaps which would prevent rank definition. Many studies have proved the efficacy of the mitochondrial cytochrome c oxidase 1 (COI or cox1) gene sequence in barcoding animal groups such as birds (Hebert et al., 2004), fishes (Ward et al., 2005), spiders (Greenstone et al., 2005), lepidopterans (Hajibabaei et al., 2006), and amphibians (Smith et al., 2008), as well as in red algae (Robba et al., 2006) and fungi (Seifert et al., 2007). In plants, the difficulty of finding a single-locus barcode has suggested a

multilocus approach, focusing on the plastid genome as currently the most effective strategy (see Hollingsworth et al., 2009, and citations therein), although there is still much debate concerning the most suitable regions to be used. From the broad pool of loci recently considered (Kress et al., 2005; Chase et al., 2007; Newmaster et al., 2008; Ford et al., 2009), the greatest interest was aroused by seven candidate plastid loci: rpoB, rpoC1 and rbcL (three easy-to-align coding regions), a section of matK (a rapidly evolving coding region), and trnH-psbA, atpF-atpH and psbK-psbI (three rapidly evolving intergenic spacers).

Various biological contexts (e.g., sampling strategies) have been used to compare the performance of plant barcoding loci, and/or the efficacy of the method. A sound assessment of the universality of regions is usually given by the "species pairs" and "floristic" approaches. The former involves analysing pairs of related species from multiple phylogenetically divergent genera and may be defined as a "methodological" protocol; the latter involves sampling multiple species within a given geographical area and represents an example of how barcoding might be applied in practice. However, only limited insights into species-level resolution is usually provided by both approaches, as individual genera are not sampled in depth to provide estimates of intra- and interspecific genetic distances to achieve species identification. Conversely, a third method, the "*taxon*-based" approach, involves sampling multiple species within a given taxonomic group, in a global geographical context. This provides limited insights into universality and local applicability, but offers more definitive information on discrimination power at species level. To date, the species pairs (e.g. Kress et al., 2005; Kress & Erickson, 2007), and the *taxon*-based (e.g. Newmaster et al., 2008; Newmaster & Ragupathy, 2009) sampling designs have provided useful insights into the potential performance of varying combinations of barcoding loci, whereas the floristic approach (e.g. Fazekas et al., 2008; Lahaye et al., 2008; Gonzalez et al., 2009), has showed strong potential applicability in as many diverse research fields as biodiversity inventories, community assembly, food and medicine identification, ethno- and forensic botany. Based on the relative ease of amplification, sequencing, multi-alignment, and on the amount of variation displayed (sufficient to discriminate among sister species without affecting their correct assignation through intra-specific variation), the most frequently recommended marker combinations for broad future applications appear to be: rbcL + trnH-psbA (Kress & Erickson, 2007), matK + trnH-psbA (Newmaster et al., 2008; Lahaye et al., 2008), rbcL + trnH-psbA + matK, and rbcL + matK (Consortium for the barcode of Life, Plant Working Group [CBOL PWG], 2009).

3.1 DNA Barcoding of forest tree species

In forestry science, DNA barcodes is highly promising for the detection, monitoring and management of biodiversity (von Crautlein et al., 2011). In addition to resolving many taxonomic uncertainties, enhancing clear and more accurate biodiversity assessments, DNA barcoding may provide a boost to efficient management and conservation practices, mainly focusing on community ecology (to describe plant-animal interactions and vegetation dynamics/changes, to discriminate native vs. alien germplasm), biodiversity inventories (aimed at habitat and species protection), silviculture (to assess forest regeneration), and nursery activities and market regulation (to establish wood, secondary products and germplasm certification). The applications might be particularly relevant to manage correctly the over-exploited and the newly identified species, to adequately protect those having limited ranges and relatively small population sizes, as well as for mending damaged landscapes by planning and monitor congruent reforestation programmes.

Indeed, one of the future challenges for DNA barcoding in plants is to increase the number of practical studies, and validation of the method for forestry purposes is still to be demonstrated. Priority should be given to the use of markers with universal primers and uniform PCR conditions. Under these criteria, the most updated recommendation from the CBOL PWG is that rbcL+matK is adopted as the core DNA barcode for land plants (CBOL PWG, 2009), with trnH-psbA (the next best performing plastid locus) as a supplementary barcode option for difficult plant groups. However, success in angiosperms is often perceived by the majority as the most important issue. For gymnosperms (and cryptogams) the universality criterion has received little consideration up to date, and clade specific/multiple primer sets were often used to evaluate matK and other putative barcode markers (including rbcL and rpoC1). For instance, in the few currently available gymnosperm-based barcoding studies, only 24% PCR success was obtained in Cycads (Sass et al., 2007) with matK universal primers, whereas Hollingsworth et al. (2009) and Ran et al. (2010) obtained 100% PCR and sequencing success in *Araucaria* and *Picea* by use of a combined set of specific primers and under non-standard PCR conditions. More recently, a *taxon* based study on *Taxus* was attempted with new matK specific primers (Liu et al., 2011). Clearly, matK universality across both gymnosperms and angiosperms is still a matter of concern, while rbcL and trnH-psbA have repeatedly shown strong rates of sequence recovery in both clades but their use still requires some technical adjustments (see for instance Hollingsworth et al., 2009).

The efficacy of the method is still under question, too. Pooled sequence data from 445 angiosperm, 38 gymnosperm, and 67 cryptogam species indicated that overall species discrimination was successful in 72% of cases (CBOL PWG, 2009), in agreement with the upper limit of ca. 70% resolution pointed out in previous studies (Fazekas et al., 2009; Hollingsworth et al., 2009). Large-scale plant diversity inventories conducted at a local or regional context matched this limit or revealed even higher percentages, although absence/scarcity of gymnosperms in their datasets is still noticeable. Irrespective to the statistical methods used to cluster sequences into taxonomic units, and to the marker combinations used, <70% of species resolution was achieved on 254 angiosperm species from an environmental sampling in Amazonia (Gonzalez et al., 2009), ca. 71% on 92 primarily angiosperm species (including 7 conifers) from selected locations of Southern Ontario (Fazekas et al., 2008), ca. 90% on 32 angiosperm species and over 1000 orchid species from two national parks (Lahaye et al., 2008), and 93-98% on 143 and 296 angiosperm species in community studies in tropical forest dynamics plots in Puerto Rico and Panama (Kress et al., 2009, 2010). However, it has been shown that woody plant lineages have consistently lower rates of molecular evolution as compared with herbaceous plant lineages (Smith & Donoghue, 2008), suggesting that the application of DNA barcoding concepts should be more difficult for tree than for non-woody floras (Fazekas et al., 2009). Moreover, the discrimination rate of plastid barcoding loci varies greatly among different plant lineages. In tree species, no resolution was achieved in 12 *Quercus* (Piredda et al., 2011), 18 *Betula* and 26 *Salix* species (von Crautlein et al., 2011), whereas 30%, 63% and 100% were achieved in *Berberis* (16 species), *Alnus* (26 species), and *Compsoneura* (8 species), respectively (Roy et al., 2011; Ren et al., 2010; Newmaster et al., 2008). In Gymnosperms, all extant five *Taxus* species (Liu et al., 2011) were fully discriminated with a non-standard barcode (trnL-F); in 32 *Picea* species (Ran et al., 2010), the highest rate of successful discrimination was 28.57% for a three-locus barcode (trnH-psbA, matK, atpF-atpH). A slightly higher percentage was obtained by Hollingsworth et al. (2009) in *Araucaria* (32%).

Available data show that some limitations are predictable, matching the view of Fazekas et al. (2009). Limitations are mostly due to polyploidy, hybridization/introgression phenomena, shares of ancestral polymorphism, which would prevent the correct match between DNA variation at the plastid level and species identity. Such phenomena probably affect many tree species; in addition, trees are known to have markedly slower mutation, nucleotide substitution and speciation rates than other plants, seemingly owing to longer generation times and slower metabolic rates (see Petit & Hampe, 2006 for a review). At the same time, biogeographic patterns of species, lineages and area relationships can strongly affect the resolution of *taxa*. Together with this assumption, the barcoding efficiency of tree *taxa* is still to be demonstrated, and it appears to be most hardly challenged by the peculiar evolutionary history and intrinsic biology of each *taxon*, and in those areas where recent explosive radiations have taken place, or where a high number of only slightly diversified congenrics co-exist.

3.2 Barcode application in the Italian flora

A summary of explorative data on the foreseeable barcoding efficacy in the Mediterranean area, with specific regard to Italian forest flora is reported in Table 2. With the aim to provide a test for future *in situ* applications of DNA barcodes by evaluating the efficacy of species discrimination under the criteria of uniformity of methods and natural co-occurrence of the species in the main forest ecosystems, we examined whether four marker regions (trnh-psba, rbcL, rpoc1, matK) proposed by the Consortium for the Barcode Of Life matched species taxonomy in a biodiversity survey of Italian forested land.

Seventy-eight species were included in a floristic study, including 53 Angiosperm and 25 gymnosperm species (trees, shrubs and vines from the Alpine timberline to the Mediterranean sea dunes; 68 native and 10 introduced/naturalized *taxa*); in addition, taxon-based studies were performed on Quercus (15 species, 30 individuals), Acer (8 species, 15 individuals) and Pinus (10 species, 30 individuals). individuals) and Pinus (10 species, 30 individuals). We observed total universality of the rbcL+trnH-psbA marker combination across all *taxa*, and an overall 78.4% of species discrimination, with 100% in gymnosperms and 66.7% in Angiosperms,whereas matK and rpoC1 showed incomplete, or limited, applicability due to some primer specificity, Differences in the biology/evolutionary history of tree genera are represented by the contrasting results obtained in the three taxon-based studies: Quercus exhibited an exceptional 0% of species resolution, whereas Acer and Pinus reached 100% discrimination success. As a main result, the barcoding approach provided molecular tools for the identification of all *taxa* co-occurring in most of the Italian forest ecosystems, from the Alpine timberline, to montane, submontane, humid/riparian, Mediterranean evergreen forest/maquis and sea dunes, including some ubiquitous vines and shrubs, with the exception of oaks and willows. The approach was also useful for the molecular identification of all the rare endemics investigated (*Fontanesia phylliraeoides*, *Acer lobelii*, *Abies nebrodensis*, *Pinus heldreichii* ssp. *leucodermis*), and all native vs. allochtonous germplasm (*Aesculus hippocastanum*, Quercus *rubra*, Acer *negundo, Abies pinsapo, A. cephalonica, Pinus radiata, P. brutia, Cupressus arizonica, Pseudotsuga menziesii, Gingko biloba*). Concerning the intraspecific *taxa*, ssp. *nigra* was clearly differentiated from all other *Pinus nigra* subspecies, as well as ssp. *turbinata* within *Juniperus phoenicea*. Lastly, two vines and four shrubs were efficiently discriminated from co-occurring arboreal *taxa*. Investigated *taxa* could be efficiently barcoded in most ecosystems, with the exception of those forests where a high number of willows and oak species co-occurred.

Among the species-rich genera, those which would benefit most from molecular identification (*Quercus*, *Salix*) because of their complex morphology, showed little or no variation at the plastid genome. Remarkably, none of the markers used could resolve 12 Italian *Quercus* species below the sectional level (i.e., Sclerophyllodrys, Cerris and Quercus), due to large haplotype sharing between closely related species. On the other hand, intra-specific variation in Italian conifers appears to correspond to some regional patterns reflecting important prints of species survival during glaciations and post-glacial recolonization (Follieri, 2010). Specific haplotypes were found in Southern Italy (Apulia), Central Italy (Tuscany, Latium), Northern Italy (Eastern and Western Alps), and main islands (Sicily), all falling within the 52 biodiversity refugia recently indicated on a regional scale in the Mediterranean basin (Medail & Diadema, 2009). Variation in the barcoding loci also evidenced the occurrence of two distinct haplotypes of *Taxus baccata* in Italy, one shared with other European provenances and a second exclusive of South East Italy. Finally, our results confirmed the genetic diversity existing between Southern and Central Italy provenances of *Cupressus sempervirens* (Bagnoli et al., 2009), and divergence between Eastern and Western Alps provenances of *Picea abies* (Collignon & Favre, 2000), as well as between Eastern and Western Mediterranean provenances of *P. halepensis* (Korol et al., 2002), all previously detected with other molecular markers.

Major Clade	Familia	Genus	Species in Italy	Species investigated	Species identification	Notes
Angiosperms	Aceraceae	Acer	7	8*	Yes	Possible haplotype sharing between A. obtusatum and A. monspessulanum
	Oleaceae	Ligustrum	1	1	Yes	
		Olea	1	1	Yes	
		Fraxinus	3	3	Yes	
		Phyllirea	3	3	Yes	Possible haplotype sharing between P. angustifolia and P. latifolia
		Fontanesia	1	1	Yes	
	Fagaceae	Fagus	1	1	Yes	
		Castanea	1	1	Yes	
		Quercus	10-14	15*	No	No species resolution at National scale
	Salicaceae	Populus	3	2	Yes	Possible haplotype sharing between P. nigra and P. alba
		Salix	>30	2	No	No species resolution at National scale (**)
	Ulmaceae	Ulmus	3	1	Yes	
	Rosaceae	Prunus	9	1	Yes	Possible haplotype sharing
		Craetegus	2-3	1	Yes	Possible haplotype sharing
		Rosa	>20	2	Yes	Possible haplotype

Major Clade	Familia	Genus	Species in Italy	Species investigated	Species identification	Notes
						sharing
		Rubus	>20	2	Yes	Possible haplotype sharing
	Betulaceae	Corylus	1	1	Yes	
		Alnus	4	1	Yes	
	Araliaceae	Hedera	1	1	Yes	
	Sapindaceae	Aesculus	0	1*	Yes	
	Cannabaceae	Humulus	1	1	Yes	
	Moraceae	Ficus	1	1	Yes	
		Morus	0	1*	Yes	
	Tamaricaceae	Tamarix	10	1	Yes	
Gimnosperms	Pinaceae	Pinus	8	10*	Yes	Possible haplotype sharing between P. mugo and P. sylvestris
		Larix	1	1	Yes	
		Pseudotsuga	0	1*	Yes	
		Abies	2	4*	Yes	
		Picea	1	1	Yes	No species resolution at National scale (**)
	Cupressaceae	Juniperus	4	4	Yes	
		Cupressus	1	2*	Yes	
	Taxaceae	Taxus	1	1	Yes	
	Gingkoaceae	Gingko	0	1*	Yes	

Table 2. Barcoding efficacy on some of the most important tree species in Italy. Asterisk indicate non native species included (*), and results implemented with literature data (**).

We therefore conclude that, despite some failures, the DNA barcoding approach will continue to be useful in some applications, especially when applied at local contexts, with some plant groups and for some peculiar investigations. Ideally, an important technological advancement to improve the method would include the achievement of primer universality for the main plastid markers, and eventually the opportunity to cope information from both organellar DNA and the more informative nuclear genome.

Organisms identification is essential to many disciplines, and the scientific community has recently come to realize the importance of integrated approaches to organism identification (Steele & Pires, 2011). Indeed, conservation planners and government agencies would need well defined species boundaries to protect ecosystems and writing effective laws (Primack, 2008), and restoration ecologists must accurately identify native plant species suitable for rebuilding damaged ecosystems (Guerrant et al., 2004). As well, conservation biologists must be able to correctly identify plant species for fighting invasive, reseeding restoration areas with appropriate species, monitor the regeneration processes of a community after their intervention, protecting native and/or threatened ecosystems by preserving all life forms. Finally, seed harvesters and germplasm traders must ensure the end-users that the right species are produced before distribution to the public. Nevertheless, the role that DNA barcoding might play in these views still relies heavily on experimentation and tests.

Our data suggest that forest biodiversity can be efficiently barcoded at a local level, or in well characterized regions of the world which have comparatively low numbers of species; conversely, the barcoding efficiency of tree *taxa* might rather be under question in large areas where peculiar genera (e.g., *Betula*, *Quercus*, *Salix*, etc.) occur with multiple species. Future large breadth *taxon*-based studies will help clarify the efficacy of DNA barcoding to inspect the biological diversity of forest tree species. However, factors suggested to contribute toward limiting the efficacy of barcoding tree species such as longevity, complex reproductive strategies, and slow mutation and speciation rates (Petit & Hampe, 2006) may not affect the barcoding efficacy at a local context.

4. From conservation to restoration: The Miyawaki method

It is widely known that global climatic changes, together with recent rapid urbanization and industrialization, have been the main anthropogenic effects worldwide in destroying natural environments, changing land use, reducing biodiversity, and modifying ecosystems. They suggest the need for performing more environmental conservation strategies, as well as using innovative environmental recovery activities. We have seen in the Introduction as *in situ* gene conservation measures ecosystem functions and species interactions, rather than individual tree species; however their conservation may require specific management measures, which could be ensured through the establishment of genetic conservation areas. From a theoretical point of view, a network of genetic resource conservation areas should be an efficient way to conserve the genetic resources of target species, if they follow the patterns of distribution of genetic variation (Eriksson et al., 1995). Practical experience suggests that sound management of genetic resources must include conservation efforts based on two overlapping strategies: management of natural forests with due respect to their genetic resources, and the establishment of networks of smaller gene conservation areas (Thomson et al., 2001). Nevertheless, it should be remembered that *in situ* conservation is only a technical option in a broader approach to conservation of the diversity between species and within species. In several cases, conserving forest trees *in situ* may be the only method that is socially and economically possible. In other cases, a combination of protected areas, managed reserves, clone banks, research plantations and breeding programmes may be better suited to different conditions and objectives.

In the last years, the greatest challenge is to move from the conservation of existing resources, toward a rationale restoration ecology, increasing efforts to rehabilitate degraded lands. Often the preliminary objective is to re-establish tree cover for environmental purposes, especially for control of soil erosion and for watershed protection. Facing these items, scientists have developed new insights both in theoretical and in practical actions for restoration and reconstruction of natural ecosystems (Clewell & Aronson, 2008; Falk et al., 2006; Jordan et al., 1987; Perrow & Davy 2002a, b; Soulé & Wilcox, 1980; Miyawaki, 1975, 1981). Natural restoration is strictly related to increased sustainability and includes rehabilitation of ecosystem functions, enlargement of specific ecosystems, and enhancement of biodiversity restoration (Stanturf & Madsen, 2004). At the ecological level, restoration is also defined as "an intentional activity that initiates or accelerates recovery of an ecosystem with respect to its health, integrity and sustainability" (Society for Ecological Restoration International Science & Policy Working Group [SER], 2002). Degraded plant communities are generally quite difficult or sometimes impossible to restore (Van Diggelen & Marrs, 2003). More than 200 years of reforestation practice has demonstrated that forest recovery takes a very long time, frequently with unsatisfying results. Nowadays, it is possible to plant

plantations of several species, but the transition from the simple plantation to a forest community able to evolve and sustain itself, according to the natural successional pattern, is still a rare event. Moreover, a number of "regreening" projects in the past have paid scant attention to the source of planting materials used and their biological requirements, and have failed because of poor species choice. Use of non-local seed sources of indigenous species can result in the contamination of genepools of nearby populations (Thomson, 2001). On the other hand, the mere superficial appearance of vegetation restoration should be avoided. It is essential to restore the natural vegetation using a combination of native species that conform to the potential trend of the habitat and to try to restore the whole specific ecosystem of a region (Miyawaki, 1992). Currently, most forest reforestation programs adopt a scheme of planting one or more early successional species; after successful establishment, they are gradually replaced by intermediate species (either naturally or by planting), until late successional species arise. This pattern tries to simulate natural processes of ecological succession, from pioneer species to climax vegetation. However, it requires several silvicutural practices and normally takes a long time; because we live in a world where industry and urbanization are developing very rapidly, improvement of an alternative reforestation technique that reduces these times could be a useful tool (Miyawaki, 1999).

One reliable forest restoration method is the "native forests by native trees", based on the vegetation–ecological theories (Miyawaki, 1993a, b, 1996, 1998b; Miyawaki & Golley, 1993; Miyawaki et al., 1993; Padilla & Pugnaire, 2006) proposed by Prof. Akira Miyawaki and applied first in Japan. Restoring native green environments, multilayer forests, and natural biocoenosis is possible, and well-developed ecosystems can be quickly established because of the simultaneous use of intermediate and late successional species in plantations. The Miyawaki method involves surveying the potential natural vegetation (sensu Tüxen, 1956) of the area to be reforested and recovering topsoil to a depth of 20–30 cm by mixing the soil and a compost from organic materials. In this way, the time of the natural process of soil evolution, established by the vegetational succession itself, is reduced. Tree species must be chosen from the forest communities of the region in order to restore multilayer natural or quasi-natural forests. For a correct choice, based on reconstructing the potential natural vegetation, several analyses (e.g., phytosociological investigation) are required. Detection of the soil profile, topography, and land utilization can improve our grasp of the potential natural vegetation. After these field surveys, all intermediate and late successional species are mixed and densely planted, with as many companion species as possible (Kelty, 2006; Miyawaki, 1998a), and soil between them is mulched. In fact, biocoenotic relationships involve autoregulations between species, favouring a dynamic equilibrium and avoiding any further silvicultural practice and need no insecticides or herbicides (with some exceptions). Indeed, in the Miyawaki method, the principles of self-organized criticality and cooperation theories have been essentially applied (Bak et al., 1988; Callaway, 1997; Camazine et al., 2003; Padilla & Pugnaire, 2006; Sachs et al., 2004). If compared to traditional methods, some known restrictions regard the requirement of specialists for botanical and ecological investigation of the sites, a higher need of manpower for planting, and higher costs of plant material due to the plant density. On the other hand, no human care is required after 1-2 years from planting, the undergrowth with late-successional species is immediately on site, and forest stands become quickly part of the natural ecosystems. Moreover, the theoretical principle at the base of the definition of Regions of Provenance might be considered almost included in the Miyawaki method, as it suggests to use seed

from the nearest natural populations. Figure 7 shows a schematic overview of the comparison between classical succession theory and the one proposed by Miyawaki.

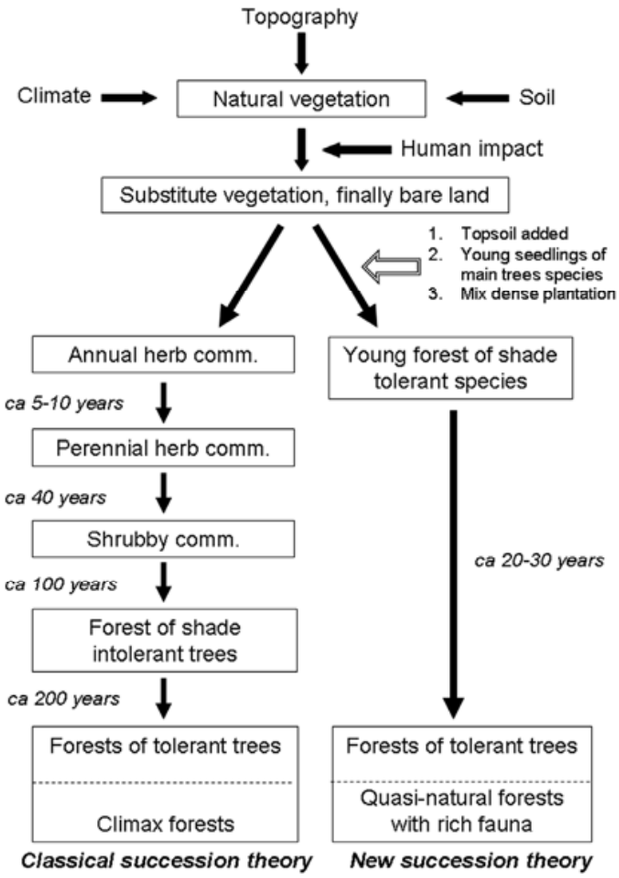


Fig. 7. Comparison between classical succession theory and the new one proposed by Miyawaki (redrawn from Miyawaki, 1999).

4.1 The adaptability of Miyawaki method to the Mediterranean environment: a case study

It has been demonstrated that multilayer quasi-natural forests can be built in 15–20 years in Japan and 40–50 years in Southeast Asia by ecological reforestation based on the system of natural forests. Results obtained by application of the Miyawaki method in about 550 locations in Japan, as well as in Malaysia, Southeast Asia, Brazil, Chile, and in some areas of China, were found to be successful, allowing quick environmental restorations of strongly degraded areas (Miyawaki, 1989, 1999). Until now, the Miyawaki method has been applied in countries characterized by cold-temperate and tropical climatic regimes, which do not experience seasonality, i.e. winter cold and summer aridity stress (cf. Mitrakos, 1980) with potential risk of desertification (increased by global change). Thus, the Mediterranean context could be considered an interesting test to assure the effectiveness of such a method in other important biomes, even with high biodiversity hotspots. Nevertheless, it could be interesting for the Mediterranean Basin, because complete environment restoration takes longer time than in tropical or cold-temperate climates. To estimate the effectiveness of

Miyawaki method in such different circumstances without altering its theoretical principles, several changes were introduced and tested in two experimental plots in Sardinia (Italy) in 1997, on target sites where traditional reforestation approaches are widely used but have mostly failed (Schirone, 1998). First, the soil condition of the planting sites was not adjusted, so no recovery of the 20-30 centimetre-deep topsoil with compost from organic materials has been done, but only a labouring of the pre-existent soil. Tillage was used to improve soil water storage over the winter and reduce water stress during the summer. Between the selected species, some autochthonous early-successional ones were planted (e.g. *Pinus pinaster* L., and shrubs) to improve plant community resilience, and no weeding after planting was done. Mulching was provided experimenting straw as in the original method, but also other types of materials (saw mill residuals, dry and green materials), and tested planting densities were assessed to 8600 and 21000 plants/hectare respectively. A particular care was dedicated to the choice of the best planting season, and watering was provided once soon after planting. Figure 8 summarizes the Miyawaki method as implemented in the mentioned experiments.

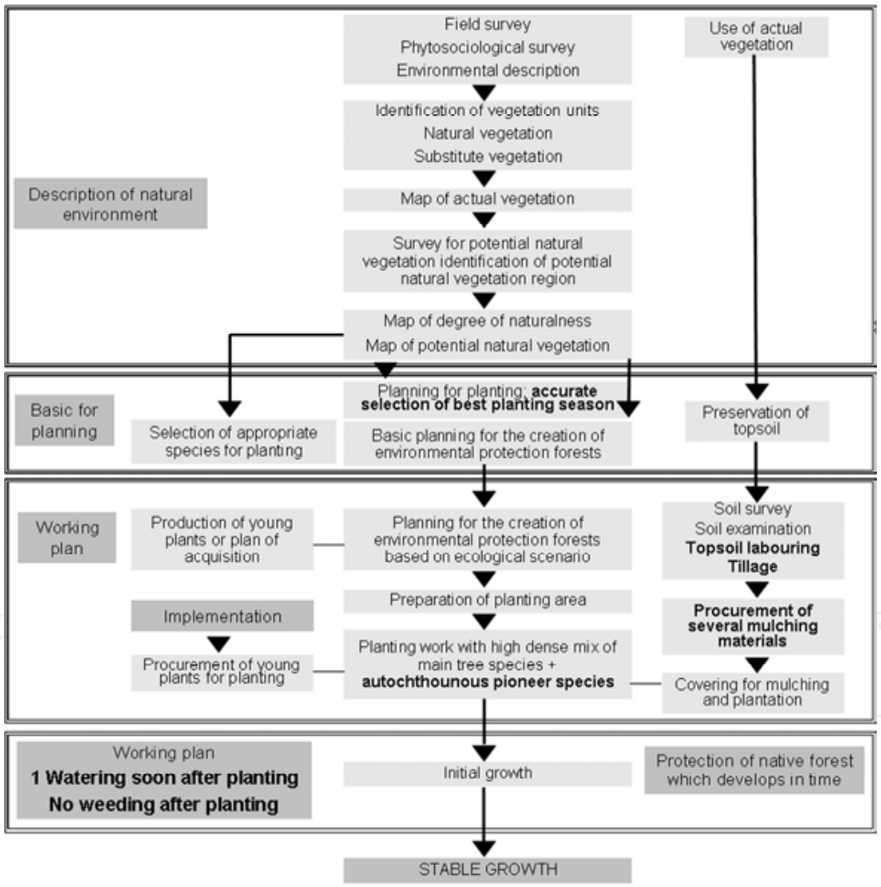


Fig. 8. Schematic overview of the Miyawaki method modified for Mediterranean environment. Dark grey text boxes describe main processes; bold texts refer to the changes to the original method.

To estimate the efficiency of this adapted Miyawaki method to Mediterranean, three surveys were performed in 1998, 1999, and 2009 in both experimental plots. Moreover, comparisons

were done with two nearby coeval sites where traditional reforestation techniques were applied to better understand the differences in plants growth, forest composition, and vegetation cover in percentage (Schirone et al., 2011). The results after 12 years from the planting showed a more rapid development of trees on the Miyawaki plots, in particular early-successional species, as well as a stable assessment of species' occurrence with high level of biodiversity (Table 3). The benefits over previous methods are remarkable and comparable with those obtained by Miyawaki in Asia and South America. At the same time, the changes made to better fit the method to the Mediterranean environment seem to be particularly useful. For instance, adding some autochthonous early successional species to the intermediate- and late-successional ones the system resilience was improved; this solution was already tested by Miyawaki in Brazil, even if no benefits were recorded (Miyawaki & Abe, 2004). Looking for an optimal high plant density, it was assessed that cooperative processes (e.g. mutual shading) prevail over competitive ones (Callaway, 1997). In fact, low plant density has been traditionally retained as appropriate in arid and semiarid environments in order to avoid competition for water resources between plants (Caramalli, 1973; Bernetti, 1995), but a higher one reduces, for instance, the impact of acorn predators, thus encouraging oak regeneration, i.e., the main late-successional forest species in Mediterranean environments (Gómez et al., 2003); high plant density can also favour root anastomosis processes, that seem to influence coenosis' stability and reforestation success (Kramer & Kozlowski, 1979). In addition, excellent plant stock remains fundamental for planting success in harsh environments (Palacios et al., 2009). Finally, these results could offer a chance to introduce a new method into the Mediterranean context that is able to reduce the time for a complete environmental restoration.

	Species survival in Miyawaki sites			**Height ± (Stand. Dev.)**			
Species	n_i	n_f	n_f/n_i (%)	MS-1	MS-2	TRS-1	TRS-2
Acer monspessulanum L.	51	2	3.92%	40 ± (14.1)	0	-	-
Arbutus unedo L.	61	41	67.21%	32.7 ± (4.1)	0	500 ± (35.8)	110 ± (20.6)
Castanea sativa Mill.	42	1	2.38%	10	0	-	-
Cedrus atlantica Endl.	-	-	-	-	-	-	162 ± (54.6)
Celtis australis L.	59	3	5.08%	26.7 ± (28.9)	-	-	-
Erica arborea L.	-	-	-	-	-	115 ± (12.7)	130 ± (18.6)
Fraxinus ornus L.	17	1	5.88%	250	-	-	-
Ilex aquifolium L.	237	23	9.70%	45.2 ± (30.6)	0	-	-
Juniperus oxicedrus L.	45	30	66.67%	-	36.2 ± (18.5)	-	-
Laurus nobilis L.	41	3	7.32%	30 ± (17.3)	0	-	-
Ligustrum vulgare L.	139	33	23.74%	32.8 ± (52.6)	30 ± (8.16)	-	-
Malus domestica Borkh.	40	7	17.50%	100 ± (45.5)	0	-	-

Myrtus communis L.	114	5	4.39%	10	10 ± (1.4)	-	-
Phyllirea angustifolia L.	1	1	100.00%	70	0	-	-
Phyllirea latifolia L.	203	0	0.00%	-	0	-	-
Pinus pinaster L.	428	288	67.29%	433.2 ± (143.6)	325.5 ± (38.6)	376.4 ± (73)	425.7 ± (25.1)
Pyrus communis L.	41	20	48.78%	71 ± (65.1)	60 ± (61.2)	-	-
Quercus ilex L.	694	255	36.74%	34.2 ± (32.1)	40.8 ± (36.2)	69.4 ± (23.2)	146.2 ± (38.1)
Quercus pubescens Willd.	361	124	34.35%	23.6 ± (27.5)	10 ± (5.3)	-	-
Quercus suber L.	632	103	16.30%	174.3 ± (49.6)	77.5 ± (51.9)	-	-
Rosmarinus officinalis L.	46	15	32.61%	89.3 ± (33.9)	0	-	80 ± (14.9)
Salvia officinalis L.	9	0	0.00%	0	0	-	-
Sorbus torminalis (L.) Crantz	42	12	28.57%	35 ± (50)	40 ± (12.9)	-	-
Spartium junceum L.	74	29	39.19%	110.7 ± (62.2)	0	-	-
Taxus baccata L.	377	9	2.39%	33.3 ± (38)	0	-	-
Thymus vulgaris L.	24	0	0.00%	-	0	-	-
Viburnum tinus L.	84	3	3.57%	10	0	-	-

Table 3. Total number of individuals in the Miyawaki sites, at the beginning of the experiment (n_i, 1997), after 12 years (n_f, 2009), percentage of species' survival (n_f/n_i), and comparison of plant height (cm) between Miyawaki sites (MS-1, MS-2) and the traditional reforested ones (TRS-1, TRS-2) in 2009. Dashes indicate species not planted, and *zero values* refer to planted species that did not survive in 2009. Successional position of each species is indicated by the row color: white (early successional), light grey (middle-successional), dark grey (late-successional).

5. Conclusion

The conservation of biodiversity has become a major concern for resource managers and conservationists worldwide, and it is one of the foundation principles of ecologically sustainable forestry (Carey & Curtis, 1996; Hunter, 1999). Many efforts were dedicated to set aside networks of reserves and protected areas advocated by scientists, governments, etc. to preserve the extraordinary biodiversity that characterizes forest ecosystems, perpetuating their integrity, their evolutionary patterns and yet providing social and environmental benefit . At the same time, a strategic value has been assigned also to biodiversity in terms of genetic resources, through the conservation of plant populations in their natural habitats (*in situ*) to better evolve and adapt to physical environmental trends and to changes in the web of interactions with other life forms. Generally, the simplest way forward in economic and political terms is for countries to locate genetic resources in existing protected areas, as this likely to provide benefits to local people communities. However, despite the critical role of conservation sites, a large debate arose about the combination of protection, management,

and restoration of forests and woodland landscapes as pivotal starting points of sustainable development in many of the world's ecoregions (e.g. Pierce et al., 2003; Norton, 2003; Aldrich et al., 2004; Loucks et al., 2004). At pan-European level, several legislative tools emphasized the need of facing habitat fragmentation, biodiversity loss, genetic pollution, and invasive species use, throughout the definition of certified basic material and ecologically homogeneous areas.

Some strategies have been included in the Directive 105/99, with the definition of Regions of Provenance and the requirements for an appropriate marketing of forest reproductive material. Unfortunatently, there was an heterogeneous achievement of the Directive by the European countries in time, as well as in adopting common methods. Mainly according to the available data, the chosen parameters for detecting the Regions of Provenance differed case by case. However, it is also evident that both agglomerative and divisive approaches could be improved by adding further variables and/or methods. Nowadays, the need for models implemented with biological parameters is suggested by a changing climate, in which bioclimatic shifts could characterize vegetation arranged along altitudinal gradients or at ecotonal boundaries (e.g. Peñuelas & Boada, 2003; Steltzer & Post, 2009). Data analysis at different temporal scales could allow to understand the effects of climate trends on species success and survival, and thus to choose the most appropriate genetic material for reforestation actions. In this view, genetic approaches must certainly be refined and made uniform through countries in order to speed up detection of diversity and comparability of results (Aguinagalde et al., 2005). At the same time, given the rapid pace of environmental degradation in many biologically species-rich parts of the world, a clear organism identification is essential for restoration experts to define species' distribution range, native plants for restoring damaged ecosystems or afforesting new ones, invasive species to fight. Moreover, it is important to check the phases of the regeneration processes of a community after an intervention, and protect native and/or threatened ecosystems. These items could be achieved by using a standardized molecular approach as DNA Barcoding, once its actual efficacy is demonstrated with preliminary study cases.

Recently, the need to understand the development and the spatial dynamics of pattern in ecological phenomena leaded to the concepts of landscape ecology, i.e. broad scale investigations strictly linked to the vegetation occurring at local scale. The Committee of Ministers of the Council of Europe adopted the European Landscape Convention on 2000, aiming to promote European landscape protection, management and planning and to organise European cooperation (European Council, 2000). The Convention is the first international treaty exclusively devoted to all aspects of European landscape, but the importance of reforestation and genetic fundamentals of landscape is not well considered yet (Granke et al., 2008).

Since the main goal is to guarantee not only simple conservation measures, but also the expansion of forest surfaces throughout reforestation actions, we need methods able to provide forest quality and reduce the time for a complete environmental restoration. This is particularly true in those areas where the environment has been modified and exploited by humans over the course of thousands of years, as in the Mediterranean Basin. In particular, forests have experienced many processes that have led to degradation and consequent soil loss as reported since the fourth century B.C. by Plato in Critias. The Miyawaki method could take up the challenge, but its effectiveness will be increased if it is joined with other tools, like well defined Regions of Provenance, *in situ* and/or *ex situ* networks of reserves for

providing the most suitable genetic resources, and DNA Barcoding to assess and monitor the trend of the intervention.

It is undoubted that we have to move toward a holistic approach, in order to improve the present methods with as many criteria as possible, and define a unique project design. For these reasons, a toolbox based on this multidisciplinary concept is presented as ideal guideline attending the gained experiences in the Mediterranean Basin (Figure 9).

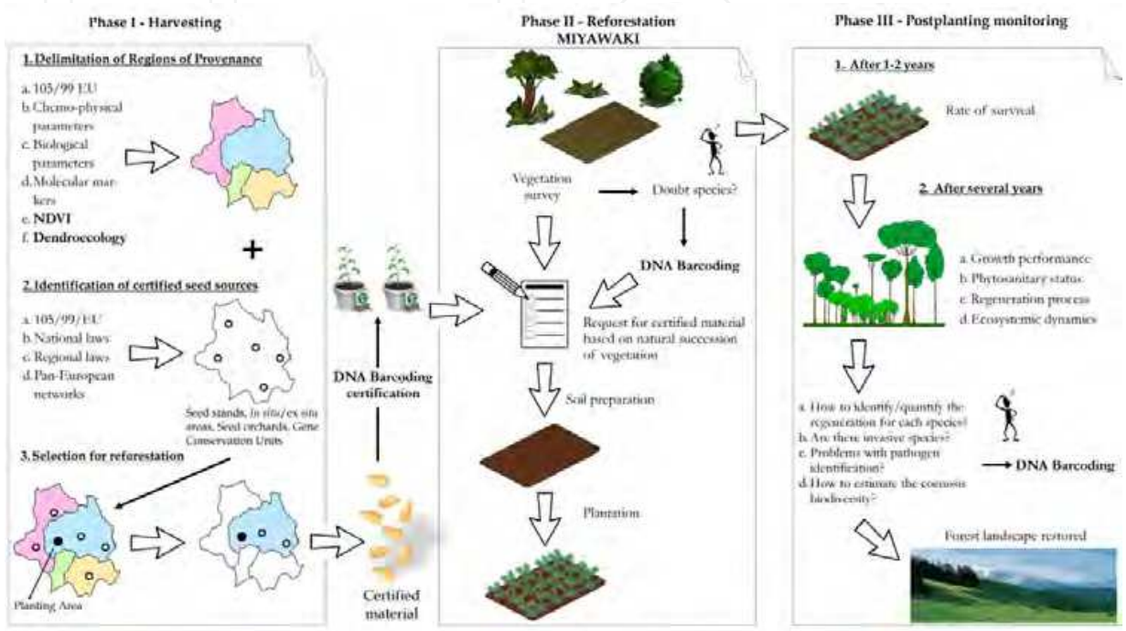


Fig. 9. Theoretical example of reforestation process implemented with the tools (bold texts) discussed in the chapter.

Computer-based methods existed since 1980 to assist tree species and provide information about uses, distribution, environment, and silviculture; nowadays The Forestry Compendium developed by CAB International (CABI) is probably the most impressive tool that has been developed (CABI, 2010). However, further developments of this tool should include information on selection systems about requirements of particular genotypes, including provenances, hybrids, clones, and genetically modified material choice. The toolbox we propose is composed of the mentioned actions and methods, including latest informatics supports, and it has been developed to be applied in reforestation activities, starting from the delimitation of the Regions of Provenance with the detection of adequate seed sources, the correct identification of plant species, the environmental and vegetation surveys, the selection of certified basic materials, up to the reforestation technique and the checks after planting. Basically, each mentioned step poses a specific question, and the toolbox would provide the answer or the best tool to achieve it. However, this proposal is a preliminary tentative to create a logic framework of actions that will require a validation measure also throughout a socio-economic analysis to estimate the costs of each step. For instance, it would be useful to understand the costs for data capture and development of further biological indexes retrieved from satellite images, the expenses for extracting and analysing DNA, including molecular markers and Barcoding of plants, the costs of manpower and plantlets for the reforestation practices.

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The book covers several topics of biodiversity researches and uses, containing 17 chapters grouped into 5 sections. It begins with an interesting chapter considering the ways in which the very biodiversity could be thought about. Noteworthy is the chapter expounding pretty original "creativity theory of ecosystem". There are several chapters concerning models describing relation between ecological niches and diversity maintenance, the factors underlying avian species imperilment, and diversity turnover rate of a local beetle group. Of special importance is the chapter outlining a theoretical model for morphological disparity in its most widened treatment. Several chapters consider regional aspects of biodiversity in Europe, Asia, Central and South America, among them an approach for monitoring conservation of the regional tropical phytodiversity in India is of special importance. Of interest is also a chapter considering the history of the very idea of biodiversity emergence in ecological researches.

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