

## THE MAIN CHARACTERISTICS OF THE GENETIC SYSTEM IN SOME FOREST TREE SPECIES

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### Abstract

*The genetic system represents the genetic pathway of organisms for organizing and transmitting genetic material that determines the balance between coherence, gene recombination, and control over the amount and type of gene combinations. The objective of this paper is to present the main characteristics of the genetic system of several species of forest trees, namely: Picea abies, Abies alba, Larix decidua, Pinus sylvestris and Quercus robur. The main characteristics of the genetic system taken into account refer to: the mode of reproduction; population dynamics type; chromosomal cycle; recombination index; presence or absence of chromosomal genetic polymorphism, etc. The used methods included searching of the various databases with the latest publications in the field and identification of some relevant results. In the case of forest trees, the genetic systems present a special situation, compared to other higher plants; is about a particular configuration of the systems components. An optimal genetic system of long-lived species, such as forest trees, is characterized by a high recombination index and cross-pollination; however it is possible to change the rate of genetic recombination through selection.*

**Key words:** genetic system, forest tree, recombination, chromosomal cycle.

### INTRODUCTION

Forests provide, directly or indirectly, important health benefits for all people. Health-enhancing qualities of forests are a result of multiple and mutually reinforcing benefits.

The conservation of forests is relevant to all aspects of sustainable development and human wellbeing; also, the forestry maintains an ecological balance (Ma et al., 2022).

The genetic system refers to all the characteristics of a natural population that determine its hereditary behavior over time periods sufficient for evolutionary (Rajora and Zinck, 2021). In another formulation, the genetic system is essentially a set of instructions stored in nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The genetic system includes the number of genes and chromosomes, the type of chromosomal structures, ploidy, the average rate of mutations, the frequency with which the crossing-over occurs, the peculiarities of the reproductive system, the type of sexual determinism, the mechanisms of the regulation of gene activity, etc.

Through the genetic system of a species it is ensured that valuable information is stored or transmitted, that other new information is created, suitable in addition to existing information or that replaces less valuable information. Genetic material, including genes and DNA, controls the development, maintenance and reproduction of organisms.

The genetic system and its components determine a population's ability to withstand evolutionary change. The evolution of the genetic system means the evolution of those mechanisms that achieve and affect genetic variability.

Being one of the most ancient groups of seed plants, the conifers are considered as a link between angiosperms and pteridosperms. The conifer genomes have a number of features that distinguish them from other plants; the most notable is their enormous genome size, which is not a result of recent polyploidization (Bondar et al., 2022).

*Picea abies*, popularly known as "spruce", is a particularly resistant and very easy-to-maintain specimen, suitable for a special garden. It adapts well to any type of soil and is equally resistant to frost and drought.

The spruce always has a pyramidal-conical crown, always green, with acicular leaves. The cones are 10-15 cm long, green or red when are young, brown when ripe, with persistent, thin, rhomboidal scales, with a truncated, ridged or even wrinkled tip.

*Abies alba*, the European silver fir or silver fir, is a fir native to the mountains of Europe. The eastern limit of the fir passes through Romania. Tree of size I/II, pyramidal crown, with acicular leaves, arranged radially, silver color with pendulous cones and parchment scales.

The European larch (*Larix decidua* Mill.) has large ecological amplitude. In the Alps and Tatra Mountains it grows in continental climates, with cold, dry and snowy winters. It grows on well-drained soils, not tolerating waterlogging, with a pH range from neutral to acid. The larch is very cold and wind tolerant during winter (dormant period), and it has a cold hardiness limit of around -30°C. Its deciduous habit confers a significant advantage by reducing desiccation damage on foliage during winter (Da Ronch et al., 2016).

*Pinus sylvestris* is a monoecious plant with pendulous blue-green or gray-green drooping cones. Leaf buds are ovoid, non-resinous. The male cones consist of an axis with scale-shaped stamens, with two pollen sacs on the upper face. The female cones are large, formed by carpel scales that present two anatropous ovules on the upper face. After fertilization the ovules become winged seeds.

*Quercus robur* (oak) is part of the *Fagaceae* family; it is widespread in the temperate climate areas of Europe, Asia and some regions of North Africa. The wood from oaks is hard and durable and valued for several purposes including for construction, furniture, veneer, fencing and firewood. It has a high tannin content, which makes it resistant to insect and fungal attacks and is particular useful for wine and spirit barrels.

Oak species also have an important ecological role, as they support insects and their acorns provide a valuable food source for many birds and mammals. The canopy of oaks allows a fair amount of light to pass through, permitting a diverse and enriched understory.

Pedunculate oak is very tolerant to soil conditions and the continental climate. It can be found in periodic wet areas by streams and

ivers, but prefers fertile and well-watered soils. Pedunculate oak is a pioneer species in plains and hills, while it is a late successional species in valleys and floodplains.

Agriculture, horticulture, forestry, economy and environmental health are interconnected and essential for identifying the best practices available (Cotuna et al., 2022a; 2022b; 2022c). The climate variability and climate changes impacting directly crops yield and indirectly the biotic constrainers might result in genetic diversity disruption (Bonciu, 2019; Velea et al., 2021), through invasion of weeds, pests and pathogens in areas where they have not been relevant before (Cotuna, 2021; Paraschivu, 2022; 2021). The conservation of the biodiversity of both agricultural and forestry ecosystems involves sustainable management measures, through the application of intensive treatments that promote the natural regeneration of species and by forest conservation (Hampe & Petit, 2005).

## MATERIALS AND METHODS

These papers present some of the main characteristics of the genetic system of several species of forest trees, namely: *Picea abies*, *Abies alba*, *Larix decidua*, *Pinus sylvestris* and *Quercus robur*.

The main characteristics of the genetic system taken into account refer to: the mode of reproduction; population dynamics type; chromosomal cycle; recombination index; presence or absence of chromosomal genetic polymorphism, etc. The research method consisted in identifying, accessing and selecting of several scientific results in the field, published in some journals indexed in WOS, Clarivate Analytics, Scopus and Springer databases.

## RESULTS AND DISCUSSIONS

### The main characteristics of the genetic system to *Picea abies*

Due to the large and complex genome of conifers, this important group of plants was, until recently, lacking species with available reference genomes (De La Torre et al., 2014). In 2013 the first draft assembly of the *P. abies* genome was published (Nystedt et al., 2013).

Norway spruce (*Picea abies*,  $2n = 24$ ) belonging to the family *Pinaceae*, is one of the most important conifers in Europe. Unfortunately, recent forest decline caused by pollution concerns mainly this species and emphasizes a necessity to study genetic background of the response to biotic and abiotic stresses.

Spruce presents a periodic flowering, the time between two abundant fruiting varying with the geographical position, determined especially by latitude and altitude. Male and female unisexual flower buds are formed in the summer before flowering, on annual spurs, and can be distinguished from September to October.

The anthesis process is influenced by air temperature; the opening of the pollen sacs takes place above 5°C, variable from one tree to another and probably genetically conditioned. It should be noted that heavy rains during the flowering period can reduce pollen production by about 25% of the potential capacity. After fertilization, the zygote divides twice and four free nuclei are formed, which are placed at the base of the archegonia, in a single plane. All cells, except those in the archegonia area, divide rapidly and are filled with substances that are nutrients for the embryo.

Genome mapping of *P. abies* genome is complicated by the biology of the species and from the complexity of the nuclear genome. One way to simplify its analysis is fractionation into individual chromosomes. The urgent need for genetic improvement contrasts with a poor knowledge of the genome (Überall et al., 2004).

The *Picea* maps were based on diploid F1 crosses with the densest composite map containing only 2,300-2,800 markers per framework map (Pavy et al., 2017).

When karyotyping the species *P. abies* var. *acuminata* it was found that 10 chromosomes have the median centromere and 2 chromosomes have the submedian centromere; also, 5 chromosomes with median centromeres showed secondary constrictions, with the possibility of forming nucleoli. Following the karyotype study of *P. abies*, it was found that their genome is very large (~20 Gbp) and contains a high fraction of repetitive DNA (Bernhardsson et al., 2019). The current

*P. abies* genome assembly covers approximately 60% of the total genome size but is highly fragmented, consisting of >10 million scaffolds (Bernhardsson et al., 2019). These authors suggest that approximately 3.8% of the anchored scaffolds and 1.6% of the gene models covered by the consensus map have likely assembly errors as they contain genetic markers that map to different regions within or between linkage groups.

According to Westin et al. (1999), some *P. abies* clones showed no consistent difference in mitotic index, either in period or in general levels (Figure 1). The response of mitotic index to temperature differed in spring and fall. Also, the differences in cold hardiness between the clones were not directly coupled to differences in mitotic index (Westin et al., 1999).

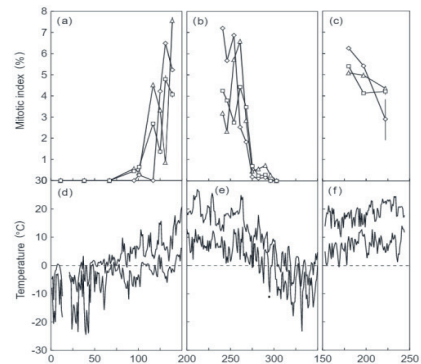


Figure 1. Mitotic indices for three spruce clones (a-c) and standard errors of the means (d-f)  
Source: Westin et al. (1999)

The use of roots offers important advantages over other systems used to isolate mitotic chromosomes. Seedlings are easy to handle, root meristems are karyologically stable and can be synchronized to obtain a high frequency of metaphase cells. In Figure 2 is selected some aspects of the *P. abies* mitosis (Bára et al., 2004).

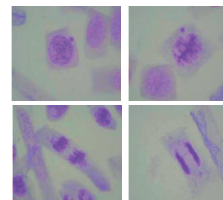


Figure 2. Aspects of the *Picea abies* mitosis  
Source: Bára et al., 2004

### The main characteristics of the genetic system to *Abies alba* (Silver fir)

Although the genus *Abies* includes over 40 species, many of them of considerable commercial importance, too few researches has been undertaken on their genetics and especially on cytogenetic relationships. With one exception (*A. firma*, in which a tetraploid with 48 chromosomes was identified), all the investigated species have  $n = 12$ , respectively 24 chromosomes in the sporophyte tissues ( $2n$ ). *A. alba* is a large conifer that can be found in central Europe and some parts of Southern and Eastern Europe. It is one of the tallest tree species of the genus *Abies* in Europe. This tree is considered an important ecological and functional balancer of European forests and a fundamental species for maintaining high biodiversity in forested ecosystems. Its future distribution is subject of a debate between palaeoecologists and modelers, with contrasting climate-response forecasts. Owing to its good cleavability and durability, especially under humid conditions, it is suitable for the production of shingles and for hydraulic engineering. Young trees of *A. alba* are very popular as Christmas trees.

*A. alba* has been showing a declining trend in its growth at its south-western limit that is related to increasing temperatures and in general in its distribution range (Dalmaris et al., 2022). Its main distribution is concentrated in Central Europe, on the Suisse plateau and in South and Eastern Germany as well as in the Czech Republic and Austria. There are conspicuous numbers in the Pyrenees, Southern Alps of Northern Italy and Ticino and the Eastern Alps, the Carpathians and Albania. It is also found more sporadically in Eastern France, on the Massif Central, and in the Apennines (Figure 3).



Figure 3. Distribution map of *Abies alba* (European Silver Fir)

Source: <https://commons.wikimedia.org/wiki/File>

*A. alba* occupies a broad range of environmental conditions and possesses a deep root system that allows to access deep water under summer drought. It is therefore considered to better cope with future drought events under climate change than *P. abies* that often occupies similar habitats (Vitasse et al., 2019).

The flowers are unisexual monoecious; the male ones are grouped in the form of thick and elongated, yellow catkins, while the female flowers are cylindrical, greenish and arranged mostly at the top of the tree's crown. The duration of the microsporogenesis phenomenon is 30-40 days. Flowering takes place from April to mid-June, depending on altitude, latitude and exposure. Unlike the spruce, the fruiting of the silver fir is more regular, more abundant and more constant. From a management perspective it is important not only to know the thresholds that determine the maximum or minimum recruitment, but also the maximum response of recruitment to a limiting factor over the gradients most frequently encountered in forest management (Trifković et al., 2023).

Silver fir is a wind-pollinated, generally outcrossing species. In dense stands with a sufficient number of mature individuals, its outcrossing rate is over 80% of all seeds produced, which is similar to many other conifer species. However, in occurrences with a reduced population size, and during years of low flower production, self-fertilization takes place (up to 95% of all seeds produced on some trees) (Wolf, 2003).

Silver fir has long been considered to be less variable than other conifers because of its low morphological variation. To preserve the population-specific genetic structures of silver fir, i.e. locally common alleles and the area-specific allele frequency distribution, many different populations from various distribution areas should be selected systematically for gene conservation purposes. The most effective way to conserve larger occurrences of silver fir and their genetic resources is through in situ conservation of stands and populations as well as their natural regeneration using long-term and small-scale regeneration methods (Wolf, 2003).

Figure 4 shows the banding and mapping of genes on chromosomes of root tip meristem

cells of *A. alba* (Puizina et al., 2008). The karyotype is characterized by three heterobranchial chromosomes, with the shortest arms, and two other chromosomes with short arms, which may or may not be heterobranchial.

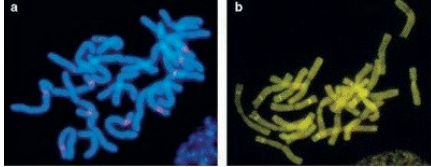


Figure 4. Banding and mapping of genes on chromosomes of root tip meristem cells of *Abies alba*  
Source: Puizina et al., 2008

The nuclear reference genome sequence of *A. alba* with an estimated size of 18.16 Gb was generated from DNA representing the adult tree AA\_WSL01 (Birmensdorf, Switzerland) in a community-based effort of the Alpine Forest Genomics Network and the chloroplast genome of this genotype was assembled (120,908 bp) (Mosca et al., 2019).

**The main characteristics of the genetic system to *Larix decidua* (European larch)**

European larch is one of the few deciduous conifers. It is one of the fastest-growing conifer species in Western and Central Europe (Figure 5), producing more than 10 m<sup>3</sup> of wood per ha annually under optimal conditions. Because of its fast juvenile growth and its pioneer character, larch has found numerous applications in forestry and agroforestry.



Figure 5. Distribution map of the native range of *Larix decidua* (European Larch)  
Source: <https://commons.wikimedia.org/wiki/File>

European larch has a high level of genetic variability for most silvicultural traits both

between and within populations. International provenance experiments have indicated the best larch populations in terms of silvicultural quality. The fastest growing populations were found among the Sudetes and Central Polish larch; Alpine populations were slowest growing. The populations from Central Europe showed also the highest stability across environments (low G x E), even across ecologically contrasting conditions, while south-western alpine larch populations performed well only at high elevation sites (Matras and Pâques, 2008).

*L. decidua* is monoecious and wind pollinated. Larch pollen is small and round without air bags. As a result it is transported by the wind over only relatively short distances of up to 300 m. Larch trees reach sexual maturity at the age of 15 years in open stands but at the age of 35-40 years in closed stands. Larch produces seeds every 3-4 years on average.

The first information on the number of chromosomes in *L. decidua* was provided by Strassburger as early as 1892 (n = 12). From the point of view of the karyotype, individual chromosomes can be classified into three groups, depending on the difficulties of individualization, as follows: easily identifiable chromosomes (I, II, III, IV and VII); chromosomes relatively easy to identify (V, VI and VIII); hard-to-identify chromosomes (IX, X, XI and XII), which practically cannot be distinguished from each other.

Of the haploid set of 12 chromosomes, six are isobranchial and six are heterobranchial. Chromosome I is the longest (144 units), with a median centromere. Of interest is the relatively long chromosome IV (118 units), which shows a satellite with a length equal to 32% of the length of the short arm and which can be very difficult to identify, because of the very narrow secondary constriction and a light strip that extends throughout chromosome width.

The satellites DNAs confer important functions with roles in cell division, chromatid separation, and chromosome stability (Jagannathan et al., 2018). Due to their fast evolution and defined chromosomal localization, may represent valuable targets to trace repeat evolution and divergence over long, evolutionary timeframes in conifers (Heitkam et al., 2021).

For *L. decidua*, idiograms have been published based on Chromomycin and DAPI banding and/or on chromosome length, arm length ratio and the visibility of secondary constrictions. But these criteria were not sufficient for an unambiguous individualization of all of the chromosomes due to similarity in size and arm length ratio, varying degrees of condensation of chromosomes between and within single metaphases and varying degrees of extension, and visibility of individual secondary constriction. Thus, Lubaretz et al. (1996) reported karyotyping chromosomes of *Picea abies*, *Pinus sylvestris* and *Larix decidua* (Figure 6), based on chromosomal localization of 18/25S and 5SrRNA genes by fluorescent in situ hybridization and on computer-aided chromosome analysis. By means of these techniques, the chromosomes of the Norway spruce and some of the Scots pine and European larch karyotype can be distinguished individually (Lubaretz et al., 1996).

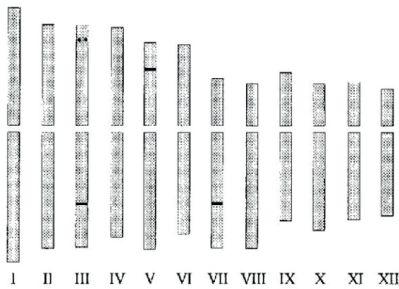


Figure 6. Chromosomes of *Larix decidua* arranged according to their relative length, arm length ratio and position of rDNA loci  
Source: Lubaretz et al., 1996

Larch seed can be stored for at least 30 years in gene banks. Pollen can also be stored *ex situ*. Cryopreservation of somatic embryogenic lines is another possibility for conservation of larch genetic resources conservation since most technical problems have been solved recently. European larch requires special management if it is to survive and flourish, especially in mixed forests. The drawing up of general rules of silviculture is necessary to ensure the establishment of progenies from the natural populations of larch and maintenance of larch stands. Assistance to natural regeneration can be provided through weed control, opening of stand canopy, complementary planting and

other management efforts (Matras and Pâques, 2008).

### The main characteristics of the genetic system to *Pinus sylvestris*

*Pinus sylvestris* (Scots pine) is a long-living, coniferous tree characterized by its orange trunk. Globally, it is the most widely distributed pine and is found throughout all of Eurasia. The genetic variety is immense and several different subspecies exist across its distribution.

Scots pine is, especially in the north of Europe, an economically important species. The wood is strong and easy to work with, making it excellent for general constructions, furniture-making and the pulp and paper industry. It is also used for stabilizing sandy soils.

This species has  $2n = 24$  chromosomes and, as with other conifers, the fact that it has long chromosomes can complicate the karyotype study for the purpose of establishing geographic variation or characterizing different taxon's. Chromosomes of medium length and those without satellites are difficult to distinguish from each other. However, the number of satellite chromosomes does not correspond to the number of nucleoli.

The karyotype of the *P. sylvestris* species highlights the 12 pairs of chromosomes of different lengths (Figure 7); 9 pairs show metacentric centromere (I-IX) and 3 pairs show submetacentric centromere (X-XII).

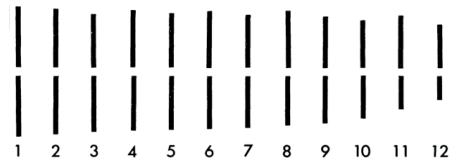


Figure 7. *Pinus sylvestris* idiogram

The formation of female and male flower primordia takes place between April and July, depending on the climate and stationary conditions. Scots pine is a normal cross-pollinated species, but self-fertilization can occur easily, and even complete self-compatibility exists. In practice, however, absolute autogamy is never achieved. Self-pollination is possible because the phenological gap between the maturation of female and male flowers is very small (usually half a day to a

day). The consequence of self-fertilization is given by the high percentage of sterile seeds and especially by embryonic mortality.

Regarding the precocity of Scots pine flowering and the formation of cone clusters, we can speak of a genetic control.

Under natural conditions Scots pine is not readily interfertile with other pine species. Spontaneous hybrids with *P. nigra*, *P. densiflora* and *P. mugo* have been reported. Towards other taxa, the species shows a robust hybrid incompatibility (Mátyás et al., 2004).

When selecting gene conservation units along a continuous cline, ecological information should be preferred to neutral markers. In the absence of drift, in a contiguous distribution range adaptively different populations may be expected at distances where annual mean temperature differs by a minimum of 1.0-1.5°C (equal to ca. 200 km in a flat landscape) (Mátyás et al., 2004). The size of gene conservation units of Scots pine should be sufficiently large to compensate for and buffer against outside gene flow: 100 ha should be considered the minimum. Nearby occurrences of genetically degraded or otherwise unsuitable stands should be either avoided or removed. A conservation unit should consist of numerous adjoining stands of various ages, provided their origin is the same. In areas of scattered occurrence, initial size may be 10 ha as a minimum, which can be increased during successive regenerations (Mátyás et al., 2004).

### The main characteristics of the genetic system to *Quercus robur* (oak)

The genus *Quercus* comprises approximately 400 to 500 species and has been divided into two subgenera: subgenus *Cerris* characterized by rugulae visible in mature pollen grains or weakly masked and subgenus *Quercus* characterized by receptor-independent sporopollenin masking rugulae in mature pollen grains.

The genus *Quercus* spreads all over the northern hemisphere in Asia, North America, Europe and Africa, down to the Equator. Over the whole genus, there are about as many species in America (200 to 245) than Eurasia (196). However, Europe comprises only 22 species and Asia +100. Diversity is highest between 15° and 30° Northern latitude in

central America (particularly Mexico) and South Asia (Figure 8) (<https://quercusportal.pierroton.inrae.fr/>).



Figure 8. Distribution map of genus *Quercus*  
Source: <https://charlois.com/en/quercus/>

Oaks are considered by many societies as sacred trees, symbols of strength and endurance, with high cultural and historical value. They provide important environmental services like carbon sequestration, reservoir of biodiversity, soil and water protection, etc. They also provide several economic and cultural services like carpentry, furniture, cabinet making, veneer, cask industry, fuel wood, hunting and fungus gathering (<https://quercusportal.pierroton.inrae.fr/>).

Oak has 24 chromosomes in its diploid phase. Extra chromosome  $2n = 24+1$ , 2 or 3 extra chromosomes have been reported as consequences of irregular segregation in mitoses (Zoldos et al., 1998).

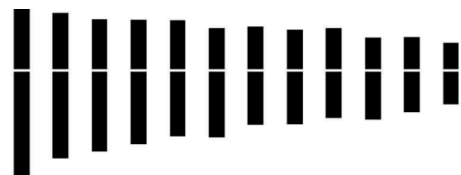


Figure 9. *Quercus robur* idiogram

Pedunculate oak (*Q. robur* L.,  $2n = 2 \times = 24$ ) is an outcrossing, highly heterozygous diploid species. Flow cytometry analysis has shown that this species has a genome of 740 Mb per  $C^{33}$ , where the C-value is the amount, in picograms, of DNA contained within a haploid nucleus (Plomion et al., 2018).

Works on the genome of *Quercus robur* tree native from Europe began in January 2012, at INRA Bordeaux. Few years later, this genome has been sequenced, assembled and annotated.

In its last version, are characterized around 26,000 genes and estimated that 50% of the 750M bp of haploid genome was made of repetitive elements (Plomion et al., 2018). This first oak genome assembled so far, provides a foundation to study the biology and evolution of these species. In particular, it will allow identifying genes essential for the adaptation of these long-lived organisms to their environment, genes involved in the symbiotic relationships between the mycelia of truffles and its root, genes responsible for the biosynthesis of wood extractives such as tannins and lactone that gives their flavor and taste to alcohol and wine ([https://www.oakgenome.fr/?page\\_id=244](https://www.oakgenome.fr/?page_id=244)). Although vegetative characters are important and frequently preferred in the identification of oaks, these are considered as quite risky because of hybridization between oak taxa. Scientific interest has recently moved from classic description to biological understanding of oak evolution by means of molecular markers (Denk and Grimm 2010; Simeone et al. 2013). However, oak taxonomy is still problematic and under debate due to often insufficient diagnostic morphological characters (Yılmaz, 2018). Being a plant with typical anemophilic pollination, the oak produces a large amount of pollen which the upward currents can climb up to about 3000 m. Self-pollination and self-fertilization are not excluded, but there is a selectivity mechanism during fertilization that ensures preferential fertilization with pollen foreigner.

## CONCLUSIONS

*Picea abies*, *Abies alba*, *Larix decidua*, *Pinus sylvestris* and *Quercus robur* are five species of *Pinaceae* that are of considerable economic and ecological importance in Central Europe. Similar to nearly all of the about 200 species of *Pinaceae* and many other gymnosperms, they have more or less symmetric karyotype consisting of  $2n = 24$  morphologically rather similar chromosomes.

The satellites DNAs confer important functions with roles in cell division, chromatid separation, and chromosome stability. Due to their fast evolution and defined chromosomal

localization, may represent valuable targets to trace repeat evolution and divergence over long, evolutionary timeframes in conifers.

The genetic systems present a special situation, compared to other higher plants; is about a particular configuration of the systems components.

The conifer genomes have a number of features that distinguish them from other plants; the most notable is their enormous genome size, and this can create some difficulties in their research due to limit of computational resources. There are several factors responsible for a large genome size in conifers, but the main is polyploidy and amplification of transposable elements. Also, these elements present a source of genetic variation, because contribute to mutations increase and affecting the gene expression.

An optimal genetic system of long-lived species, such as forest trees, is characterized by a high recombination index and cross-pollination; however it is possible to change the rate of genetic recombination through selection. The priorities for specific gene conservation to conifers measures differ regionally. Preservation of genetic resources depends of the forest management practices, the extent of protected or unmanaged areas and the fragmentation of the species. Of course, the urgency to set up gene conservation units is much higher in an area with fragmented populations than in a region which sustainable forestry.

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