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PhD THESIS

The study of genetic variability for different larch provenances (*Larix decidua* Mill.) from Baciu Orchard, Cluj

Summary of the PhD Thesis

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Introduction

The doctoral thesis, "The study of genetic variability for different larch provenances (*Larix decidua* Mill.), from Baciu Orchard, Cluj Forestry" aims to study phenotypic, genotypic and molecular variability for diferrent larch provenances.

Because of the biological material analysed and the work methods, the study distinguishes as original, since such an evaluation of phenotypic and molecular variability has been little studied in Romania. Even more, the theme of the study contributes to the researches that impose in the last years in order to reduce the erosion of the genetic diversity and to consolidate the stability of ecosystems.

In the first stage of the thesis there was considered the thorough study of researches in the field and also the profound investigation of specialty literature, of researches conducted and the results obtained nationally and globally.

In my researches, there was conducted an exhaustive study, at a phenotypic and molecular level, for seven larch areas in Romania and for descendents obtained through breeding, located in Baciu Orchard, Cluj-Napoca.

Being known the fact that larch seeds have low germination capacity, of only 30-50%, in order to see if seed germination can be stimulated, treatments with different substances, on more variants, were performed.

Most studies on larch seed germination suggest recommendations for different treatments (WITOWSKA, 2009; GRODNITSKAYA, 2008; KOVYLINA, 2008; HANSEN, 2008).

Based on these reasons, within the study it was conceived and created a data basis with the phenotypic traits of larch seeds from Baciu Orchard, which is useful in identifying genotypes and for offering relevant information concerning the phenotypic correlations between seed traits and their percentage of germination.

The results can be extremely useful in improving the process of obtaining new genotypes and the judicious choice of genitors for breeding methods.

Also, the study followed tree resistance to pest (*Coleophora laricella*, *Adelges laricis)* and disease (*Hipodermella laricis*) attacks, besides the study of germination faculty and germination capacity of larch seeds under laboratory conditions.

Molecular characterization of these larch provenances was made with the help of RAPD (Randomly Amplified Polymorphic DNA) markers. This stage was conducted in the Department of Biotechnologies of UASVM Cluj-Napoca. The results obtained through RAPD method allowed the estimation of genetic correlations among the studied larch provenances, and made possible to project the dendogram for the studied genotypes.

The study of phenotypic and genotypic variability for the larch provenances considered, allows the selection of valuable genotypes, with the purpose of using them as potential genitors in future breeding works.

Larch breeding program started in Romania in 1963-1965, once with the process of seed carting for the most valuable trees, and their designation as seed sources.

In Romania, European larch (*Larix decidua* Mill.) occupies approximate 30 000 ha (STĂNESCU, 1979), surface out of which approximate 4 000 ha are natural brushes and the rest are artificial brushes, largely created with seeds imported from Austria. Although the percentage of participation of this specie in forest structures is small, larch is still appreciated for wood, for its rapid growth and for the contribution it could offer in increasing the stability of spruce brush, in the composition of which it can be inserted.

This also explains the interest for extending this specie through heavily cultivation, resulted also in the delimitation of approximate 970 ha of seed reservations, from which 507 ha are occupied by larch reservations and 134,6 ha are larch orchards that assure the necessary seed for the production of seedlings (MIHAI, 2007). Although there were taken many measures, the

demand of seeds is still covered, mostly, from imports, which denotes that the created seed basis do not produce enough seeds.

The insufficient production can be caused by many factors, among which the most important are late frosts, which, often, completely destroy the flowers of this specie very early, (OLENICI, 2004).

Orchards represent plantations of untested plus trees, selected upon phenotypic traits and breaded by vegetative methods. Orchards have reached the age of 30 – 40 years and it is necessary to test the genetic value of plus trees in order to achieve a first selection according to traits related to the objectives of breeding.

In consequence, orchards offers the opportunity not only of obtaining genetic improved seeds, of conservation "*ex situ*" of the most valorous genetic forest resources in Romania, but, in the same time, they constitute a wide genetic basis for obtaining advanced generations for breeding programs.

The current PhD thesis is structured on seven chapters and contains 220 pages, 54 tables, 44 figures and 184 bibliographic titles.

Part of the results obtained through the 3 years of study were, or are to be, published in specialty publications, indexed in recognized international data basis (BDI). Two representative papers were presented at the international symposium "Prospects for the 3rd Millennium Agriculture", UASMV, 2011, and they are published in "Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture" (index CABI, ISI-MJL). Other articles are the ones published in „Journal of Forest Science" and respectively „Agricultura" magazine, while one article is prepared for an ISI publication.

The importance of larch in forest economics

Larch, also known as tamarack, is one of the indigene conifers that present a special importance in national economy, due to its wood superior qualities, great productivity and longevity, but also because of its decorative characteristics.

Among all the other conifers cultivated outside the natural area with the purpose of increasing forest productivity, the larch has enjoyed a great deal of attention from the scientific world, due to the contradictory results obtained in artificial cultures. In Germany, the European larch has been heavily extended in forests in the XVIII and XIX century; many trees had dried because of the *Dasyscypha wilkomii* fungus attack. In the same time, other healthy trees reached old ages and recorded remarkable growth.

Deepen knowledge of biological and forest traits of larch, considered to be „an enigmatic specie" (DENGLER, 1935) determined, once with the promotion of the modern spirit „multiple use of the forest", acquiring new valences and the growth of the percentage used in afforestation.

From an economic point of view, in Europe and especially in our country, natural larch tree presents little importance due to its low spread. Larch becomes valuable specie from an economic point of view outside the natural area, where in certain stationary conditions and mixing with other species, trees are much more productive than the local types of natural forests: a greater volume of wood and more ranges obtained.

Its wood is one of the most precious woods that our forests produce; it is for this reason that it has been called the oak of the mountains. Its complete lignification and its richness in resin assures a longer duration, while the regularity and tighten growths (altering rough and soft areas) provide remarkable strength and flexibility. Larch wood is resistant, hard, heavy, very elastic and resistant even in water.

Larch, (*Larix decidua* Mill.), although with a more limited natural distribution and with a lower percentage in artificial cultures compared to the pine and to the spruce, it has

been the subject of numerous researches and studies, so that, as ROHMEDER and SCHONBACH (1959) claim, if we were to establish the importance of the specie according to the number of published papers then it should be considered among the most valuable species in Europe.

The tamarack presents a series of particular biological traits (early fructification, disjoint area, a great genetic diversity), doubled by important forestry traits (growth speed, easiness in cultivation outside the area, superior wood quality, relatively high resistance to adversities).

Due to its light behavior and also to the high resistance to cold and heat, larch can be cultivated in open areas, after deforestations. This is in favor of larch expansion in artificial cultures.

In fields where natural regeneration is not sufficient, it can be successfully used for filling. Rapid growth in youth, and also until the ages of 40-60, makes possible its further installation in natural seedlings.

In perspective, larch will play a more and more important role in Romanian forestry. In the range of extending of the conifers, in many stations it will represent the source specie, especially when we consider the easiness in cultivation, the fact that the litter decomposes rapidly and produces a humus of mull type, the stability of high yields (reduced interaction between genotype x environment), etc.

Its mixed rooting determines a higher resistance than the one of spruce to the action of powerful winds. Due to this, it is common to increase the resistance of spruce to wind breaks by associating it with larch.

Also, on soils less profound it seems to be more resistant to wind breaks and snow ruptures than the pine, because, compared to this, it has a denser crown and, usually it becomes leafy after the fall of snows. As fire wood, the larch has the inconvenient that it throws sparks more than any other resin species. The coal that is obtained from larch wood is more appreciated than the one from spruce or pine. It produces important quantities of resin, which is rich in turpentine (20-22%); this turpentine being is clean and of higher quality than the one from the pines. Rough resin is used in the production of paints and varnishes, in enameled colors and red wax in papermaking, patches and ointment. Once with the resin there are also eliminated soluble starches (a viscous liquid which thickens very quickly) which is used in textile industry in the process of thickening the colors of different fabrics, in the production of paints, medicinal products, etc.

Distribution area for larch throughout Europe

Even though larch is a European tree, its distribution is restricted to small areas. The most important and large area is in the Alps, starting with the region of the city Nyssa, close to Vienna, following high altitudes.

The larch is also met in Czechoslovakian and Carpathian Sudetens, of Poland (Tartan) and Romania. In Poland, except for the Carpathians Mountains it can be found also on the mountain Chelm, close to Nowa Slupia. Its spread is limited to mountain regions, of high and middle latitudes, without extending, such as the spruce in north plains. The European larch appears at heights that vary between 200 and 2400 m (VIDAKOVIC, 1991) in pure stands and mixed with the Norwegian spruce (*Fagus sylvatica*), *Abies sp.* and *Pinus mugo* Turra (MØLLER, 1965).

ABAIMOV (1998) claims that at high altitudes, the maximum of distribution doesn't represent the maximum of vegetation, but more a recourse of where it was castoff by other species. At a height of 1500 m, the growth of the larch diminishes and weather accidents lead to many deformations of the trunk.

In the Alps and in the Carpathians larch is a tree of high altitude. The center of the area is considered to be Austria where it is commonly found, except for the region from the left of Danube. The altitude limits present significant variations: in the French Alps 1000 (exceptionally 1600m-2000m) -2000 m (exceptionally 2600 at Queyras and Tinee); in the Italian Alps it goes up to 2400-2500 m and down to 250 m in Lombardy, 300 m in the province of Venice and 350 m in Trenton; in Switzerland it vegetates between 600 and 2400 m. In the Sudeten Mountains the most important areas can be found between the altitudes of 300 and 800 m and in Tartars (Slovakia), and also between 1100 and 1300 m; in Poland it vegetates at altitudes of 600 m in the south of the country and at 450 m in the north.

Larch distribution area in Romania

In Romania, the larch spontaneously vegetates in five centers (HARALAMB, 1967):

- In Ceahlău Mountains, in Poliţa and Crini (1400-1450 m altitude), Piatra cu Apă (1500 m) and under the rocks Piatra Neagră.
- In Ciuca Mountain, on Zăganul (1000-1450 m altitude) and Tigăile.
- In Bucegi, in many points: Furnica, Piatra Arsă, Mălăieşti, and in Gârbova, but also on the valleys Ciuta and Adâncă (1200-1550 m altitude).
- In Lotru Mountains, near Târnovul Mare and Târnovul Mic as important points and in the surrounding mountains (Gorgani, Voineasa, Vânata, and others).
- In Apuseni, in many points: Gilău, the mountain Scărişa, Piatra Vulturesei, Trascău Mountains and in some spots on the rivers Arieş and Geoagiu (600-1200 m altitude).

Normally, larch is situated at the altitude limit of vegetation, where it forms pure trees or mixed with spruce, pine (Coştila, Bucegi), beech, fir, ash and birch (Târnovul). In the past, in our country the larch was more spread than nowadays, when the specie occupied by this specie diminished until approximately 4500 ha. This drastic reduction of the surface is due to intense exploitations along the years, as a consequence of the special wood qualities (ŞOFLETEA and CURTU, 2001).

The center of maximum spread is in Bucegi, where the area afforested with larch is of approximately 1570 ha, from which 470 ha are disseminated. Lotru Mountains are next, where it can be found in natural stands on approximately 1380 ha, from which disseminated 620 ha (GUREAN, 1990, 2000).

Most of these natural stands are situated at over 1000 m altitude, reaching the limit of the forest area (1700-1750 m) or even beyond it (in Lotru Mountains up to 1900 m, in Bucegi up to 2050 m), but only in bush samples. For a long time it was thought that the larch, being mountainous specie, can not be found under 1000 m of altitude, due to cancer and wood depreciation. Researches made in Romania showed that larch has been cultivated in proper stations records with active growth and significant accumulations of biomass up to altitudes of 300 m. The most active growths and the largest productions, that justify framing larch in the category of quick growing species, were obtained in hill regions and middle mountains, in mix with spruce, fir, ash and oak, where its productivity reaches 15 m^3/ha.

The morphological traits of the larch

The larch belongs to the family of *Pinaceae,* is an indigene tree, with a height of up to 65 m and a diameter of 2 m.

The stem is straight, slender, slightly cylindrical, well shaped, sometimes it can deform. In the opinion of some researchers, this shape could be hereditary, while others consider that it could be due to the dominant wind or the direction from which snow is coming. In our country,

in the valleys of Latorița and Ialomița, larch has reached heights of 60- 65 m and diameters of 2-2, 5 m.

Rooting is pivoting at the beginning and later it forms deep lateral roots, well anchored into the soil. On clay soils roots become superficial. The form and the size of the root system vary between certain limits, according to age and stationary conditions (STĂNESCU, 1979).

The bark, when young is smooth, grey, formed very early and that can reach more than 10 cm, it will break into irregular pieces. The wood is very precious, distinct heartwood, of superior qualities, compared to other conifers and many deciduous species. The bark contains tannin, in a percentage of 7-16% (REMINGTON, 1995).

The crown is narrow and with a long, pyramidal shape, formed by thin, long branches, opened and reverberated at the extremities, without whorls. The branches which form the crown are numerous, long and thing. Due to the crown shape and density, the light easily gets through the branches, the soil hardly being covered.

The sprouts are of two kinds: long- thin, pendent, yellowish, and furrow because of the pillows of the leaves and short sprouts, dark brown, with a bug at the end and; in spring, a couple of weeks after flowering, some short sprouts develop, transforming in long sprouts.

The shoots have a form almost spherical, brown color, and the surface covered with yellow lyme, they contain resin. The shoots on the short sprouts are the ones that first start the vegetation, and only later the shoots from the long sprouts.

The needles are obsolete, soft, 1-3 cm length and with stomata lines only on the back; on long sprouts they are solitary and spirally arranged, and on the short ones they stay grouped in 30-40. After fall, the needles leave on the sprouts a triangular scar. In autumn, the leaves color in yellow-orange, thus giving a decorative aspect to the tree. The fallen needles decompose very quickly, forming very convenient humus.

The flowers are unisexual, monocots and scattered around the entire crown; the male ones have an ovoid form, of yellow color, while the female ones are ovoid-spherical, erect, red-purple or green with many scales.

It flowers and leafs at the same time, very early in spring, at the end of April, beginning of May. It fructifies early, especially when it is cultivated in temperate regions but, in this case the seeds are dry. Only around the age of 30 years they start to fructify on a regular basis and have fertile seeds; they fructify isolate at the age of 15-20 years.

The cones, ovoid-long up to 4 cm, with skin scales, rounds and flat, have short bracts, with sharpen ends, are visible only after the dissolution of scales. The cones are attached to a stem and after the seed is disseminated they remain on trees for other 2 up to 4 years.

The seeds, long of 3-4 mm, overgrow with a wing of 7-8 mm, and do not have resin bags. A kilogram of seeds contains approximately 160000 seeds without wings. The maturation of seeds takes place in the autumn of the first year, in October- November, and the dissemination in the following spring. Fructification takes place every 3-5 years and at high altitudes at 6-10 years. The germinative capacity is reduced, of only 20-45% and can be maintained for 3-4 years.

Biological material

The biological material used in the research is represented by different provenances of the specie *Larix decidua* Mill. In Baciu Orchard, the material for the study was formed by larch clones obtained through grafting from plus trees that were selected from seven different natural or artificial larch populations from Romania.

Methods

Evaluation methods for the main traits of the trees from origin areas

The phenotypic analysis of the biological material studied implied 50 trees from each forestry department: Gura Humorului, Braşov-Valea Cetății, Săcele, Braşov-Valea Popii, Sinaia, Anina and Latorița.

There were studied the traits that, based on specialty literature (SAVATTI, 2005), are considered to be significant in any variability study. These traits are: trunk height (m), basis diameter (measured at 1,30 m from ground) (cm), diameter at 1 m (cm), and form defects such as taper (cm), ovality (cm), curvature (cm) and sprawling (cm).

The realization of the observations and measurements for these traits were made accordingly to the following:

- Measurement of tree *height* is very important in determining the volume and other form parameters. In this operation it was used the hypsometer, determining for each tree the height after well-established procedures. In order for the measurements to be as exact as possible, the operator was situated at a distance close to a presumed tree height.

- Measurement of tree *diameter* was made with the help of a forest-die at a height of 1, 30 m from ground, diameter also called „at the base". There were made two perpendicular measurements in order to obtain medium diameter, because the majority of the trees have irregularities and only in ideal situations their diameter is a perfect circle.

- *Curvature* (Cu) consists in the curve deviation of the trunk axe, from a straight line, in one or more plans. The curvature is due to large burdens or tree symmetry, caused by wind, snow, crown irregularity, etc. It was calculated using the formula: Cu=s/L (cm/m), where "s" represents the arrow (cm) and "L" represents the length of the curve portion (cm/m). The curvature is measured by placing a bar at both ends of the curvature and with a reglet the maximal arrow will be measured.

- *Abnormal taper* (AbT) is continuously increasing, more pronounced for the ranges of round wood (1 cm/m) of the diameter from basis to the peak of the trunk. Abnormal taper is specific to all forest species. It was determined by using the formula: AbT=D-d/L (cm/m), where "D" represents the diameter at the thick end (cm), "d" represents the diameter at the thin end (cm) and "L" represents trunk length between these two diameters, measured in cm or m.

- *Sprawling* (Sp) represents a pronounced, abnormal thickening of the trunk basis for a certain portion from the bale up. Sprawling was calculated using the formula: Sp=D-d/L, where "D" stands for diameter at base trunk in cm, "d" represents diameter at 1,00 m distance from the base in cm, and "L" the length between the two sections in cm or m. The defect leads to wood loose and to the bearing down the processing of wood.

- *Ovality* (Ov) represents the oval form of the transversal section of the trunk. Often, ovality also implies the drift of the wood marrow from the center of the section towards the exterior. The defect is associated with the action of dominant wind, and in other situations with the asymmetric development of the crown or the tree's root system. Ovality was calculated with the formula: Ov=D-d/D (cm/m), where "D" represents the ellipse's large diameter, in cm, "d" small diameter in cm. The measurement of the two diameters was made using the forest-die, at the same height, using two diameters on different directions.

Evaluation methods of the main traits for larch clones in Baciu Orchard

These studies meant taking into consideration 50 trees from each provenance. At the clones from the seven provenances there were made numerous calculations, observations and measurements and field analysis for each tree, all this data being centralized for each clone

separately. The following traits have been studied, traits which based on specialty literature are considered to be extremely important in the variability studies. These traits are: tree height (cm), diameter at 1,30 m from the ground, (cm), diameter at soil basis (cm), diameter at one meter from the ground (m), height at first ramification (m), crown diameter (m), crown height (m), number of whorls, distance between whorls (cm), number of branches, branch thickness (cm), insertion angle (degrees), volume (m^3) and form defects such as taper (cm), curvature (cm), sprawling (cm), ovality (cm).

The observations and measurements for these traits were made in the same way as those in origin areas:

- Measurement of *crown diameter* was made using a reglet, using the horizontal projection of the whorl from the trunk basis. In order to obtain very exact values, two perpendicular measurements were made.
- The *number of branches* was determined by counting each ramification. In order to obtain a value close to the real one, two counts were done for each tree.
- The measurement of the *insertion angle* was made with a protractor in order to underline the angle formed by the branches with the trunk of the tree, because it is well-known the fact that this angle influences flowering and fructification.
- *Branch thickness* was measured with a reglet. In order to determine this trait there were taken into consideration only the branches at the tree basis, the result being the arithmetic average of the measured branches.

Evaluation methods for the main traits of seeds and cones from Baciu Orchard

The larch cones were harvested from Baciu Orchard at the end of 2010. The harvest was made manually using poles with hooks; in case of taller trees there were used double stairs with the height of 4 m.

The cones were harvested and transported in wooden cases, separated for each provenance in Baciu Orchard. After harvest, the cones were stored in special magazines. Because they are not that easy to break, the extraction process was made in dryers at a temperature of 35˚C, up to maximum 40˚C, for 48 hours.

Conifer seeds, in their majority, have wings, which facilitates the process of natural dissemination, through air currents. At larch, the wings overgrow with the tegmen seeds.

The breaking of larch seeds was made manually, by gentle rubbing between hands. The result was a mixture of seeds (different dimensions and qualities) and impurities, vegetal remains (pieces from the wings). The cleaning system of the seeds implied their fan in an air current produced by a ventilator.

The cones and the seeds of each clone were analyzed from the point of view of the main morphological traits.

The analyzed traits for larch seeds and cones, considered to be illustrative for the variability of each clone, were the following:

- Cone weight (g), using the analytical balance with four decimals;
- Cone length (cm) calculated with the caliper;
- Cone width (cm) calculated with the caliper;
- Seed weight (mg), using the analytical balance with four decimals;
- Seed length (mm) calculated through measurement, using the reglet;
- Seed width (mm) calculated through measurement, using the reglet.

Methods of determining seed germination

Breeding through seeds is a special and important method because it maintains the genetic diversity of different larch populations.

The seed is considered sprout if specific biochemical and physiologic processes can take place, process that can trigger embryonic activity and determine the growth and development of a new plant. The germination capacity of seeds can be established, first after the healthy appearance and also after the reaction to different physical and chemical treatments.

In general, larch seeds have low germination capacity. At a quantity of 85-95%, the germination is only 30-50% (DAVID, 2002).

The majority of the studies on larch seed germination suggests and recommends different treatments (WITOWSKA, 2009; GRODNITSKAYA, 2008; KOVYLINA, 2008; HANSEN, 2008). Seed germination was determined under two points of view: germinate energy and germinate faculty. Germination was studied under the influence of six treatment variants. So V_1- water, V_2- concentrated hydrous solution that also contains active substance- sodium, V_3- concentrated hydrous solution, with a content of auxines of 9%, V_4- concentrated hydrous solution that also contains active substance of copper (2,5 g/0,5 l water), V_5- concentrated hydrous solution that also contains active substance potassium permanganate (1,25 g/0,5 l water), V_6- concentrated hydrous solution that also contains active substance calcium hydroxide (lime-20,0 g/0,5 l water).

Determining tree behavior to diseases and pests in Baciu Orchard

Tree behavior to disease attack

In the orchard there was noticed the attack of *Hypodermella laricis* Tub. (the yellowing of larch needles). The disease manifests itself through the coloring of the needles in yellow and ultimately drying. On the dried needle, peritheciums appear, in a median longititudinal line, in the needle ascospores, in the shape of a tear. The ascospores get into the cuticle of the soft needles and the mycelium attacks (sub) epidermal tissues. During summer, the areas bleach, grey, yellowish and light-brown spots appear.

The recording method of the intensity of the attack (I%) was made on 30 trees from each clone in Baciu Orchard.

Attack appreciation for *Hypodermella laricis* was realized according to the classification of the attack in a system of 6 classes (Table 2.1.); the noting system was adapted after the Forest Protection, 2000.

Tabelul 2.1./*Table 2.1.*

Aprecierea atacului în funcție de intensitate
Attack appreciation according to intensity

Aprecierea atacului/ *Attack appreciation*	Intensitatea atacului (%)/*Attack intensity(%)*
Fără atac / *No attack*	(I% = 0)
Atac foarte slab / *Very weak attack*	(I%=0,1-1)
Atac slab / *Weak attack*	(I%=1,1-5.0)
Atac mediu/ *Average attack*	(I%=5,1-15)
Atac puternic / *Strong attack*	(I%=15,1-30)
Atac foarte puternic / *Very strong attack*	(I%>30,1)

Tree behavior to pest attack

At the trees from Baciu orchard there were studied the effects of two pests which attack larch leaves: *Coleophora laricella* Hb. (miner mol for larch needle) and *Adelges laricis* Vall. (Larch louse), in 2010-2011.

Coleophora laricella Hb. is a pest that attacks larch needles, the butterfly having wings wide 9-10 mm, the female being smaller than the male.

Adelges laricis Vall. is a small insect (louse), larva sting and suck the sap of the needles, this specie having a development cycle that comprises 6 generations and lasts two years, using as main host the spruce and as secondary host the larch.

In order to determine the degree of infestation of the trees in the orchard, for the pest *Coleophora laricella* there were considered the caterpillar stage (from October to March).

In order to have a better precision of population density, there were taken and analyzed 12 branches per tree (4 branches at the tierce of the crown peek, 4 at the tierce in the middle and 4 at the tierce at the basement, at each level there were chosen branches from all 4 cardinal points.

Reporting the number of larva to the number of short sprout, it was determined the medium number of caterpillars per sprout, respectively the degree of infestation. According to this, it was determined the percentage of defoliation. (Table 2.2.).

Tabelul 2.2./*Table 2.2.*

Stabilirea gradului de infestare în cazul dăunătorului *Coleophora laricella*
Determining the degree of infestation for the pest Coleophora laricella

Nr. mediu de omizi/lujer scurt/ *Average number of caterpillars/ short stalk*	Infestare/ *Infestation*	Procent probabil de defoliere/ *Estimative procent of exfoliation*
<0,12	Foarte slabă/*Very weak*	<10%
0,13-0,30	Slabă/*Weak*	11-25%
0,31-0,60	Mijlocie/*Average*	26-50%
0,61-0,90	Puternică/*Strong*	51-75%
0,91-1,20	Foarte puternică/*Very Strong*	76-100%

După ICAS, Câmpulung Moldovenesc/*According to*: *ICAS, Câmpulung Moldovenesc*

The branches that were selected in order to establish the density of the *Coleophora* population were also used for establishing population density for *Adelges,* but this time by reporting the number of *Sistens* adults to the number of short sprouts. According to this, it was calculated the percentage of defoliation (Table 2.3.).

Tabelul 2.3./*Table 2.3.*

Stabilirea gradului de infestare în cazul dăunătorului *Adelges laricis*
Determining the degree of infestation for the pest Adelges laricis

Nr. mediu adulți/lujer scurt/ *Average no. of adults/short stalk*	Infestare/ *Infestation*	Procent probabil de defoliere/ *Estimative procent of exfoliation*
<0,03	Foarte slabă/*Very weak*	<10%
0,04-0,08	Slabă/*Weak*	11-25%
0,09-0,17	Mijlocie/*Average*	26-50%
0,18-0,25	Puternică/*Strong*	51-75%
0,26-1,33	Foarte puternică/*Very strong*	76-100%

După ICAS, Câmpulung Moldovenesc/*According to*: *ICAS, Câmpulung Moldovenesc*

The method RAPD - Random Amplified Polymorphic (randomly amplified DNA)

RAPD technique was first described by WILLIAMS and colab. (1990). It is based on the polimorfism of the parental forms at an allelic level concerning the presence or absence of amplification products, as a result of using only one oligonucletodic decamer primer which randomly connects in the genome, where it finds homology.

In general, each primer will determine the amplification of some sequences from more spots in the genome, the method proving to be an efficient way of investigating DNA polimorfisms between individuals (TINGEY and DEL TUFO, 1993).

What was intended by RAPD was to determine the profile of the bends of the amplification products from electrophoresis in agarose gel. The amplification with the same primer, achieved for different genomes, can reveal differences of the bends profile in gel when electrophoresis, examining the reaction products. These differences can be due to the absence of some bends or to the presence of additional bends, in comparison with the profile with which the comparison is being made.

The vegetal material was constituted by 77 samples from different provenances. A part of the vegetal material came from Baciu Orchard, another part from the areas in the country where the clones originate. From these genotypes, there were gathered young sprouts which were immediately introduced in liquid azote, after which they were transferred in the freezer, for conservation until the moment when the extraction of DNA was done. The protocol used for DNA extraction was the one of LODHI and collaborators (1994), improved by POP and collab. (2004).

Results and discussions

Results obtained on trait variability for larch trees of origin areas

After statistically processing the data on trunk traits, through tree height, the provenances from Bv. Popii and Anina distinguished. Opposite, with a low height of trees were noted the provenances Săcele, Latorița and Sinaia.

When classifying provenances according to trunk diameter (diameter at sol base, at 1 m and at 1,3 m), values which were expressed in cm, it is noticeable that valuable provenances from the point of view of wood production were the following: Gura Humorului, Anina and Bv. Valea Popii. The smallest diameters were recorded at the trees from Bv. Valea Cetății and Săcele.

As for the trunk form, the most obvious defects were taper and curvature. KARLMAN (1998) reveals the fact that defects such as curvature are pretty common for larch and are often influenced by genetic factors, but they can also be caused by environmental factors such as frost, wind and snow pressure.

Results obtained on existing variability for larch, after the study of the main traits for seeds and cones

In the present study there were analyzed seeds from the 7 larch clones from Baciu Orchard. The following traits were studied, which based on specialty literature (NEGRUȚIU and ABRUDAN, 2004; HOLONEC, 2007) are considered to be important: cone weight (g), cone length and width (cm), seed weight (mg), seed length and width (mm).

The study of the main traits of seeds and cones present a significant importance for identifying different larch provenances and also in obtaining information on possible correlations between seed traits or the way these influence the germination capacity and rise of plants.

In the conditions of medium variability of cone traits (length, width and weight) we can conclude that for the provenances of Valea Popii and Sinaia, the quality of the cones is the best. Large weight of cones (4,79 g and 4,93 g) is directly proportional with the weight of the seeds and implicitly with their quality.

The seeds with the largest weight belong to the following provenances: Sinaia (74, 4 mg), Anina (67,7 mg), Bv. Valea Popii (67,0 mg), all presenting superior differences, statistically assured, compared with the experience average (61,9 mg). The seeds with the smallest weight belong to the provenances of Latorița (46, 2 mg) and Bv. Valea Cetății (54,4 mg).

Analyzing seed variability from the point of view of their weight, length and width, we can notice that the best results were recorded at Valea Popii and Sinaia. Comparing these results with the ones obtained through the study of cone traits variability, we can conclude that in terms of seeds and cones weight, the provenances Valea Popii and Sinaia are the most valuable.

From the results obtained, the high value of breeding for the main traits of tree fructification allows that, through a careful selection of the genitors, there can be obtained generative descendents, facilitating the spring of new valuable genotypes.

Results obtained on improving seed germination capacity and the possibility of applying growth treatments

Propagation by seeds is a reproduction method with a special importance because it sustains the genetic diversity for different larch provenances. Studies regarding seed viability and longevity and also germination treatments are recently new (SORG, 2010). Knowing the fact that larch seed has a lower germination capacity, of only 30-50%, in order to see whether the germination can be stimulated different treatments were applied.

Seed germination was determined under two aspects: germinative energy and germinative faculty.

Studies showed that at larch, no matter the provenance, treating seeds with auxines (ANA) 9%, copper sulphate şi potassium pergamanate greatly improves the germinative energy and faculty.

Sinaia, no matter the treatment, recorded the weakest percentages in the case of germinative energy but also for germinative faculty.

The provenances Bv. Valea Cetății, Bv. Valea Popii and Latorița recorded the highest percentage of germination, in the case of germinative energy but also germinative faculty.

For larch seed, in order to have a better germination, it is recommended to use the treatment with auxines (ANA) 9%, copper sulphate şi potassium pergamanate.

The lower percentage of seed germination can be explained through the high percentage of empty seeds. The results obtained are valuable and useful in the production of seeds, but also for a better germination, issues that are problematic for this specie.

Results obtained on trait variability for larch clones in Baciu Orchard

Following observations, measurements and determinations for three from the seven clones of Baciu Orchard (Gura Humorului, Bv. Valea Cetății, Săcele, Bv Valea Popii, Sinaia, Anina and Latorița), there were obtained important data, which were statistically assured, as medium values for each character, including also the differences between variants, and the comparison with the control variant.

Trunk variability was analyzed through tree height, trunk diameter (at 1,3 m from ground and at sol basis), height at first ramification and tree volume.

After statistically processing the data on tree height, from all the seven clones, four presented statistically assured differences in comparison with the medium average of trait per

experience (12,03 m), average considered control variant. With a high height stood out the clones from Gura Humorului, Anina and Bv. Valea Cetății. Opposite, with a low height the following clones were recorded: Latorița, Bv. Valea Popii and Sinaia.

The largest diameter per tree was recorded at the clones from Gura Humorului and Bv. Valea Cetății, and the smallest diameter at the clones from Bv. Valea Popii and Sinaia.

The highest tree volume was recorded at Gura Humorului (14,23 m^3), Bv. Valea Cetății (13,43 m^3) and Anina (13,30 m^3), and the smallest volume at Bv. Valea Popii (11,52 m^3) and Sinaia (11,16 m^3).

As potential genitors for trunk traits were remarked the origins Gura Humorului, Anina and Bv. Valea Cetății. These clones can be successfully recommended for forestation works, some of them being appreciated also for good seed germination.

Crown trait variability was analyzed in terms of whorls number and branches, insertion angle, branch thickness, crown diameter and distance between whorls. For the clones that were studied the number of whorls and the number of branches presented superior values, positive, statistically assured for the Anina provenance. Inferior values to the control variant were recorded at the clones from Săcele and Latorița for the number of whorls and for the number of branches. Results on insertion angle were not statistically assured for any provenance.

On branch thickness we could say that the trees from Gura Humorului and Anina form the thickest branches in whorls.

Superior values for crown diameter compared with the experience average (6,71 m) presented the provenances: Gura Humorului (7,78 m) and Sinaia (7,22 m), and the smallest crown diameter was recorded at the clones from Latorița.

The variability of trunk form defects was analyzed in terms of taper, curvature, sprawling and trunk ovality.

As for trunk form, the most pronounced defect was curvature, with statically assured differences at the clones from Anina and Bv. Valea Cetății. For defects such as taper, sprawling and ovality were not registered statistically assured values.

If we try a classification of the seven origins from Baciu Orchard we can say that superior values for all the productivity elements were recorded at the clones from Gura Humorului and Anina. These clones can be recommended for a future selection in order to obtain a higher productivity. The clones from Bv. Valea Popii and Latorița recorded the highest values for the traits studied.

Besides the fact that these provenances present superior values of all productivity traits, they also present a medium towards high variability for most of the traits studied.

The limits of variability coefficients for the traits analyzed are situated between 4,6 % for the insertion angle and 60,6 % for ovality.

Within the study, there were recorded direct correlations, significant, positive between tree height and all the other traits, with the exception of insertion angle and form defects. There were also obtained positive correlations between the number of whorls and the number of branches, positive correlations between the number of verticals, crown height and distance between whorls.

Between the insertion angle of branches and all the other traits there were recorded statistically assured values, but correlation coefficients were negative.

Within the experience, the majority of the analyzed traits presented a significant genetic determinism, trunk and crown traits being influenced by genotype, and form defects by environmental factors.

The values of heritability coefficients underline a genetic control, from moderate to strong, for the traits analyzed. It results that all the traits have a powerful genetic determinism, being influenced more by the genotype than by the climate conditions.

Results obtained on larch clones behavior to pest and disease attacks in Baciu Orchard

The behavior to the attack of *Hipodermella* (the yellowing of larch needles) was different from one clone to another. According to the data obtained, the reaction to the attack of *Hipodermella* was different, in regard to the six classes of noting, the seven clones were registered in three classes: very weak attack (I%=0,1-1), weak attack (I%=1,1-5,0) and medium attack (I%=5,1-15).

No matter the position of the attack in the crown, with very weak attack there were recorded the clones from Latorița, Gura Humorului, Bv. Valea Popii and Bv. Valea Cetății, and with medium attack the clones from Săcele and Sinaia.

No matter the clones analyzed, high intensity of *Hipodermella* attack by the position in the crown was registered at tree base, north exposition.

Results underline the fact that within these clones there are gene sources for improving larch resistance to the attack of *Hipodermella*. In this sense, the clones analyzed in Baciu Orchard, have potential to be recommended as potential genitor for new breeding works.

From among the most dangerous pest for larch needles are *Coleophora laricella* and *Adelges laricis*.

From the data obtained, the most resistance clones at the attack of *Coleophora* were recorded at Latorița and Gura Humorului, and the most sensitive at Bv. Valea Cetății, Sinaia, Anina and Bv. Valea Popii.

No matter the clones analyzed a high intensity of *Coleophora* according to the position in the crown was recorded in the peak of the trees, in east, vest and south parts. According to data, the reaction to the attack of *Coleophora* was very weak.

The provenances of Latorița and Gura Humorului, in term of resistance to the attack of *Coleophora*, can be recommended as potential genitor source in future breeding works.

According to the presented data, the reaction of clones to the attack of *Adelges* was diverse, in regard to the six classes of grading, the seven clones were classified in two classes: weak attack (I%=0,04-0,08) and medium attack (I%=0,09-0,17).

From the results we can notice that, no matter the position of the pest in the crown, with weak attack there were recorded the trees of Latorița.

High intensity of *Adelges* attack was registered at the peak and the middle of the crown, in east, vest and north parts.

From all the Baciu clones, the trees from Latorița, from the point of view of their resistance to the attack of *Adelges*, can be recommended as potential genitors for breeding works.

Specialty literature underlines possible connections between tree behavior to the attack of pests and diseases, respectively between sensibility/resistance to pathogen agents and/or insects; it was considered useful to verify some correlations, with the purpose of using them in breeding works.

Within this experience there was identified a significant positive correlation between the intensity of the attack of the two pests, *Coleophora* and *Adelges*.

Heritability coefficients had considerable values for the intensity of the attack for pests and diseases, showing a consistent contribution of the clones to the phenotypic behavior of tree response to the diseases and insects analyzed.

The data obtained illustrate the fact, that in the given conditions, all the traits analyzed concerning the reaction of genotypes to the attack of main pests and diseases presented a strong genetic determinism. In consequence, within the experience, larch sensibility or resistance was strongly influenced by the genotype and in a lower percentage by the climatic and culture factors.

Results obtained on larch variability, by molecular marking methods

The molecular technique used was the technique of RAPD markers.

The medium quantity of DNA was of 104, 30 ng/µl. The quantity of DNA varied from 30, 47 ng/µl (Gura Gumorului) up to 1634,07 ng/µl (Săcele).

There were used 15 primers, with a content of guanine and citozine between 70%-80%, for all the 77 samples. From the 15 primers used for amplification, only 5 primers generated polymorphic bends: OPAL-20, OPC-15, OPA-03, OPAB-11 and OPA-18. In the case of RAPD analysis, the ***dendrogram*** represents very well the phylogenic relations between different provenances of *Larix*. The analysis of the ***dendrogram*** reveals also a diversification in three large groups, noted from „A" to „C".

Group "A" included three subgroups, grouping five areas from the country and four provenances from Baciu Orchard. According to the ***dendrogram***, with the biggest share were the provenances of Anina and Gura Humorului.

Group "B" included two subgroups, grouping four areas from the country and one provenance from Baciu Orchard. According to the ***dendrogram***, with the biggest share were the provenances of Latorița and Sinaia. We can assume that the traits these provenances have in common have been inherited from a common genitor.

Group "C" reunites the provenance from Bv. Valea Popii, having the biggest share in this group. According to the ***dendrogram***, genetically tied are also the provenances of Săcele and Sinaia.

The closest provenances from the genetic point of view, analyzed through RAPD technique, according to the genetic distance between them, are the following provenances: Anina and Valea Cetății (0, 25); Latorița and Anina (0,23); Gura Humorului and Valea Cetății (0,29); Latorița and Valea Cetății (0,28). The biggest genetic distance was between Sinaia and Anina (1, 00); Gura Humorului and Anina (1, 00), being the farthest from the genetic point of view.

For a better interpretation of the results, there were used molecular analyses by DNA bulk. The primers OPAL-20, OPC-15, OPA-03, OPAB-11 and OPA-18 were tested, generating a strong polymorphism for all genotypes.

With the help of the five primers used there were obtained a number of 40 polymorphic bends, the average being 8 bends/ primer.

The genetic approach from origin areas and Baciu Orchard, established on the matrix of genetic distances and the algorithm of Neighbor Joining Tree, is represented by a ***dendrogram***.

The analysis of the ***dendrogram*** obtained through DNA mix reveals a uniform diversity between the origin areas and Baciu Orchard, noted with the letters „a" for origin area and „b" for Baciu Orchard.

It results that the majority of the areas are grouped in the same branch with the provenances from Baciu, Latorița area „a" with Latorița area „b", Anina area with Anina, Sinaia with Sinaia, except for the provenances Săcele and Bv. Valea Popii, which are in the same branch. An explanation could be the short geographical distance between the two provenances.

RAPD technique through DNA mixing proved to be a valuable instrument in determining the phylogentic relations within larch provenances, conclusion underlined also by precedent studies.

Conclusions and recommendations

Because of the many uses of larch trees, the enrichment of the genetic fond existing with new genotypes is absolutely necessary. In our country, breeding works for larch were not that common, so that with this thesis it was indented an enrichment of specialty knowledge in genetics and breeding for the specie *Larix decidua*.

The results obtained lead to several conclusions on the study of genetic variability for different larch provenances, for the trees from the origin areas and also for the descendents obtained through grafting in Baciu Orchard.

Conclusions and recommendations after the evaluation of trees in origin areas

After the statistical processing of data on trunk traits, in terms of tree height, good results were observed at Bv. Valea Popii (33,46 m) and Anina (33,14 m). Opposite, with a small height of trees were recorded the provenances of Săcele, Latorița and Sinaia.

By classifying provenances according to trunk diameter, we notice that the most valuable provenances from the point of view of wood production were: Gura Humorului, Anina and Bv. Valea Popii. The smallest diameters were recorded at Bv. Valea Cetății and Săcele.

As for trunk form, the most pronounced defects are taper and curvature. The most sensitive trees to taper were the ones in Gura Humorului, and the most resistance in Anina. In case of curvature the straightest form was found at the trees from Valea Cetății, and the most affected by this defect were the ones from Valea Popii.

Recommendations

As potential genitors for breeding works the trees from Gura Humorului, Anina and Bv. Valea Popii are recommended in regard to height and tree diameter.

Conclusions and recommendations after evaluating the main traits of seeds and cones for the clones in Baciu Orchard

The study of the main traits of cones and seeds presents a high importance in identifying different larch provenances, and also in obtaining information on correlations between seeds traits and the way these influence the capacity of germination and spring of plants.

In conditions of medium variability of cones (length, weight, width) we can conclude that in the case of the provenances Valea Popii and Sinaia, the quality of the cones is the best. Heavy cone weight (4,79 g respectively 4,93 g) is directly proportional with seed weight and implicitly with their quality.

The seeds with largest weight were in the provenances: Sinaia (74, 4 mg), Anina (67,7 mg), Bv. Valea Popii (67,0 mg), all of them presenting superior differences, statistically assured in comparison with the experience average (61,9 mg). The seeds with the lowest weight have been recorded in the provenances of Latorița (46,2 mg) and Bv. Valea Cetății (54,4 mg).

From the main traits of cones and seeds, the smallest value of variability coefficient was recorded for seed weight (CV=16,7%).

Analyzing seed variability in terms of weight, it was noticed that the best results were recorded at the provenances Valea Popii and Sinaia. Comparing these results, we could say that in terms of dimensions and, especially weight of seeds and cones, the provenances of Valea Popii and Sinaia are the most valuable ones.

Conclusions and recommendations on improving seed germination capacity and the possibility of applying growth treatments

Studies revealed that at larch, no matter the provenances, treating the seeds with auxine (ANA) 9%, copper sulphate and potassium permanganate, improves both germinative energy and capacity.

Sinaia provenance, no matter the variant of treatment, recorded the smallest percentages for both these traits.

The provenances Bv. Valea Cetății, Bv. Valea Popii and Latorița recorded the highest germiantion percentage.

The lower percentage of seed germination for larch seeds can be explained through the high percentage of empty seeds. The results obtained can also be useful in producing seeds with a better germination.

Conclusions and recommendations on traits variability for larch clones in Baciu Orchard

The study of the main traits allowed the evaluation of the biological material used in the experience, the realization of a synthetic description of the clones, but also the identification of possible genitors, in the purpose of using them in breeding works.

The variability of trunk traits was analyzed in terms of height, trunk diameter (at1, 30; at 1 m from the sol and at sol base), height at first ramification and tree volume.

After statistically processing the data on tree height, from the seven clones, four presented statistically assured differences in comparison with the medium average of the trait per experience. (12, 03 m), considered control variant. With a high height were recorded the clones from Gura Humorului, Anina and Bv. Valea Cetății. Opposite, with a low height were the trees from: Latorița, Bv. Valea Popii and Sinaia.

The largest volume per tree was recorded at the clones from Gura Humorului and Bv. Valea Cetății, and the smallest diameter at the clones from Bv. Valea Popii and Sinaia.

The largest volume per tree was recorded at the provenance Gura Humorului (14,23 m^3), Bv. Valea Cetății (13,43 m^3) and Anina (13,30 m^3), and the smallest volume at Bv. Valea Popii (11,52 m^3) and Sinaia (11,16 m^3).

Recommendation

As potential genitors for trunk traits were remarked the provenances Gura Humorului, Anina and Bv. Valea Cetății. These clones can be succesfully recommended. Besides the fact that these facts present superior values of all productivity traits, they also present a medium to high variability for the majority of the traits analyzed.

The variability of crown traits was analyzed in terms of the number of whorls and branches, insertion angle, branch thickness, crown height, crown diameter and distance between whorls. Fro the clones studied the number of whorls and branches presented superior values, statistically assured for the trees of Anina. Values inferior to the control variant were recorded at the clones from Săcele and Latorița fro the number of whorls and the branches. The insertion angle did not present statistically assures results for any provenance.

On branch thickness we can say that the clones from Gura Humorului and Anina from the thickest branches in whorls.

Superior values for crown diameter compared with the experience average (6, 71 m) presented the provenances: Gura Humorului (7,78 m) and Sinaia (7,22 m), and the smallest crown diameter was recorded at the clones from Latorița.

Variability of trunk form defects was analyzed in terms of trunk taper, curvature, sprawling and ovality.

As for trunk form, the most pronounced defect was curvature, with statistically assured differences for the clones from Anina and Bv. Valea Cetății. At defects such as taper, sprawling and ovality did not record statistically assured values.

If we try a classification of the seven provenances from Baciu, we can say that superior values for all productivity elements were recorded at the clones from Gura Humorului and Anina. These clones can be recommended for a future selection in order to obtain a high productivity. The clones from Bv. Valea Popii and Latorița recorded the smallest values for the traits analyzed.

Conclusions and recommendations on trees behavior to the attack of pests and diseases

The behavior to the attack of *Hipodermella*, (the yellowing of larch needles) was different according to larch provenances. According to data, the reaction of clones to the attack of *Hipodermella* was diverse, in regard to the six noting classes, the seven clones classifying in three classes: very weak attack (I%=0,1-1), weak attack (I%=1,1-5,0) and medium attack (I%=5, 1-15).

No matter the position of the attack in the crown, with a very weak attack, were recorded the trees from Anina, weak attack for the clones of Latorița, Gura Humorului, Bv. Valea Popii and Bv. Valea Cetății, and medium attack for the clones from Săcele and Sinaia.

No matter the clones analyzed, a high intensity of *Hipodermella* attack according to the position in the crown was recorded at the base of the trees, northern exposition. The results underline the fact that within the clones there are gene sources for improving larch resistance to the attack of *Hipodermella*. In this sense, the clones from Anina are recommended as potential genitors.

The most dangerous pests of larch needles are *Coleophora laricella* and *Adelges laricis*, which defoliate the larch trees.

No matter the position of the pest in the tree crown, the most resistance clones to the attack of *Coleophora* were in Latorița and Gura Humorului, and the most sensitive clones in Bv. Valea Cetății, Sinaia, Anina and Bv. Valea Popii.

No matter the analyzed clones, high intensity of *Coleophora* attack according to the position in the crown was recorded in the peak, in east, vest and north parts. According to data, clone reaction to the attack of *Coleophora* was classified as a weak attack.

Recommendation

From the larch clones in Baciu Orchard, the provenances Latorița and Gura Humorului, due to their high resistance to the attack of *Coleophora*, can be recommended as potential genitors for new breeding works.

According to the data, clones reaction to the attack of *Adelges* was diverse, as the seven clones classified in two classes: weak attack (I%=0,04-0,08) and medium attack (I%=0,09-0,17).

From the data it can be noticed that no matter the position of the pest in the tree crown, with weak attack were recorded the trees from Latorița.

High intensity of *Adelges* attack was recorded at the peak and the middle of the crown, in the directions E, V and N.

From the clones analyzed in Baciu Orchard, the trees from Latorița can be recommended as potential genitors for new breeding works.

Specialty literature remarks possible connections between tree behavior to pests and diseases, respectively between their sensibility/resistance to pathogen agents and/or damaging insects. It is for this reason that it was considered useful the verification of correlations, in the purpose of using them in breeding works.

Within the experience, there was identified a positive correlation between the intensity of the attack between the two pests, *Coleophora* and *Adelges*.

Heritability coefficients in broad sense had considerable values for the intensity of the attack to pest and diseases, showing a consistent contribution to phenotypic manifestation of the response of trees' behavior to the analyzed diseases and insects.

The data shows that, in the given conditions, all the traits analyzed upon the genotype reaction to the attack of the main diseases and pests present a strong genetic determinism. In consequence, within the experience, the resistance or the sensibility of clones to diseases and pests was strongly influenced by genotype and less by climatic and culture conditions.

Conclusions and recommendations on molecular marker methods

Knowing the efficiency of molecular markers in the analysis of plant diversity, within the present experience it was considered useful the verification of phylogenic relations, represented by genotypes belonging to the specie *Larix decidua* from the seven different areas in the country and descendents obtained through grafting, situated in Baciu Orchard. In this purpose, there were effected studies at molecular level and there was made the phylogenic characterization of the provenances.

The molecular technique used in order to identify genetic relations between populations for the studied specie was RAPD marker technique.

The ***dendrograms*** resulted after computer processing the data are a faithful representation of the phylogenic relations between the different larch provenances. According to the dendrogram obtained through RAPD technique, the provenances were classified in three distinct groups, noted A, B and C.

Group "A" included three subgroups, grouping five areas from the country and four provenances from Baciu Orchard. It worth to remark the fact that this group also includes trees from Baciu Orchard. According to the ***dendrogram***, with the biggest share were the provenances of Anina and Gura Humorului.

Group "B" included two subgroups, grouping four areas from the country and one provenance from Baciu Orchard. According to the ***dendrogram***, with the biggest share were the provenances of Latorița and Sinaia. We can assume that the traits these provenances have in common are inherited from the same genitor.

Group "C" reunites the provenance from Bv. Valea Popii, having the biggest share in this group. According to the dendrogram, genetically tied are also the provenances of Săcele and Sinaia.

The closest provenances from the genetic point of view, as analyzed through RAPD technique, according to the genetic distance between them, are the following: Anina and Valea Cetății (0,25); Latorița and Anina (0,23); Gura Humorului and Valea Cetății (0,29); Latorița and Valea Cetății (0,28). The biggest genetic distance was recorded between the provenances Sinaia and Anina (1,00); Gura Humorului and Anina (1,00).

For more conclusive results there were made molecular analysis through DNA mix (bulk).

The analysis of the ***dendrogram*** obtained through DNA mix reveals an uniform diversity between origin areas and Baciu Orchard, notated with the letters „a" for origin area and „b" for Baciu Orchard.

From the ***dendrogram*** obtained through DNA mix, we can notice that the majority of the areas are grouped in the same branch with the provenances from Baciu Latorița „a" with Latorița „b", provenance Anina with Anina, Sinaia with Sinaia, except for the provenances of Săcele and Bv. Valea Popii, which are on the same branch. An explanation could be the small geographical distance between these two provenances.

The closest provenances, obtained through Nei genetic distances, based on the analysis from DNA mix are the following: Anina „a" and Bv. Valea Cetății „a" (0,18), Gura Humorului „a" and Bv. Valea Cetății „a" (0,14); Anina „b" and Anina „a" (0,12); Săcele „b" and Săcele „a" (0,11).

The largest Nei genetic distances were recorded at the provenances Sinaia „a" and Bv. Valea Cetății „b" (0,42), Valea Popii „a" and Bv. Valea Cetății „a" (0,40), Bv. Valea Popii „b" and Gura Humorului „a" (1,50) these being the farthest from the genetic point o view.

RAPD technique through DNA mix proved to be a valuable instrument to determine phylogenic relations within larch provenances.