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STUDIES ON THE PHENOTYPIC EVALUATION OF SOME ROMANIAN VARIETIES OF APPLE CONCERNING THE RESISTANCE TO SCAB

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Abstract

The apple scab produced by the Ventura inaequalis pathogen is a serious disease affecting the leaves, flowers and fruits. In Romania, the disease is present every year in all fruit trees. The negative side effects of large-scale, sometimes abusive, use of pesticides in agriculture, and in particular environmental pollution and residual accumulation, as well as increasing practical difficulties in tackling combating problems through the emergence of disease-resistant breeds and pests, have led research to find new solutions to limit their use. In support of this idea, research into obtaining new varieties with genetic resistance to scab is a basic premise. For this, identifying new sources of scab resistance is a major concern for breeders. This paper proposes the evaluation of native varieties with natural genetic resistance to Venturia inaequalis by phenotypic techniques. For this purpose the monitoring of the growth peculiarities, the fruiting of some old resistant apple varieties: 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă', as well as the phenotypical evaluation of these old varieties following artificial infections with Venturia inaequalis. The apple varieties taken in the study 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă' were laid at 4^0C and after emergence and repaired the highest percentage of raised plants were observed at 'Tari de Ghindă' variety with 47.5% followed by 'Călugăreşti' variety, with 45.6%. The lowest percentage was recorded for the 'Moţi' variety, by 22.5%. Following the artificial infections with Venturia inaequalis, the highest percentage of plants resistant to infection was recorded in the 'Călugăreşti' variety with 88.0%

Key words*: resistance, scab, varieties, apple, phenotyping.*

INTRODUCTION

The apple's biggest problems were and are given by two mycosis that causes the popularly known diseases *Scab of the apple* and *Apple oidium*.

The more valuable varieties have been created, cultivated in higher-intensity plantations, the higher the damage they have caused. The costs of preventing and stopping the attack of these diseases are very high, and sometimes in the years favorable to their attack, with all these costs, the attack still manifests, reducing the quality and volume of production (Lespinasse et al., 1990). The disease is manifested on leaves, floral pedunculos, fruits and even shoots. The attacked leaves are covered with brownish-brown, velvety spots, which in time grow and unite (Balaci & Ivan, 1998). The strongly attacked leaves are dried, fall early and reduce the possibility of feeding the tree, which results in the decrease of the harvest, as well as the differentiation of a low number of buds for the next year (Gladieux et al., 2008).

The form of attack on the peduncle and sepale occurs in years of warm spring, when the first infections have occurred since the onset of inflorescence. Fruits are attacked from training until late autumn. They remain small and have brown spots on the oval, velvety. Attacked fruit deforms, cracks and most fall before maturation (Sestraş, 2004).

The form of attack on shoots is encountered in untreated trees. In the years with frequent rain in May, June, July, there is a strong attack on shoots (Hugh et al., 1953).

They have spots similar to those on the leaf. Over time, the part of the attacking spatula crackles, browns and finally one part dries out in summer, some frosts in the winter, and the less attacked, whose wood is baking normally, ensures the viable mycelium which in the following spring will generate primary conidia (Day et al., 1956).

Since the *Malus* species carry many resistance genes, it has been assumed that several hosts exert selective pressures on *V. inaequalis* populations as demonstrated by the Rvi6 gene

(White et al., 1990). Breeding of fruit is very time-consuming and costly. It takes at least 20 years from the first crossing to a commercial apple cultivar (Becker & Burr, 1990). The flower is emasculated and pollen from a known parent (father) is transferred. The selection of parents is very important. The flower is protected in a small bag after the pollination and later the fruit is collected (Carisse et al., 2000) The next season the seeds are sown, and thereafter selection is made among the seedlings. The juvenile period is long, at least 4 years and sometimes up to 7-8 years. Sometimes backcrossing is necessary to obtain the right characteristics. If the desired trait is under polygenic control, there will be a range of variation. In this case a progeny of several thousands of seedlings would only yield one seedling with a combination of these characters (Janick & Moore, 1996).

MATERIALS AND METHODS

Material plant and pathogen

The plant material used in this study is the old Romanian varieties, identified and collected in the southern area of the country, such as: 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă'. The pathogen is composed of a mixture of *Venturia inaequalis* strains colected in the southern area of the country, and prepared in conidia suspensions.

Methods of work

Methods of phenotyping consisted of:

• controlled pollinations that were manually performed by isolating the female genitor in paper bags, pollination being done with the brush according to the pollination scheme;

• the inoculum was prepared in the laboratory according to the protocol (Bénaouf & Parisi, 2000);

• artificial infections were made under greenhouse conditions by fine spraying on young plants in the 4-5 leaf stage;

• the reading was performed 14 days after the infection, where each hybrid according to the symptoms presented was classified on classes of resistance starting from class 0 to class 4 with subunits 3a, 3b and 3c (Day et al., 1956). It was used an inoculation mix. The young plants of 4-5 true leaves were sprayed with a suspension of conidia of *Venturia inaequalis* CKE.

The plants were incubated for 48 hours at 18°C and relative humidity of 100% air. Symptoms of the disease were assessed macroscopically after 21 days of inoculation in a greenhouse. Following the reading, the plants were divided into 5 classes. Class 0 plants were free of infection symptoms. Class 4 plants had complete spore lesions. PCR plants have been selected for plants with no symptomatology on leaves 0 to 3.

RESULTS AND DISCUSSIONS

Following artificial infections with *Venturia inaequalis*, the largest number of plants resistant to infection was recorded in the 'Călugăreşti' variety with 22 resistant plants from 25 inoculated plants, the success rate being 88.0% followed by the variety 'Tari de Ghindă' with 73.6%, followed by 'Moți' variety with 68.7%. Among the varieties studied the worst resistant is the 'Ouţe' variety with a percentage of 53.8 (Table 1).

Table 1. Resistance of apple varieties to scab

No.	Combinations	Parameters			
		Total hybrids	Number of inoculated plants	Number of resistance plants	% of resistance plants
1	Călugăreşti	25	25	22	88.0
2	Moți	16	16	11	68.7
3	Ouţe	26	26	14	53.8
4	Mere Tari	23	23	14	60.8
5	Tari de Ghindă	38	38	28	73.6

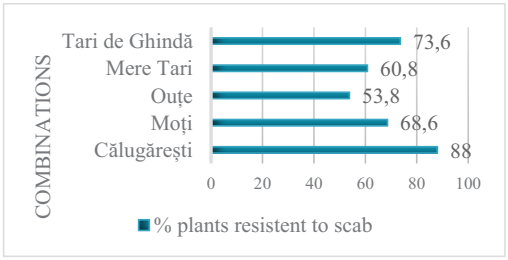

Figure 1. Number of plants resistant to scab after artificial inoculation (%)

Following artificial infections with *Venturia inaequalis*, placentas were classified as classes of resistance and the Class 0 of resistance, meaning no symptoms, with 26.6%, followed by Class 2 showing mild infection patterns of 29.8%. Class 1, as well as the most important forms of resistance of scab, registered 9.6%. Of the 124 young plants, 12.9% were died (Table 2, Figures 2 and 3).

Table 2. Results on plants infected with *Venturia inaequalis* to establish scab resistance classes

No.	Combinations	Clases of symptoms							
		0	1	2	4	3A	3B	Dead	Total
1	Călugărești	9	1	7	0	5	0	3	25
2	Moți	4	1	1	0	3	2	5	16
3	Ouțe	5	1	8	0	5	4	3	26
4	Mere Tari	6	2	6	0	5	3	1	23
5	Tari de Ghindă	9	6	13	0	5	1	4	34
Total		33	12	37	0	23	10	16	124
Value [%]		26.6	9.6	29.8	0	18.5	8.0	12.9	100

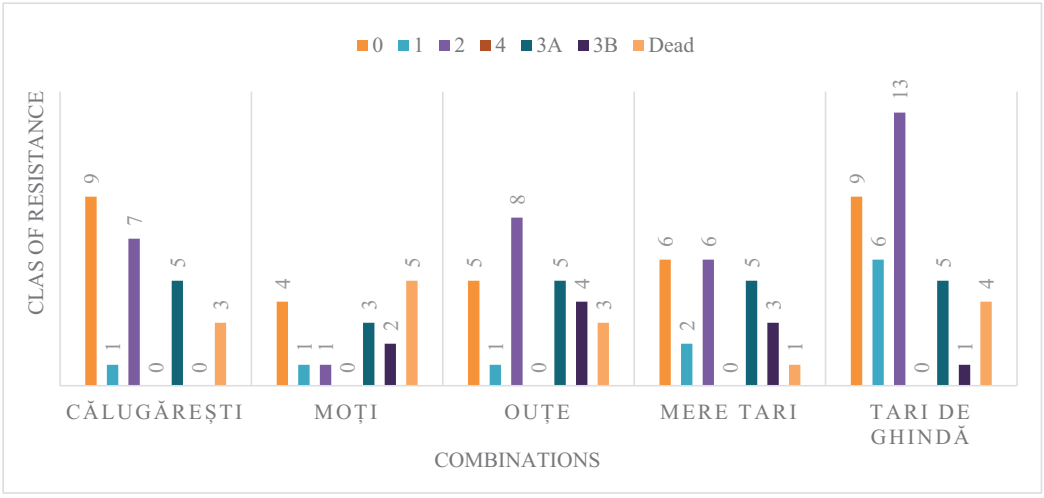

Figure 2. Results on plants infected with *Venturia inaequalis* to establish scab resistance classes

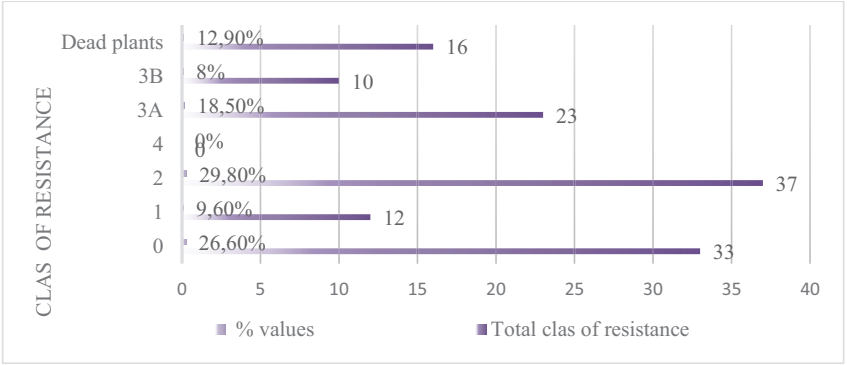

Figure 3. Total resistant plants to scab

Figure 4. Aspects during infection in the greenhouse and in the reading of infections

The classes 0 to 3 are considered to be resistance responses while class 4 is a susceptible response (Figures 4 and 5). Interestingly some of the susceptible cultivars also demonstrate variable extent of resistance against isolates of the pathogen.

The matured leaves of apple demonstrate ontogenic resistance because of which the pathogen growth is suppressed immediately after cuticle penetration and appearance of disease symptom gets delayed.

The strengthened cell wall and cuticular membrane along with sub-cuticular pH of such leaves are speculated to play a role in governing such resistance. A breakdown of ontogenic resistance revealed by restored growth of the pathogen is observed in the old senescing leaves of apple. Detailed studies are needed to elucidate the functionality of such resistance and understand its breakdown mechanism.

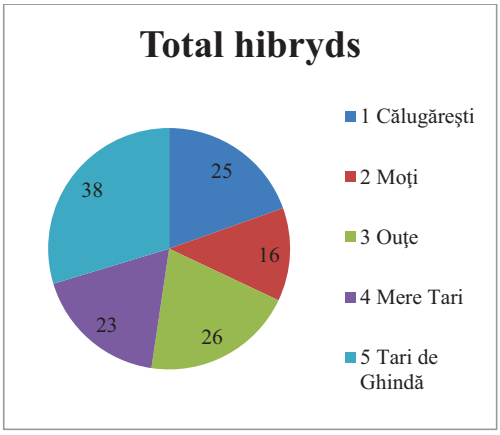

Figure 5. Total apple hybrids

Figure 6. Scab symptoms on the leaves

The artificial infections were made in greenhouse according to the protocol presented by Chevalier et al. (1991). Young plants with 4-5 true leaves were sprayed with a conidia suspension of *Venturia inaequalis* CKE. They were incubated for 48 hours at 18°C and 100% relative humidity. Symptoms on the leaves were evaluated visually (reading) at 21 days after inoculation, and the young plants were divided into 5 classes. Class 0 includes plants without visible symptoms on the leaves, Class 4 include plants with lesions on leaves and strong sporulation. For PCR analysis only the plants without symptoms or in classes 0-3 were used.

Figure 7. 'Moți' variety

Using in the breeding program the domestic apple varieties well adapted to the climate in Romania can be an interesting premise on inducing natural genetic resistance to *Venturia inaequalis*. In this regard, scab races, the inoculation method, environmental conditions and culture and defining characters were not the same in all of these studies. The artificial infections in this varieties and theur hybrids can provide some information on plant defense mechanism against attack pathogen agent. Support and promote in the culture the apple varieties with genetic resistance to diseases constitute, for new plantations, technological links with economic performance, with immediate effect on total or partial removal treatments with fungicides, representing the main factor in obtaining organic production.
In the Romanian breeding program recovery of the old local apple varieties, varieties that are best accommodated to the climatic conditions of Romania, was used like a natural source of resistance to pathogen attacks. The purpose of this paper is to carry out a screening of the presence of gene VF (a gene involved in genetic resistance to *Venturia inaequalis*) in some local varieties of apple.

Figure 8. 'Mohorât' variety

Change of the assortment at shorter time intervals is requirement by increased susceptibility to diseases and pests in some apple varieties, the emergence of new varieties showing superior qualities of existing varieties, adaptation to new crop technologies and systems, adapting assortment climate changes - resistance or tolerance to climatic stress factors.

Figure 9. 'Mere tari' variety

CONCLUSIONS

Regarding the study of the behavior of old, native varieties of apple with genetic resistance to diseases in terms of growth and fertility, a number of varieties have been revealed that did

not show moderate symptoms on fruits and leaves such as varieties: 'Călugăreşti' 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă'. As a result of artificial infections, we notice that the varieties 'Călugăreşti' and 'Tari de Ghindă' have shown some genetic resistance to *Venturia inaequalis*, belonging to class 0 of resistance.

For better expression of resistance characters, artificial infections are recommended under controlled conditions and in a known controlled infection pressure so that the selected individuals (genotypes) express these characters in the short time, and then improve other valuable plant features.

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