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MOLECULAR ANALYSIS OF SCAB RESISTANCE IN APPLE CULTIVARS AND HYBRIDS FROM TRANSYLVANIA

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Abstract. Scab, caused by *Venturia inaequalis*, is the most important apple disease worldwide, therefore all major apple breeding programs have included disease resistance as a primary goal. In Romania, especially in Transylvania climatic conditions are extremely favorable for disease development and spread. In addition, the vast majority of the apple cultivars in these areas are susceptible to the infection. In this study, we analyzed 64 apple plants (cultivars and hybrids) cultivated in Transylvania in order to genotype for the *Vf* locus. Plants were screened using specific DNA markers for the presence of *Vf* resistance gene.

INTRODUCTION

Apples are the most important fruit species in the temperate zone which contain dietetically important substances, thereby playing an important role in human nutrition. Unfortunately, most of the commercial cultivars are susceptible to the fungus *Venturia inaequalis*, the causative agent of apple scab, which attacks both leaves and and fruit reducing yield quality. Apple scab is currently controlled by frequent application of fungicides, in consequence increasing production costs as well as raising ecological and health concerns (Tartarini, S. et al. 1999). For these reasons, apple breeding programs have been orientated to create new scab-resistant cultivars. The main source of scab-resistance originates from *Malus floribunda 821* encoded by the *Vf* gene. In cultivated apple varieties this resistance has been acquired by introgression of the Vf resistance gene.

In *Malus* there are two types of scab resistance: monogenic and polygenic (Dvorak et al., 1976). Polygenic resistance occurs mainly among older apple tree cultivars. The first commercial cultivar carrying Vf gene of resistance was Prima, a fourth generation of descendants of *Malus floribunda* 821, in 1970 in the USA (Dayton et al. 1970).

This resistance has been utilized in apple-breeding programmes throught the world for more than 40 years and has been incorporated into a substantial number of apple cultivars (Crosby et al., 1992).

MATERIAL AND METHOD

Plant material was represented by four different apple cultivars (Starkrimson, Golden Spur, Florina and Liberty) and sixty hybrid apple plants grown in Transylvania (Fruit Reseach Station in Cluj-Napoca). Hybrids were obtained by combinations between Liberty and Florina, Starkrimson and Golden Spur, Starkrimson and Florina, Strakrimson and Liberty, Golden Spur and Florina, Golden Spur and Liberty cultivars. Leaves from each plant were collected in plastic bags, brought immediately to the laboratory and stored at -80^{o}C.

DNA extraction was performed from leaf material according to Lodhi's et al. 1994 protocol. Primers used for the PCR amplifications were obtained from Microsynth (Switzerland). PCR reactions were carried out in 25µl volumes containing: 5X Green GoTaq Flexi reaction buffer (Promega), 1.5mM $MgCl_2$, 100µM of each dNTP, 0.2µM of each primer (AL07F-5'-TGGAAGAGAGATCCAGAAAGTG-3'andL07R-5'-ATCCCTCCACAATGCC-3'; AM19 F- 5'- CGTAGAACGGAATTTGACAGTG-3' and AM19 R- 5'GACAAAGGGCTTAAGTGCTCC-3') and 1U of GoTaq polymerase (Promega). The amplification was performed on a Mastercycler Gradient (Eppendorf) programable thermal cycle. Cycling parameters were set as described by Tartarini at al. 1999 with some modification as follows: one cycle of denaturation at 94^{0}C for 2 min and 30 s, annealing at 60^{0}C for 1 min, extension at 72 ºC for 2 min, and 35 cycles of 30 s denaturation at 94^{0}C, 1min annealing at 59^{0}C, and 2 min extension at 72^{0}C finalized by a final extension step 10 min at 72^{0}C.

PCR products were run in 1.5% agarose (Sigma) gel, 1 hour at 90V in TAE (Sambrook et al. 1989) buffer and visualized by ethidium bromide (0.5µg/ml) staining. 100 bp Step Ladder (Promega) was used as a size marker. Images were aquired using a ALPHA IMAGE 2200 system under UV light.

RESULTS AND DISCUSIONS

After the DNA extraction from four apple cultivars: Starkrimson, Golden Spur, Florina and Liberty and sixty hybrid apple plants obtained by combinations between Liberty and Florina, Starkrimson and Golden Spur, Starkrimson and Florina, Strakrimson and Liberty, Golden Spur and Florina, Golden Spur and Liberty cultivars, I have obtained different concentration. The concentration was between 15,4 and 2857,9 ng/µl, and the purity was between 1,5 and 2,03. DNA concentration was mesured throught the spectofotometric method with the Eppendorf Biophotometer.

Al-07 is a codominant primer, while AM-19 is a dominant primer. Both of them are specific primers for the *Vf* gene.

Specific primer AL-07 produced a clear length polymorphism in different genotypes with two products of 724 and 466 bp, and specific primer AM-19 produced one products of 526 bp.

Fig. 1

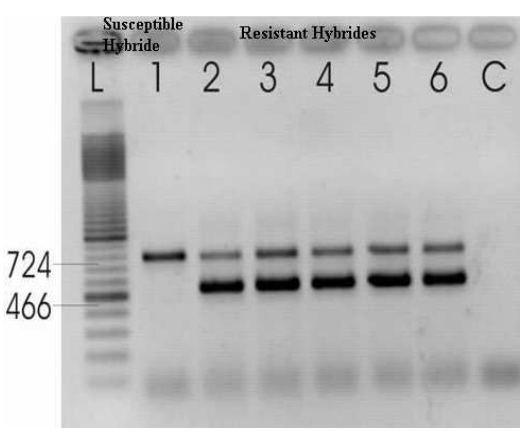

Fig.2

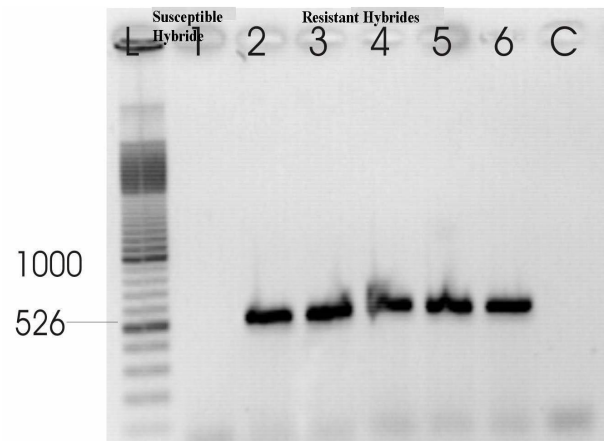

Fig.1 describe the Al-07 466 bp (A) and 724 bp (a) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. L is a 100 bp ladder molecular weight marker (Promega).
Fig.2 describe the 526 bp fragment of AM-19 (A allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance.

The diffrent genotypes of the four apple cultivars and sixty hybrid apple plants obtained with this two specific primers (AL-07, AM-19) are presented on table 1.

Sample No.	Combination	Phenotype	Primer AL-07	Primer AM-19	Genotype
1	Starkrimson	Susceptible	+	-	aa
2	Golden Spur	Susceptible	+	-	aa
3	Liberty	Resistant	+ +	+	Aa
4	Florina	Resistant	+ +	+	Aa
5	Liberty x Florina	Susceptible	+	-	aa
6	Liberty x Florina	Susceptible	+	-	aa
7	Libertyx Florina	Susceptible	+	-	aa
8	Liberty x Florina	Susceptible	+ +	+	Aa
9	Liberty x Florina	Susceptible	+ +	+	Aa
10	Liberty x Florina	Resistant	++	+	Aa
11	Liberty x Florina	Resistant	+	+	AA
12	Liberty x Florina	Resistant	+	+	AA
13	Liberty x Florina	Resistant	+ +	+	Aa
14	Liberty x Florina	Resistant	+ +	+	Aa
15	Starkrimson x Golden Spur	Susceptible	+	-	aa
16	Starkrimson x Golden Spur	Susceptible	+	-	aa
17	Starkrimson x Golden Spur	Susceptible	+	-	aa
18	Starkrimson x Golden Spur	Susceptible	+	-	aa
19	Starkrimson x Golden Spur	Susceptible	+	-	aa
20	Starkrimson x Golden Spur	Resistant	+	-	aa
21	Starkrimson x Golden Spur	Resistant	+	-	aa
22	Starkrimson x Golden Spur	Resistant	+	-	aa
23	Starkrimson x Golden Spur	Resistant	+	-	aa
24	Starkrimson x Golden Spur	Resistant	+	-	aa
25	Starkrimson x Florina	Susceptible	+	-	aa
26	Starkrimson x Florina	Susceptible	+	-	aa
27	Starkrimson x Florina	Susceptible	+	-	aa
28	Starkrimson x Florina	Susceptible	+	-	aa
29	Starkrimson x Florina	Susceptible	+	-	aa
30	Starkrimson x Florina	Resistant	+ +	+	Aa
31	Starkrimson x Florina	Resistant	+ +	+	Aa
32	Starkrimson x Florina	Resistant	+ +	+	Aa
33	Starkrimson x Florina	Resistant	++	+	Aa
34	Starkrimson x Florina	Resistant	+ +	+	Aa
35	Starkrimson x Liberty	Susceptible	+	-	aa
36	Starkrimson x Liberty	Susceptible	++	+	Aa

37	Starkrimson x Liberty	Susceptible	+ +	+	Aa
38	Starkrimson x Liberty	Susceptible	+	-	aa
39	Starkrimson x Liberty	Susceptible	+ +	+	Aa
40	Starkrimson x Liberty	Resistant	+ +	+	Aa
41	Starkrimson x Liberty	Resistant	+	-	aa
42	Starkrimson x Liberty	Resistant	+ +	+	Aa
43	Starkrimson x Liberty	Resistant	+	-	aa
44	Starkrimson x Liberty	Resistant	+ +	+	Aa
45	Golden Spur x Florina	Susceptible	+	-	aa
46	Golden Spur x Florina	Suceptible	+	-	aa
47	Golden Spur x Florina	Susceptible	+	-	aa
48	Golden Spur x Florina	Susceptible	+	-	aa
49	Golden Spur x Florina	Susceptible	+	-	aa
50	Golden Spur x Florina	Resistant	++	+	Aa
51	Golden Spur x Florina	Resistant	+ +	+	Aa
52	Golden Spur x Florina	Resistant	+ +	+	Aa
53	Golden Spur x Florina	Resistant	++	+	Aa
54	Golden Spur x Florina	Resistant	++	+	Aa
55	Golden Spur x Liberty	Susceptible	+	-	aa
56	Golden Spur x Liberty	Susceptible	+	-	aa
57	Golden Spur x Liberty	Susceptible	+	-	aa
58	Golden Spur x Liberty	Susceptible	+	-	aa
59	Golden Spur x Liberty	Susceptible	+	-	aa
60	Golden Spur x Liberty	Resistant	+ +	+	Aa
61	Golden Spur x Liberty	Resistant	++	+	Aa
62	Golden Spur x Liberty	Resistant	+ +	+	Aa
63	Golden Spur x Liberty	Resistant	+ +	+	Aa
64	Golden Spur x Liberty	Resistant	+ +	+	Aa

Legend
+ - genotype aa
++ + genotype Aa
+ + genotype AA

CONCLUSIONS

Molecular markers linked to important phenotypic traits may become an important tool in shortening the length of the selection process by reducing time and costs of the entire breeding programme. Availability of molecular markers linked to different resistance genes against the same pathogen and their map position can also be used to estimate the possible relationship among various, apparently unrelated resistance sources. In fact, it has been demonstrated that markers linked to a specific gene (*Vf* or *Vm*) are not present in selection carrying other resistance genes.This marker-specificity can be used to easily select plants carrying multiple resistances against the same pathogen.

Marker assisted selection of juvenile plants can facilitate the breeding programme for apple scab resistance. The codominant marker can identify plants homozygous for the resistance gene which is important in selection of parent for the next crossing

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