Scientific Papers. Series B, Horticulture. Vol. LXII, 2018
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE ISOLATION OF GENOMIC DNA FROM OLD APPLE VARIETIES

Ligia ION

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: ionnagyligia@yahoo.fr

Abstract

The apple scab disease has probably evolved over a long time along with the apples. The disease is caused by the fungus Venturia inaequalis (Cke.) Wint, anamorph Spilocaea pomi Fr. The aim of this study were the isolation and quantification the genomic DNA on old Romanian varieties in order to select the most important to them for the Marker Assisted Selections (MAS) on apple trees. This paper presents the results of DNA isolation by exploring local populations of apple like 'Prescurate', 'Seghese', 'Vieşti' and 'Kniş' apples old varieties, that are well adapted to the conditions in Romania and that are un interesting genetic potential for resistance to scab. Only the old apple varieties were used in the study. The implementation of marker selection depends on the quality and quantity of isolated genomic DNA. The results of the quantification performed following genomic DNA isolation show a large variability in the amount of DNA in each old apple variety. The DNA concentration in apple varieties both parents and some descendants have shown that the values are higher in parents than hybrids, with the highest value for 'Turnu' variety with 179.1 ng / µl, followed by 'Calvil alb' variety with 161.1 ng / µl, then 'Varga' variety with 155.0 ng / µl.

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

INTRODUCTION

Apple (*Malus x domestica* Borkh.) is one of the most cultivated fruit crops in temperate climates. The major constraint of apple cultivation is the apple scab, a fungal disease caused by *Venturia inaequalis*, which can lead to important crop losses if not properly controlled (Holb et al., 2003).

The 17 major resistance genes in apple (*Rvi1* to *Rvi17*) against *V. inaequalis* have been found (Bus et al., 2011).

Only *Rvi6* (previously *Vf* from *Malus floribunda* 821) has been extensively used for resistance breeding to date (Gessler et al., 2012).

Since the breakdown of the *Riv6* gene in the early nineties (Parisi et al., 1993), new breeding programmes have started to investigate other resistance genes for future resistance breeding (Gygax et al., 2004; Gaecia-Ruiz and Murphy, 2001).

An increase in resistance with increasing apple leaf age (ontogenic resistance) has been observed in all apple genotypes and is known to act against all known *Venturia inaequalis* strains. Up to know, no report of the breakdown of this type of resistance has been found in the literature; thus, ontogenic resistance is considered durable (MacHardy et al., 2001). Goethe (1887) and Aderhold (1900) are believed to have been the first researchers noticing age-related resistance in the *Malus-Venturia* pathosystem.

The authors observed a decrease of leaf susceptibility with increasing tissue age. Nearly three decades later, Keitt and Jones (1926) showed an increase in incubation period and a decrease of disease severity by increasing leaf age.

Following these observations, many researches have been carried out on *Malus-Venturia* interaction during leaf infection. Gessler and Stumm (1984), Li and Xu (2002), and Gusberti et al. (2012) showed that the fungus grew faster in young leaves compared to old ones.

The first unfurled and expanding leaf is considered susceptible to the apple scab disease, while the fifth leaf (starting from the top of the shoot) is considered fully resistant (MacHardy, 1996).

Disease resistance mechanisms during tissue ontogenesis have been studied in different plant pathogen systems and some factors have been

suggested to be correlated to the observed age-related resistance.

Among them, the most important appears to be chemical compounds such as salicylic acid (Kus et al., 2002; Hugot et al., 1999) and pathogenesis-related proteins (Wyatt et al., 1991), physiological barriers like the cuticle (Peries, 1962), lenticels (Kennelly et al., 2005), restricted phloem movement (Garcia-Ruiz and Murphy, 2001) or a limiting nutritional substrate for fungal infection (Juen and Hwang, 1991).

However, since a different mechanism for age-related resistance is described in each crop plant, much work remains to unveil the mechanism underlying this type of resistance in other plants. In apple, several aspects have been investigated in order to unveil the nature of ontogenic resistance.

Physiological barriers like the cuticle and papillae (Stadler, 1988), were not correlated to the age-related resistance.

The objectives of this study were the isolation and quantification the genomic DNA on old Romanian varieties in order to select the most important to them for the Marker assisted Selections on apple trees.

MATERIALS AND METHODS

Plant materials

The old Romanian apples genotypes ('Prescurate', 'Gurguiate', 'Vieşti', 'Kniş', 'Turnu', 'Moharat') were tested for their ability to support the artificial infection to scab. All this material was inoculated (by spreeding) with fiels races of *Venturia inaequalis*.

DNA isolation and SSR analysis

Genomic DNA was isolated from fresh apple leaves using the hexadecyltrimethylammonium bromide (CTAB) protocol described by. DNA concentrations were measured by a NanoDrop. Working of dilutions of genomic DNA at 100 ng/µl in TE buffer (pH 8.0) were prepared for analysis. Fresh leaf tissue (<100 mg) was sampled from inoculated and uninoculated leaf samples at 72 and 96 hpi and collected in 2 ml Eppendorf tubes (Eppendorf, Germany), previously prepared with 5 to 10, 2 mm sterile glass beads, immersed in liquid nitrogen immediately after sampling and stored at -80°C until processing. Tissues were ground twice with the FP 120 Fast-Prep machine (Bio 101 Savant Instruments Inc., Qbiogene, France) for 30 s at a speed of 5.5 m s^{-1} with an intermediate immersion in liquid nitrogen between the two grinding steps. Total RNA Isolation System (Promega Corporation, USA) and column purified following the manufacturer's instructions.

After RNA isolation and quality assessment, samples were stored at -80°C until cDNA library construction and transcriptomic assay the each population were carried out according to conditions specified in Zhebentyayeva et al., 2003.

RESULTS AND DISCUSSIONS

The results on the concentration in genomic DNA of the studied apple varieties showed that the highest concentration was recorded in 'Turnu' variety with 179.1 ng / µl, followed by the 'Calvil alb' variety with values of 161.1 ng / µl, then 'Varga' variety with 155.0 ng / µl, compared to 12.6 ng / µl 'Rosu Marin', followed by 'Sălciu' with 51.2 ng / µl (Table 1).

Table1. DNA concentration in apple varieties

No.	Cultivars	Conc DNA (ng/µl)	Raport 260/280	Raport 260/230	Absorbance x260
1	Prescurate	121.3	2.21	0.23	2.425
2	Turnu	179.1	2.11	0.29	3.582
3	Sălciu	51.2	2.50	0.14	1.028
4	Venchi	76.9	2.30	0.15	1.537
5	Gurguiate	114.6	1.88	0.60	2.291.
6	Iridium	55.2	2.54	0.12	1.104
7	Calvilalb	161.1	2.16	0.31	3.222
8	Vânători 48	87.2	2.23	0.17	1.744
9	Varga	155.0	2.04	0.29	3.099
10	Renet Portocaliu	143.7	1.35	0.56	2.874
11	Costat de Albeşti	46.8	2.00		0.454
12	Andrifişer	97.8	2.04	5.57	1.957
13	Roşu Marin	12.6	2.38		
14	Sângeriu	66.5	1.91	4.55	1.330
15	Roşu de Cluj	59.6	2.04	11.83	1.192

The results of the quantification performed following genomic DNA isolation show a large variability in the amount of DNA in each old apple variety (Figures 1-4).

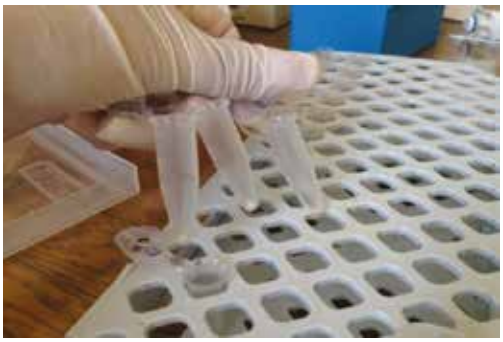

Figure 1. Apple DNA isolation

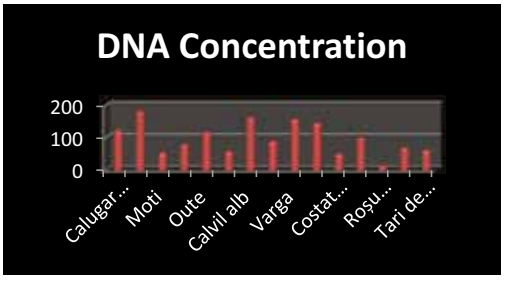

Figure 2. DNA concentration on apple cultivars

DNA purification involves the removing from the lysate all components except DNA, dividing the DNA, that is, separating the different DNA species into distinct categories. The two aspects are not necessarily constituted in separate events.

In fact, DNA purification can still begin at the stage of cell lysing.

Figure 3. Aspects of DNA isolation

Removal (after lysate formation) of all biomolecules and contaminants of DNA is accomplished by several methods: denaturation and precipitation of proteins with organic solvents such as phenol and chloroform; Following centrifugation, the precipitated proteins can be separated from the DNA, which remains in the supernatant.

The implement of resistance older apple varieties of Romanian origin could be a promising way for a viable breeding program in Romania.

First step in this work was the identification and collection (from different parts of the country) and evaluation an important number of old local varieties (Ion L. et al., 2016)

Figure 4. Apple DNA for absorbance 260

Using DNA markers of resistance to *V. inaequalis* will be based on the polymerase chain reaction (PCR).

Recovery in the Romanian breeding program the old local varieties, best suited to the climatic conditions of Romania was used like a natural source of resistance to pathogen attacks.

CONCLUSIONS

During three years of field-controlled pollination for new hybrids, other sources of resistance such as some old apple varieties, such as 'Amelie Wacsman', 'Gurguiate', 'No Name', 'Mohorât', 'Nobile de Geoagiu', 'Plocsay's favorite' etc.

The final number of signatures was relatively low.

Regarding the DNA concentration in apple varieties both parents and some descendants have shown that the values are higher in parents than hybrids, with the highest value for 'Turnu' variety with 179.1 ng / µl, followed by 'Calvil alb' variety with 161.1 ng/µl, then 'Varga' variety with 155.0 ng / µl.

REFERENCES

Aderhold R., 1900. Die Fusicladien unserer Obstbäume. Landwirtschaftliche Jahrbücher, 29, 541–588.

Bus V.G.M., Rikkerink E.H.A., Caffier V., Durel C.E., Plummer K.M., 2011. Revision of the nomenclature of the differential host-pathogen interaction of *Venturia inaequalis* and *Malus*. Annu Rev Phytopathol, 49, 391-413.

Garcia-Ruiz H., Murphy J.F., 2001. Age-related resistance in bell pepper to Cucumber mosaic virus. Ann Appl Biol, 139, 307–317.

Gessler C., Pertot I., 2012. Vf scab resistance of Malus. Trees Struc Funct, 26(1), 95-108.

Gessler C., Stumm D., 1984. Infection and stroma formation by *Venturia inaequalis* on apple leaves with different degrees of susceptibility to scab. J Phytopathol, 110, 119–126.

Goethe R.,1887. Weitere Beobachtungen ueber den Apfel und Birnenrost, *Fusicladium dentriticum* (Wallr.) Fuckel und *Fusicladium pyrinum* (Lib.) Fuckel. Gartenflora, 36, 293–299.

Gusberti M., Patocchi A., Gessler C., Broggini G.A., 2012. Quantification of *Venturia inaequalis* growth in *Malus* x *domestica* with quantitative Real-Time Polymerase Chain Reaction. Plant Dis, 96, 1791–1797.

Gygax M., Gianfranceschi L., Liebhard R., Kellerhals M., Gessler C. et al., 2004. Molecular markers linked to the apple scab resistance gene Vbj derived from *Malus baccata jackii*. Theor Appl Genet, 109, 1702–1709.

Holb I.J., Heijne B., Jeger M.J., 2003. Summer epidemics of apple scab: the relationship between measurements and their implication for the development of predictive models and threshold levels under different disease control regimes. J Phytopathol, 151, 335–343.

Hugot K., Aimé S., Concord S., Poupet A., Galiana E., 1999. Developmental regulated mechanisms affect the ability of a fungal pathogen to infect and colonize tobacco leaves. Plant J, 20, 163–170.

Ion L., Badulescu L., 2016. Studies of The Old Romanian Apple Varieties Involved in New Breeding Programs for Resistance to *Venturia inaequalis*. Journal of Agricultural Science. Vol. 8, No. 5.

Juen Y.C., Hwang B.K., 1991. Carbohydrate, amino acid, phenolic and mineral nutrient contents of pepper plants in relation to age-related resistance to *Phytophthora capsici*. J Phytopathol, 131, 40–52.

Keitt G.W., Jones L.K., 1926. Studies of the epidemiology and control of apple scab. Agricultural Experiment Station, University of Wisconsin, Research Bulletin, 73, 104.

Kennelly M.M., Gadoury D.M., Wilcox W.F, Magarey P.A., Seem R.C., 2005. Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. Phytopathology, 95, 1445–1452.

Kus J.V., Zaton K., Sarkan R., Cameron R.K., 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell, 14, 479–490.

Li B., Xu X., 2002. Infection and development of apple scab (*Venturia inaequalis*) in old leaves. J Phytopathol, 150, 687–691.

MacHardy W.E., 1996. Ontogenic resistance to scab in *Mauls* Apple Scab Biology, Epidemiology, and Management: APS Press, St. Paul, Minnesota, 104–116.

MacHardy W.E., Gadoury D.M., Gessler C., 2001. Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. Plant Dis, 85, 1036-1051.

Parisi L., Lespinasse Y., Guillaumes J., Krüger J., 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the *Vf* gene. Phytopathology, 83, 533-537.

Peries O.S., 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr, ex Fries) Jaczewski-II. Host-parasite relationships on foliage of strawberry varieties. Ann Appl Biol, 50, 225–233.

Stadler B., 1988. Quantitative Untersuchungen der varietalen und ontogenetischen Resistenz der Apfelblätter von Golden Delicious, Liberty und *Malus floribunda* gegenüber *Venturia inaequalis*: Swiss Federal Institute of Technology ETHZ; Ph.D Thesis no.8685: 72.

Wyatt S.E., Pan S.Q., Kuc J., 1991. β-1,3-glucanase, chitinase and peroxidase activities in tobacco tissues resistant and susceptible to blue mould as related to flowering, age and sucker development. Physiol Mol Plant Pathol, 39, 433–440.

Zhebentyayeva T.N., Reighard G.L., Gorina V.M., Abbott A.G., 2003. Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot germplasm. Theor Appl Genet 106:435–444.