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DOCTORAL SCHOOL OF ENGINEERING RESOURCES PLANT AND ANIMAL

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Eng. ANDREEA MARIA SASU (LAZAR)

SUMMARY OF PhD THESIS

CREATING GENETIC VARIABILITY AT APPLE TREE AND SELECTION OF VALUABLE GENOTYPES, WITH DIFFERENT INCREASE, PRODUCTIVITY AND HIGH QUALITY AND RESISTANCE AT CERTAIN DISEASES

Scientific coordinator:

Prof. univ.dr.eng. AURELIAN ADRIAN BACIU

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ABSTRACT

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The apple tree is the pomiculture plant cultivated with the highest importance for the temperate climate area of Terra, as for production and surface (over 80 million tons and over 4.75 million ha).

Throughout the hundreds of years of culture, an impressive number of varieties (more than 10 000) was created, each with differentiated genotypic and phenotypic characteristics, as well as with different behaviour in areas of the culture and for various culture systems.

The general trend is to increase the genetic variability atMalus variety and to create new genotypes to meet the requirement of the society, especially of the future generations.

In my own researches, in the conditions from theUniversity of Craiova SCDP Valcea, having the available genetic basis, I proposed the creation of a large genetic variability, particularly with consecrated apple tree varieties, but also withcolumnar forms, the selection of valuable genotypes, the proliferation and monitoring in competition comparative culturesand some of these, that have outstanding characteristics, to promote at new varieties.

Objectives of the own researches

The general objective of the research was to obtain and promote 1-2 genotypes as new varieties.

The specific objectives of the work refer to:

1- Assessing accessions of the Working Collection and selection of genitors in view of using them for the creation of genetic variability;
2- Creating a large genetic variability using different improvement methods (hybridization, mutagenesis, individual and clonal selection);
3- Setting up the field of hybrids and mutants, assessment of main phenotypic characteristics and selection of most valuable ones;
4- Clonal proliferation and setting up a comparative competition culture with valuable selection and their assessment;
5- Determining the way of transmitting in descendance of certain important characteristics for the apple tree culture;

6- Phenotypic characterization of main selections object of the proposals for homologation and/or patenting as new varieties.

Biological material

The biological material used in the researches carried out by the University of Craiova (Valcea Pomiculture Research and Development Station) consisted of accessions existing in the Working collection for apple tree, in a number of 125.

Most accessions are formed of new varieties and hybrids and present a large genetic diversity. Plants are grafted on rootstock M9. Their age is comprised in between 5-15 years. The research period was during years 2010-2015.

Research methods

Various research methods were used in the work, according to the scope and the assessment of genetic variability obtained:

Obtaining genetic variability selection of genitors according to their main characteristics (geographic origin, genetic origin, plant vigour, yield, fruit quality, etc.). In the paper, part of hybridizations carried out by SCDP Valcea were taken over from previous years (2010). The research carried out previously for this species were sporadic at this station (natural hybridization).

The sexuate hybridization was natural as type, following the classic methodology where only the maternal genitor was known.

In total, hybrid seeds were obtained from: 9 natural hybrid combinations; 16 artificial hybrid combinations, out of which – 6 presented in the work; in hybrid stages in year I-10.

Artificial hybridization since 2013-2014 gave birth to artificial hybrids that were at present transferred in the hybrid field, but as they could not be assessed, therefore they were not included in this paper work.

During the sexuate hybridization stage, the proceedings aimed also at obtainingmutant plants by treating certain annual branches of Granny Smith with Deuterium(D2O) in a concentration of 3% and 20%.

Transmission in descendance of certain features obtained at descendants: columnar character; semi columnar character; standard character;

Recording of observations was done on the field and in the laboratory, using the method of laying in randomized blocks, mono-factorial type, with 4 to 10 repetitions, according to the character pursued.

Own research results

Genetic resources, assessment and selection of the genitors. In the Working collection of Valcea SCDP, 125 accessions were made available.

It should be noted the presence of apple tree varieties, of international circulation, and especially of varieties of columnar type Wijcik, Telamon, Trident, KV8, KV42, KV7, etc.

The assessment of genetic resources and their valutation allowed for selection and use of genitors: **standard** type: Florina, Idared, Granny Smith, Starkrinson, Wagener Premiat, Generos, Liberty and Prima; **columnar** type: Telamon (sin Waltz), Wijcik, KV8, KV42 and Trident

Creating genetic variability at the apple tree

In this work paper, the following methods of improvement were used: natural hybridization, artificial hybridization, mutagenesis and clonal selection.

With the help of 13 genitors and 21 hybrid combinations, out of which 14 derived from natural hybridization, 9 are presented in the paper, the remaining 6 being artificial combinations.

The descendants obtained from 15 hybrid combinations present a large variability.

The total number of hybrids studied in the field of hybrids is of 325; with columnar port - 118 hybrids(34.76%); with **semi columnar** port - 28 hybrids (11.70%); with**standard** port - 179 hybrids (53.54%). The hybrid combinations where at least one genitor was columnar,produced columnar descendants in different percentages. Combinations with varieties: Starkrimson, Granny Smith and Idared, with free pollination did not give birth to columnar descendants.

The character of columnar port of plants is determined by the following features:

The plant growth under the form of axis, singular, rarely 2-3 erect branches started at 25-50 cm from the soil, on the main axis; the elements on the central axis are very dense, presenting themselves as a compact growth with no side branches; the foliage is dense and compact; internodes are very short; they tend to fruiting spur (on skewers or directly on the axillary buds on the vertical axis); they form very small fruits (25-50g) or averagefruits (rarely exceeding 150-200), generally asymmetric, round-conical and with a relatively fine taste.

The descendants are distinguished one form another by architectonicspecific features of the plant: columnar, semicolumnar andstandard.

Variability relating to growth at genotypes on their own roots:

The trunk sectional area (SST); SST, the basic element in determining the vigour of the plants recorded the following values (age 5-7 years): at **columnarhybrids**– average SST =

39.9cm^3, depending on the hybrid consistency; coefficients of variation average to high; **semicolumnarhybrids**-average SST = 44.0cm^2; coefficients of variation average to high; **standardhybrids**-averageSST = 55.3cm^2; coefficients of variation highto average.

SST values of columnar and semicolumnar hybrids are very significant negative as compared to SST at standard hybrids.

Semicolumnar mutant plants presentSST = 45.2cm^2, and those standard type 64.4cm^2, with a variability coefficient s% = 21.4%.

Columnar, semicolumnar and standard descendants be do not differ only by the plant rootstock, but also by the value of SST.

Crown diameter, plant height and crown volume

Descendants obtained from different hybrid combinations present a large variability regarding the growth elements:

Crown diameter:**at columnar hybrids:**average = 119cm, **at semicolumnar hybrids**: average= 241cm;**at standardhybrids**:average= 293cm,

Plant height:**at columnar hybrids:**average= 356cm,**at semicolumnar hybrids**: average= 288cm;**at standardhybrids**:average= 334cm.Columnar hybrids tend to grow erectly and therefore have the greatest height.

Crown volume: **at columnar hybrids:**average= 3.51m^3/tree;**at semicolumnar hybrids**: average = 11.40 m^3/tree;**at standardhybrids**:average= 20.05 m^3/tree.

The crown diameter, the plant height and the crown volume are very different also at descendants from each hybrid combination.

Correlation and regression between SST and crowns volume

Determination of correlation coefficients (r) and of regression equations were done according to the type of descendant plants. Regression straight lines at standard hybrids and semicolumnar ones have a high slope, while at columnar ones the slope is slightly negative, and the division degree of values is very compact.

Columnar hybrids present shorter and thicker annual branches (6.1mm), as compared to the semicolumnar ones (5.8mm) and standard ones (5.7mm).The wood of these branches is of 4.9mm at columnar hybrids, of 4.5 mm at the semicolumnar onesand of 4.0mm thickness at standard ones.

Considerable differences were recorded for the size of wooden vessels: 22,0μ at standard hybrids, 18,4μ at semicolumnar ones and 16,8μ at columnar hybrids. Such differences are recorded also in terms of distance between the medullary raysand the thickness of the medullary rays.

Variability of genotypes descending on their own roots, regarding the fruiting characteristics:

a) Blossoming period (2011 - 2014) between the 4th of April and the 5th of May, but with differences between descendants relating to the beginning of blossoming: - columnar hybrids 07 – 12 ofApril,standard hybrids04-14 of April;

b) Fruit ripening period.The hybrid combinations were conceived using genitors that have a late fruit maturation period.

Tardive maturation of fruits is recorded at all the columnar and semicolumnar hybrids (212), and 13 hybrids with maturation in the 1st decade of September are the standard type.

Entering into fruiting for most of hybrids occurred beginning with year IV.Some hybrids yield fruits (2-5 pieces) also in year III, but the quantity was not economically insufficient.

At the hybrid combinations (Telamon x free pollination, Generos x KV8, Liberty x Wiycik and Granny Smith x free pollination) there were hybrids that started fruiting in year V.

Fruit production at hybrids on their own roots

Fruit production has proved to be variable both for hybrid combinations, and among their descendants.

The maximum yields ranged between 16.0 kg/tree and 24.0kg/tree.

By reporting to the density of 2222 t/ha (3.0x1.5m), there results that some hybrid combinations give birth to very productive descendants: Trident x freepollination(36.4t/ha) at Telamon x freepollination(21.5t/ha).

Fruit yield was directly influenced by the formation way and by the number of fruit formationsper tree. Fruits fromdescendantcolumnar hybrids are very different in size and weight (38-195g), but most descendants have fruits of 55-105 g. The fruits are larger at standard hybrids (120-180) similar to those from the parents.

Hybrid resistance at Venturia inaequalis

Apple tree resistance at scab is an important objective in all breeding programs.Out of the 325 hybrids studied on their own roots, it was found that 69 present resistance (21.23%), and the rest of 256 have no resistance at scab (78.77%).

The hybrid combinations in which one or two of the genitors present resistance at scab, produced a total number of 217 hybrids, but out of these only 69 are resistant (31.79%) and the rest of 108 hybrids have no resistance. Hybrids with resistance at scab are grouped as following: columnar - 50 (72.46%); semicolumnar - 10 (14.49%); standard - 9 (13.05%).

Genitors KV8, KV42 and Trident transmit relatively easily the characterof resistance at Ventura inaequalis.

Identification of transmitting way in descendance for the "columnar port" character

Determining the transmission way of the port character of columnar, semicolumnar standardplant is very important for an apple tree breeding program. To this end,the fixed model and the random model were used to calculate estimated variants (s2) and expected variants (S2) (Ghidra, 1996; Botu 1999).

At the hybrid combinations at descendants in F1, the character transmission ratio for the columnar port is present intensely. This transmission ratio of the character is very different from one combination to another, oscillating between: 1.3:1 at Wijcik x free pollination; 11.99:1 at KV8 x Wijcik; 2628:1-average ratio at all combinations.

The columnar character is transmitted relatively easy in descendance, once with the reduced plant vigour and short fruiting formations. The selection of certain elites with valuable characteristics in the hybridfield in order to promote them at proliferation and testing in comparative competition cultures.

There were selected 16 elites belonging to 5 hybrid combinations and to a mutation from the Granny Smith variety.

a)Selection according to the plant port

Selected elites are grouped as follows: **columnartype** (11 hybrids out of 4 hybrid combinations) - 68.7%; **semicolumnar type** (1 hybrid from a hybrid combination) - 9.1%; **standard type** (3 hybrids+ 1 mutant - a hybrid combination and a Granny Smith plant - 22.2%.

b) Selection of elites according to the growth vigour.

Establishing the growth vigour of elites selected was based according to the values of the elements of growth of plants aged 6-8 years on their own roots: SST; crown diameter; plant height; crown volume.

Columnar and semicolumnarselections (12) have the vigour: **low** (below 10 pts) - 4 selections; **average to low** (11.45 to 15.44 pts) - 8 selections (7 columnar and one semicolumnar); **high** (> 21 points) - 4 selections from descendants with standard port.

The classification of selections taking into account the plants vigour has a determining role in establishing the planting distances and the density of plants per hectare.

c) Morphological and anatomical structure of annual branches.

Annual branches at elite selections present a different morphological and anatomical structure: **columnar** hybrids have an average distance between buds on the annual branch of 17.8 mm; **standard**hybrids have an average distance of 24.6mm, **semicolumnar** hybrids present intermediate values between the columnar and the standard ones.

The annual branches differ from anatomical point of view at the selections studied: **columnarselections** - present wooden vessels (17 μ), thickness of medullary rays (15.1 μ) and the distance between the medullary rays (18.2 μ) lower than at standard selections; **standard selections** - presentwooden vessels (21.7 μ), thickness of the medullary rays (19.6 μ) and the distance between the medullary rays (20,7μ).

Fruit yields at hybrid selections on their own roots

Fruit yield is the major object ofdescendants' selection. Entry into economic fructification occurred: in the 3rdyear, at the columnar selections; in the 4thyear, at the standard selection.

Fruit yields per tree and calculated per hectare (2222 pl/ha) was on average: 12.9 kg/tree, respectively 28.7 t/ha, at columnar selections; 11,5kg/tree,respectively 25.6 t/ha, at standard selections.

Fruitsquality at elite selections

Fruits produced from hybrid descendants (F1) are different in size, weight and colour. The size index (Im) at fruits of the 16 selections is comprised between 60.4mm (H4-1-2007) and 78.0 mm (H1-16-size 2007).

The average weight of fruits ranges between 134g and 195 at the selections. The covering colour is different, those with bicolour fruits being dominant. The shape is specific, slightly flattened, sometimes with small peaks. The pulp presents a high firmness, is juicy, tasting average to good. The total sugar oscillated between 11.9% and 13.9%. The acidity is 0.58 to 0.92%;

Apples from these selections fulfil the commercial conditions for fresh consumption and for industrialization.

These are included in the group of winterapples, to be stored in natural conditions (without cooling rooms) for 3-4 months (December-January).

The resistance of elite selections at attack by Venturia inaequalis

Out of the 16 hybrids selected for their performance as plants and fruits: 12 selections present resistance at scab; 4 selections are without resistance. Out of the 12 columnar and semicolumnar selections, 9 are with resistance at scab and 3 without resistance.

Out of the 4 standard selections, only 2 have resistance at scab; columnar selections resistant at scab have maternal genitors from varieties: Trident (5), Kv8 (1), KV42 (2) and Wijcik (1). The standard selections present a ratio of 2/2 = 1 and the columnar ones 9/3 = 3. The higher ratio is due to the genitors with a resistance that were at the basis of the hybrid combinations,

The behaviour of elites in the competition culture grafted on rootstock M9

In the growth process, the hybrid selections (16) were grafted in year 2010 on rootstock M9, from where they were planted in the spring 2012 in the competition culture (density = 3125 pl/ha, planting distance 3.2 x 40cm). After 3 years, SST oscillatedbetween 7.04 cm^2 (H1-30-2007) to 14.48cm^2 (M8-9-2006). As compared to the average SST of the 16 selections, all values of the columnar elites were insignificant, while the standard selections had significant valuesto very significantly positive.

Selections of apple treesdiffered among them also as to the plant height (120.2-176.8 cm at columnar selections and 170.4 - 184.0 cm at standard selections), crown diameter (16-26 cm at columnar ones and 52 - 71 cm at standard selections).

Columnar selections present a large number of spikes on the axis and give the aspect of a tight crown, and at the standard selections 5-8 branches, with 3-4 spikes and with a more obliquegrowth were formed, giving the aspect of lax crown.

The leaves are more in number, dense (compact) and dark green at the columnar selections, as compared to the standard selections that are lighter green and rarer.