Thesis summary entitled

" EVALUATION OF LOCAL VARIETIES OF OLD APPLE IN TERMS OF RESISTANCE TO DISEASES"

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1.1. Introduction

One of the most dangerous diseases is present in the apple orchards is the scab caused by (*Venturia inaqualis*). This disease can cause crop losses of over 30% in neglected orchards which he creates favorable conditions for development. The apple orchards are numerous treatments each year to avoid material losses caused by the refusal to export fruit attacked by this fungus. (Albert F., 1993).

To control this major fungal pathogen of fruit genetic strategy used is the selection of species resistant to scab Venturia inaequalis induced (Cke.) Wint. There is an assumption that the *Vf* gene conferring inherited from class 3 and that side to reach the reactions of class 1 and 2 are other additional genes that increase the *Vf* effect, based on various observations as a result of several crosses with parents *Vf* gene. *Vf* resistance was interpreted by Tenzer I., et al in 1993 as "the result of hypersensitivity reactions and the intensity of hypersensitive response is reinforced by quantitative gene inherited cumulative".

1.2. Objectives

The proposed objective is to study some aspects of the behavior of old varieties with partial resistance to diseases indigenous to support small producers of fruits especially not greater financial resources and sometimes no expertise in plant protection to obtain new disease resistant varieties better adapted to conditions in our country with an efficiency cost / higher productivity in culture. We have established a series of synthetic targets to promote their re-evaluation and improvement processes in support of this major objective, including:

- studies of the fructifications of old apple varieties resistant and growth monitoring features: “Turnu”,” Salciu”, “Venchi”, “Iridium”, “Vanatori 48”, “Mohorat”, “Fară nume”, “Călugaresc”, “Ouț alb”, etc.

- studies of fruits quality;

- to used an new processes of hybridization of the most interesting old local varieties;

- isolation of genomic DNA from the most of the varieties studied;

- after *Venturia inaequalis* artificial infections were evaluated phenotypically the new progenies.

- the old varieties of apple taken studies performed molecular marker assisted selection.

In recent decades, both worldwide and in our country, obtaining biological or ecological beneficial fruits consumers have become the main concerns of researchers.

The need to study the processes involved in the improvement of these apple varieties with genetic resistance to disease of good behavior even under untreated with fungicide and therefore a significant reduction in pesticide use and environmental pollution, is entitled to obtain genotypes of increasingly responsible for the new vision of biological agriculture competitive on the world market.

1.3. Material and methods

The **plant material** used in the study consists of:

► old Romanian varieties in a sufficiently large number of identified and collected from different areas of the country such as: "Venchi", "Iridium", "Prescurate", "Gurguiate", "Roşu Marin", "Calvil alb", "Transimre", "Andrifişer", "Renet portocaliu", "Varga", "Sălciu","Turnu", "Vânători 48", "Prescurate", "Gurguiate", "Vieşti", "Cormose", "Seghese", "Trotuse", "Fără nume", "Măr Orbai" etc.

► pollen from some apple genotypes such as apple and some local populations "Prescurate" and "Gurguiate", "Trotuse" , "Mohorat", "Nobile de Geoagiu", *Malus pumila* and, *Malus floribunda.*

Material pathogen

The fungus *Venturia inaequalis* producing disease called scab. The disease can occur on leaves, sepals, the fruit, the stalk, and rarely on the shoots. Pete characteristic brown - olivacee cover infested leaves on both sides, which are blackish brown with time, with velvety; are small and many, confluence, reducing the possibility of feeding the tree and occupying a large area of the leaf.

Working methods

Once the relation: G / V, (g or kg) / (ml or l) was done to determine the **specific weight**.

The amount of **total solids** is represented by a weight percentage in the first case or the second case, the difference between the weight of the material to be analyzed and the water lost on drying, and between 100 and the percentage of water found.

The substance **soluble solids** was determined by refractometry way.

Determination of **total titratable acidity** is achieved by current methods which rely on direct titration of the product, or an extract or dilution thereof at the expense result, with an alkaline solution of known concentration.

Use volumetric methods (titration) neutralizing the practical application of this principle, and according to them, the way their mutual neutralization is achieved quantitative determination of acids or bases.

Determination of polyphenolic about permanganometrică indirect method is provided as follows: it first determines the amount of potassium permanganate oxidation of all the materials necessary (phenolic nefenolice) of the product under review. Then, using activated carbon, the material is removed by filtration, tanning and coloring substances and is determined in the filtrate, the amount of permanganate oxidation of other substances necessary, the nefenolice.

Determination of **ascorbic acid** in vegetable products can be considered as the determination of vitamin C, as a result of this, it is heated to 60 ^{0} C in the presence of 25 ml of 2% oxalic acid and a pinch of calcined quartz sand, an amount product (5-20 g according to the riches of its vitamin C), weighed technical balance with an accuracy of 0.01 g, well cut and grind to obtain a very fine paste. In a flask of 100 ml filtered extract obtained.

For the phenotyping in greenhouse conditions were made artificial infection with the scab according to the protocol presented by Chevalier et al. (1991). A mixed inoculum was used for inoculation. With a suspension of conidia of *Venturia inaequalis* was sprayed the young seedlings of 4-5 true leaves.

For genotyping, prior the DNA isolation was performed for genotypes which did not show symptoms in the phenotype of the plant. The NanoDrop DNA was quantified and then was revealed in elictroforeză gel 2%. A kit from Applied Biosystems amplification with primers specific for detecting *Vf* gene was used to perform the PCR reaction.

1.4. Results

Using the old apple varieties (best adapted to the climatic conditions of Romania) for the improvement processes can be an interesting premise for inducing genetic resistance to *Venturia inaequalis*, and other diseases. Artificial infection of these varieties and hybrids can provide information on plant defense mechanism against pathogenic infections.

The most important results emerging from this work are:

1. With regard to studying the behavior of old varieties, domestic apple with genetic resistance to disease in terms of growth and fruiting capacity in order rewording and promote the breeding programs most valuable for obtaining new varieties better adapted to the specific conditions of our country were further highlighted a number of varieties not had symptoms on fruits and leaves are the varieties: : “ Kniș”, „Favoritul lui Polocsay”, „Wachsman Amelie”, „Wachsman Sammeling”, „Trotușe”, „Mohorât”.

Another group of varieties under field conditions showed *Venturia inaequalis* average resistance are: „Anisovska”, „Dulci de Rădășeni”, „Seghește”, „Pokomake”, „Roșior Călugăresc”, „Mere Tari”, „Cernenko”, „Măr Orbai”, „Verzi de Rădășeni”, „Nobile de Geoagiu”, „Roșii de Geoagiu”, „Trotușe” etc. The most valuable of these were used in breeding programs to obtain hybrid progeny to show their valuable characters and especially *Venturia inaequalis* resistance.

2. In terms of fruit quality assessment to determine the most viable scheme of pollination results were found:

- the largest fruit weight was registered by the varieties "Renet Orange» with 165.47 g, followed by the variety "Turnu" with 158.70 g and variety "Venchi" with 156.45 g. The fruits were lowest registered the variety "Prescurate" with 73.37 g;

The old fruit varieties are large, for the variety "Kniş" with 298.6 g followed by the variety "Mar Orbai" with 268.2 g followed by variety 'Ancuța "with 222.6 g and" Fara Name "with 210.2 g, but are fizzy, and ride to show their valuable characters and especially *Venturia inaequalis* resistance.

3. Regarding the **soluble dry substance** highest value recorded a variety "Wachsman Amalie" by 28.5%. followed by variety 'Roșu de Cluj "27.1% and" Fară Nume "by 24.2%

- In terms of fruit soluble solids content is observed that the highest values are recorded in variety "Coadă scurtă" with 20.6%, followed by variety 'Roșu Marin "19.0%" Renet Portocalu "with 18.7% of group varieties in 2013, the highest value recorded a variety "Wachsman Amalie" by 28.5%. followed by variety 'Roșu de Cluj "27.1% and" Fară Nume "by 24.2%.

- In terms of content in vitamin C is observed that as the fruit progresses during their retention, vitamin C drops dramatically every 10 days. The highest value in terms of vitamin C content was recorded by the variety "Andrifişer" with 36, 63 mg / 100 g pp, followed by the variety "Iridium" with 36.37 mg / 100 g pp.

Vitamin C is very unstable and its presence is also an indication of the freshness of fruits. The content of vitamin C in variety "Gurguiate" was less than 14.52 mg / 100g pp than the variety "Trotuse" at the end of the shelf with a value of 35.32 mg / 100g pp.

4. As regards the involvement of old varieties of apple breeding process research was conducted during 3 years of field controlled pollination, hybridization with us and explore other sources of resistance like some old apple varieties, they being “Amelie Wacsman” , “Gurguiate”, “Fără nume”, “Mohorât”, “Nobile de Geoagiu”, “Favoritul lui Plocsay” etc. All these results demonstrate that despite a large number of flowers pollinated seed number obtained was very very low, which is related to agricultural technique applied during the growing season. Therefore

location where pollination is done is extremely important developments in research and achieving results.

5. From the results of the phenotyping made after artificial infection with *Venturia inaequalis* both old apple varieties and the hybrid progenies note that:

- that have been made resistant to scab progenies from crosses a variety sensitive (such as 'Idared') and an old variety ("Poloscay's favorite") demonstrates that the variety "Poloscay's favorite" recessive resistance alleles exhibit. So if local variety "Mar Orbai" which was crossed with a resistant variety "Generos". It raises several assumptions about genetic resistance to scab control in the apple species.

- after artificial infection notice that a number of varieties such as "Prescuratele", "Turnu", "Venchi", "Gurguiate", "Roșu Marin" and "Sângeriu" showed some genetic resistance to *Venturia inaequalis*, falling class 0 for resistance;

6. With regard to the concentration of DNA in apple varieties both parents and offspring have shown that some values are higher for parents to hybrids, with the highest value of the variety 'Florina' to 2402.5 ng / µl, followed by the variety "Gurguiate" with values of 1937.9 ng / µl, then the variety "Prescurate" with 829.9 ng / µl compared with values hybrids mere hundreds of 205.0 ng / µl the combination HCl 1-5, and the lowest was recorded in the combination 1-2 HCl 64.0 ng / µl. Among old varieties, variety "Turnu" has 179,1 ng / µl, followed by the variety " Calvil alb" with values of 161.1 ng / µl, then the variety "Varga" with 155.0 ng / µl versus varieties "Roșu Marin" 12.6 ng / µl followed by "Salciu" variety with 51.2 ng / µl. The very close value of genomic DNA concentrations were recorded for variety 'Nobile de Geoagiu " with 179, 1 ng / µl, followed by the variety "Dulci de Rădăşeni" with values of 161.1 ng / µl.

7. In order to improve the apple breeding efficient programe, with the completion of phenotypic selection made with the molecular level (MAS selection - Marker Assisted Selection), special offers safety and efficiency. MAS selection can be performed at any stage of plant growth, including very young phase, when hybrids are in the greenhouse, so that much of the biological material can be removed and transferred into the field only plants showing the desired characteristics and economically effects are time plots of land, labor, respectively considerable amounts of money. PCR detection success for *Vf* gene was confirmed that depends on the quality and quantity of DNA isolated during procedures.

Vf heterozygous gene structure was confirmed between varieties. The reason manifestation of heterozygous genotypes studied where *Vf* gene is probably due to a high degree of open pollination in the genus *Malus*. The structure was detected in homozygous clearly a large number of lineages with different levels of resistance. It is important to use more PCR's with all four primers (A, B, C, D) mades in pool.