AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic ISSN 1842-4309

Not. Bot. Hort. Agrobot. Cluj 37 (1) 2009, 273-277

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Use of Phenotypic and MAS Selection Based on Bulk Segregant Analysis to Reveal the Genetic Variability Induced by Artificial Hybridization in Apple

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Abstract

Phenotypic selection in apple seedling populations (F_1), derived from semidiallel hybridizations between genitors with different peculiarities ('Florina' and 'Liberty', resistant to apple scab attack; 'Starkrimson' and 'Golden spur', with spur ideotype) was completed with Marker Assisted Selection (MAS), based on Bulk Segregant Analysis (BSA) technique. Molecular analyses performing by Bulk Segregant Analysis were intended to indentifying markers for scab resistance, powdery mildew resistance and architectural tree structure (ideotype) with bulks of five to six plants per groups, and testing the bulks with RAPD polymorphic primers. The polymorphic bands between the bulks were mostly associated to apple scab resistance genes. Concerning powdery mildew resistance and architectural tree structure analysis the results are inconclusive. Although it seems that the primer 54 showed a polymorphic band in a resistant to powdery mildew hybrid, which is missing in the sensitive hybrids, the result is only apparently favourable since this band is present in both parental forms ('Starkrimson', tolerant to powdery mildew and 'Liberty', relative susceptible). Molecular profile of the standard ideotype and spur ideotype, resulted in hybrids originated in standard parental form ('Florina') and spur parental form ('Golden spur'), were usually monomorphic. Genetic association among the parental molecular polymorphism for scab resistance and the other two important traits (powdery mildew resistance and ideotype), was not conclusive.

Keywords: apple hybrids, breeding, BSA technique, Marker Assisted Selection

Introduction

Apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) are two of the major diseases of apple in Transylvania, Romania. Consequently, releasing new apple cultivars resistant to these diseases represent an important goal for breeders (Sestras, 2004; Sestras *et al*., 2005; Bodea *et al*., 2008).

Resistance of apple to scab and powdery mildew attack are both monogenically and polygenically inherited (Crosby *et al*., 1992), this hypothesis being suggested even for architectural ideotype of apple tree (Sestras *et al*., 1998). For apple, gene tagging turns to be efficient in evaluating the resistance to apple scab, powdery mildew and other favourable features, including type of growth and tree fructification (Kellerhals *et al*., 2000; Bus *et al*., 2000; Botez *et al*., 2002; Stankiewicz *et al*., 2002; Huaracha *et al*., 2004 etc.).

Gene tagging represents a compulsory phase integrated in apple breeding programs in the context of using molecular markers for selection stages, on the basis of Marker Assisted Selection (MAS). A number of genetic markers for the well-known genes for resistance to apple scab introgressed from *Malus floribunda* 821 (i.e.*Vf*) are available on the market thus MAS selection has become a new successful breeding tool for scab, completing or replacing the classical phenotypic selection (Garnier *et al*., 1996). In apple breeding programs, Tartarini *et al*. (2000, 2002, and 2003) consider that phenotypic selection does not prove sufficient accuracy for plant resistance to apple scab, compared to the MAS selection.

Using two specific molecular markers, AL-07 (SCAR) and AM-19 (SCAR), for screening the presence of *Vf* gene, the apple cultivars 'Florina' and 'Liberty' were identified by Bodea *et al*. (2008) as heterozygous genotype (*Vfvf*), and 'Starkrimson' and 'Golden spur', scab susceptible cultivars, as recessive homozygous (*vfvf*). In experiments presented in this paper, phenotypic selection of F_1 hybrids applied to these cultivars was completed with MAS selection using polymorphic primers, in order to eventually find polymorphic bands associated to important traits for apple breeding.

Materials and methods

Phenotype selection in the F_1 apple offsprings, obtained by semidiallel hybridizations between different apple cul-

tivars used as genitors ('Florina' and 'Liberty', apple scab resistant due the presence of *Vf* gene in their genomes; 'Starkrimson' and 'Golden spur' characterised by spur ideotype), was completed by molecular Marker Assisted Selection (MAS).

Within each hybrid combination, the response of F_1 apple seedlings to natural infection with apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) under no fungicide treatment condition was noted on 50 randomly chosen hybrids, using a scale from 1 (no attack) to 5 (very strong attack). According to their architectural ideotype, the F_1 hybrid trees were scored using the following scale (Lespinase, 1992): 1 = columnar; 2 = spur; 3 = standard; 4 = weeping. Data obtained in each combination, were processed as mean of the analyzed trait and processed by using ANOVA ("t" test).

The offsprings were classified in six groups, based on the different ideotype (spur, standard), response to apple scab (resistant, sensitive) and to powdery mildew attack (resistant, sensitive). Thus 36 variants of hybrid combinations resulted and four variants represented by the cultivars that were used as genitors (Tab. 1).

BSA (Bulk Segregant Analysis) protocol was applied, using young leaves from 5-6 hybrids, belonging to extreme groups of seedlings (standard / spur ideotype, resistant / sensitive to apple scab, and to powdery mildew, respectively) from each hybrid combination. For RAPD analysis there were used thirteen decamer primers (Tab. 2).

Results and Discussion

The phenotypic means for analysed characteristics and the coefficients of variability (s%) for the specific features of the of F_1 hybrids habit, their response to apple scab and powdery mildew are shown in Tab. 3.

The average score for ideotypes of trees was comprised between 2.56, in 'Starkrimson' x 'Golden spur' combination, and 2.78 in 'Starkrimson' x 'Liberty' combination, without presenting statistical differences from the mean of experiment, considered as control. The mean of score for apple scab attack was comprised between 1.06, in 'Liberty' x 'Florina' descendants, and 1.48 in 'Starkrimson' x 'Golden spur' progenies. As expected, the cultivars resistant to scab ('Liberty'' and 'Florina') produced many seedlings with no attack, while 'Golden spur' produced the most

Tab. 2. Decamer primers used for RAPD analysis

Primers		Nucleotidic sequence
P 52	70.03	5` - ACG GTG CCT G - 3`
P 53	70.04	5` - CGC ATT CCG C - 3`
P 54	70.08	5` - CTG TAC CCC C - 3`
P 56	MIC-13	5` - TTC CCC CCA G - 3`
P 59	270- UBC	5` - TGC GCG CGG G- 3`
P 44	OPA- 17	5` - GAC CGC TTG T - 3`
P 45	OPA-18	5` -AGGTGACCGT
P 51	OPC-20	5` -ACTTCGCCAC
P 55	MIC-07	5` -TGTCTGGGTG
P 57	MIC-14	5` -TGAGTGGGTG
P 60	HBC-534	5` -CACCCCCTGC
P 61	HBC-563	5` -CGCCGCTCCT
P 63	HBC-570	5` -CCCCCCTAAT

Tab. 1. The apple hybrid combinations, their genitors and their phonotypical features

Var. No.	Hybrid combination or parental cultivar	Features of F_1 hybrids (phenotypic selection) and genitors
1	'Starkrimson' x 'Golden spur'	Ideotype – Standard
2		Ideotype – Spur
3		Apple scab – Resistant
4		Apple scab – Sensitive
5		Powdery mildew – Resistant
6		Powdery mildew – Sensitive
7	'Starkrimson' x 'Liberty'	Ideotype – Standard
8		Ideotype – Spur
9		Apple scab – Resistant
10		Apple scab – Sensitive
11		Powdery mildew – Resistant
12		Powdery mildew – Sensitive
13	'Starkrimson' x 'Florina'	Ideotype – Standard
14		Ideotype – Spur
15		Apple scab – Resistant
16		Apple scab – Sensitive
17		Powdery mildew – Resistant
18		Powdery mildew – Sensitive
19	'Golden spur' x 'Liberty'	Ideotype – Standard
20		Ideotype – Spur
21		Apple scab – Resistant
22		Apple scab – Sensitive
23		Powdery mildew – Resistant
24		Powdery mildew – Sensitive
25	'Golden spur' x 'Florina'	Ideotype - Standard
26		Ideotype - Spur
27		Apple scab - Resistant
28		Apple scab - Sensitive
29		Powdery mildew - Resistant
30		Powdery mildew - Sensitive
31	'Liberty' x 'Florina'	Ideotype - Standard
32		Ideotype - Spur
33		Apple scab - Resistant
34		Apple scab - Sensitive
35		Powdery mildew - Resistant
36		Powdery mildew - Sensitive
37	'Starkrimson'	Spur - tolerant to powdery mildew
38	'Golden spur'	Sensitive to apple scab
39	'Liberty'	Resistant to apple scab (*Vf*)- semi spur
40	'Florina'	Resistant to apple scab (*Vf*) - standard

Tab. 3. Phenotypic differences among hybrid combinations, for the architectural ideotype and the response of F_1 apple seedlings to natural infection with apple scab and powdery mildew

Combination	Mean of scores, significance[1] and the coefficients of variability (s%)		
	Architectural ideotype	Apple scab	Powdery mildew
'Starkrimson' x 'Golden spur'	2.56(-); 32.7%	1.48(x); 34.1%	1.76(-); 39.0%
'Starkrimson' x 'Liberty'	2.70(-); 24.0%	1.30(-); 35.6%	1.54(-); 39.8%
'Starkrimson' x 'Florina'	2.78(-); 23.3%	1.34(-); 35.7%	1.58(-); 31.6%
'Golden spur' x 'Liberty'	2.68(-); 25.5%	1.38(-); 35.5%	1.52(-); 38.2%
'Golden spur' x 'Florina'	2.64(-); 27.3%	1.40(-); 35.3%	1.56(-); 43.3%
'Liberty' x 'Florina'	2.76(-); 23.8%	1.06(ooo); 22.6%	1.80(-); 52.6%
Mean of experiment (Control)	2.69; 26.1%	1.33; 35.4%	1.63; 42.1%

[1] "t" test – x, xx, xxx/o, oo, ooo Significant, positive or negative, for $P<0.05$, 0.01 and 0.001

susceptible ones. For powdery mildew response, the mean of scores was comprised between 1.52 in 'Golden spur' x 'Liberty' combination and 1.80 in 'Liberty' x 'Florina' combination.

The coefficients of variability of these marks exhibited large values in all combinations (23.3-32.7% for architectural ideotype; 22.6-35.7% for response to apple scab attack; 31.6-52.6% for response to powdery mildew attack), and offered a good background for an efficient phenotypic selection in each F_1 hybrid population.

After phenotypic selection applied in F_1 seedlings, young leaves were picked from 5-6 hybrids, belonging to extreme groups (standard / spur ideotype, resistant / sensitive to apple scab and to powdery mildew, respectively) and used for DNA analysis (BSA).

Among genitors, for some primers, an obvious polymorphism was noticed (Fig. 1 and 2).

These results, at the molecular level, confirmed the phenotypic variation of the studied characters presented in Tab. 3.

The existence of the molecular polymorphism in parental forms is a first condition for a BSA successful analysis. Taking into account that RAPD markers are dominant, these markers should be linked in a coupling phase, in the segregant hybrids, with the genes for the analyzed traits, as a second condition. Such a condition was exhibited only for scab resistance in apple and for very few primers. Such a favourable situation was shown in the hybrid combinations 'Liberty' x 'Starkrimson', for MIC-07, UBC 534, UBC570 primers (Fig. 3) 'Florina' x 'Starkrimson', for OPC-20, and MIC-07 primers (Fig. 4) and 'Florina' x 'Golden spur', for UBC-534 primer (Fig. 5).

In spite of the fact that in the hybrid combination 'Liberty' x 'Golden spur' a significant polymorphism was observed both among hybrids and their parental forms, the required condition for the coupling state of RAPD markers and Vf gene was not fulfilled (Fig. 6). It has to be mentioned that even in hybrid combinations originated in two susceptible parental forms (i.e. 'Starkrimson' and 'Golden spur'), which are supposed to lack Vf allele, there were noted several scab resistant hybrids.

Concerning powdery mildew resistance and architectural tree structure analysis the results are inconclusive (Fig. 7 and 8). Although it seems that the primer 54 showed a polymorphic band in the resistant to powdery mildew hybrids, which is absent in the sensitive hybrids (Fig. 7), the result is only apparently favourable since this band is present in both parental forms (37 - 'Starkrimson', tolerant to powdery mildew and 39 - 'Liberty', suscepti-

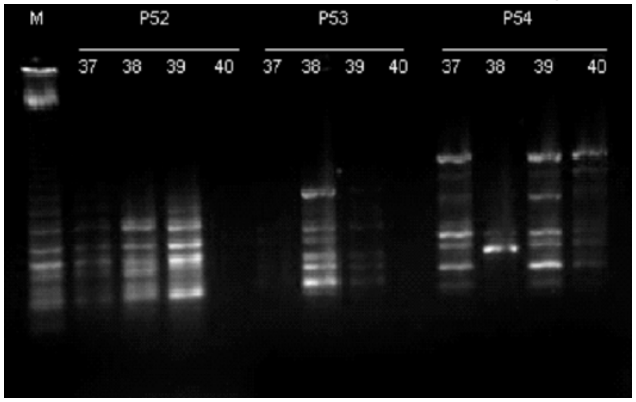

Fig. 1. RAPD amplification products obtained for the four genitors with P52-70.03, P53-70.04 and P54-70.08 decamer primers (37-'Starkrimson'; 38-'Golden spur'; 39-'Liberty'; 40-'Florina'). M-100 bp ladder

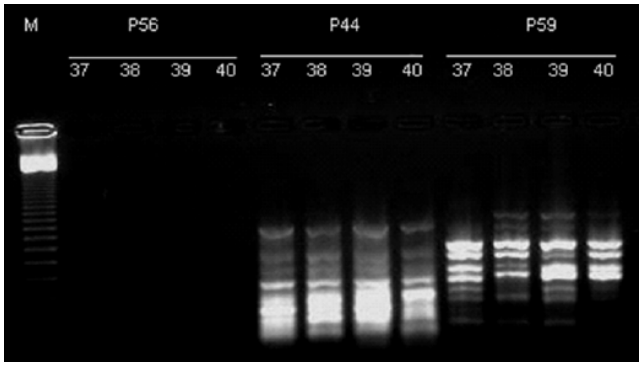

Fig. 2. RAPD amplification products obtained for the four genitors with P56-MIC-13, P44-OPA-17 and P59-270-UBC decamer primers (37-'Starkrimson'; 38-'Golden spur'; 39-'Liberty'; 40-'Florina'). M-100 bp ladder

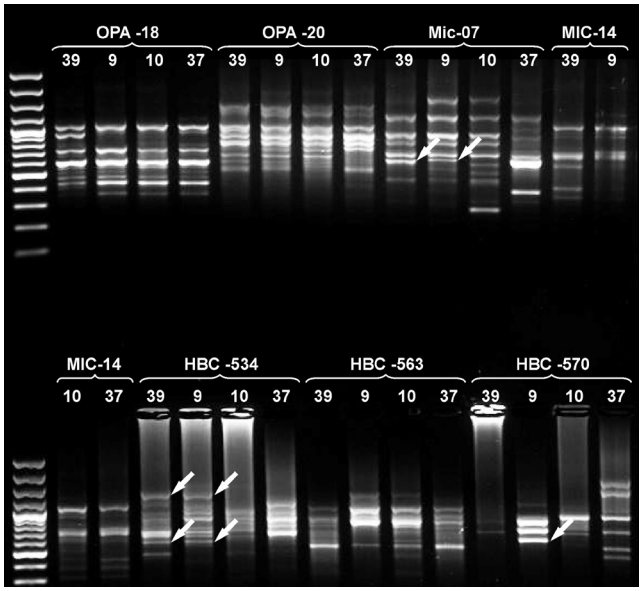

Fig. 3. RAPD amplification products obtained in 'Liberty' (39) and 'Starkrimson' (37) parental forms and their F1 hybrids (9 and 10). L-100 bp ladder

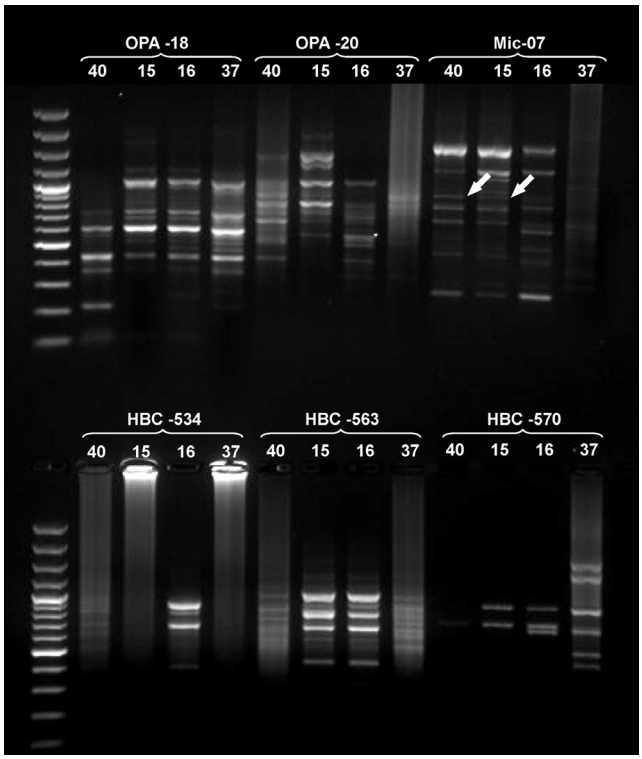

Fig. 4. RAPD amplification products obtained in 'Florina' (40) and 'Starkrimson' (37) parental forms and their F_1 hybrids (15 and 16). L-100 bp ladder

ble), as it has been presented in Fig. 1. Molecular profile of the standard ideotype and spur ideotype, resulted from standard parental form ('Florina') and spur parental form ('Golden spur'), were usually monomorphic (Fig. 8).

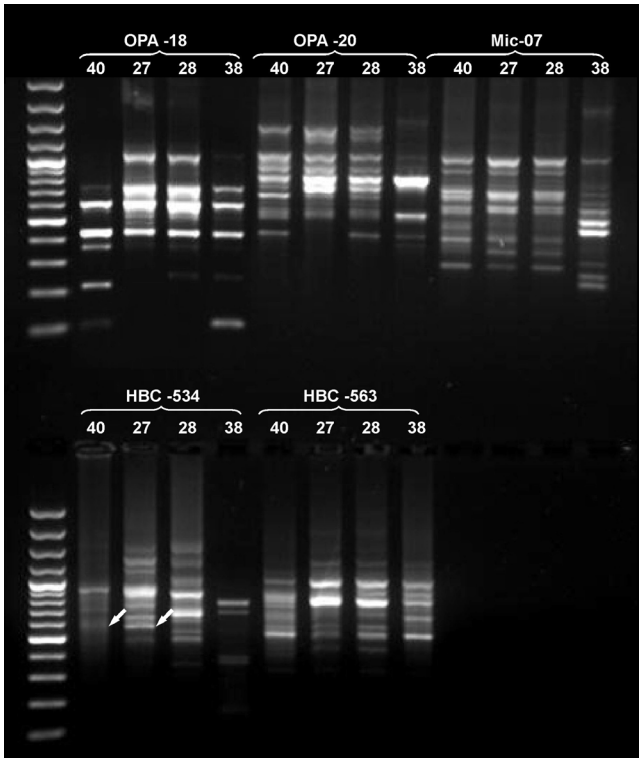

Fig. 5. RAPD amplification products obtained in 'Florina' (40) and 'Golden spur' (38) parental forms and their F_1 hybrids (27 and 28). L-100 bp ladder

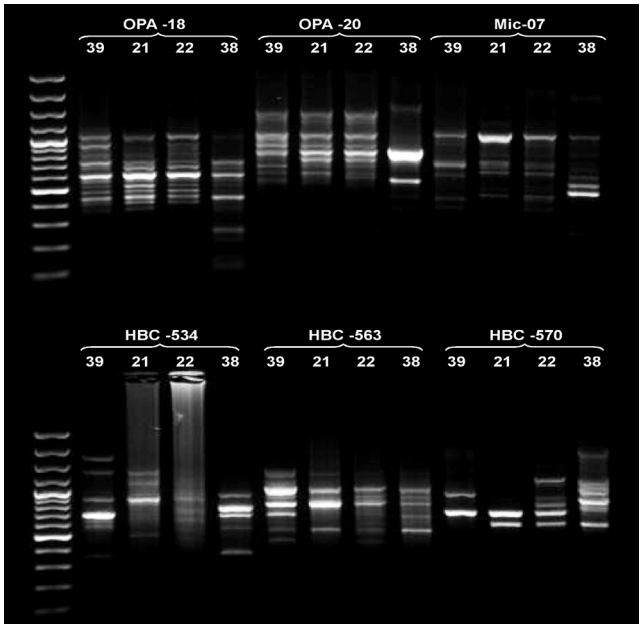

Fig. 6. RAPD amplification products obtained in 'Liberty' (39) and 'Golden spur' (38) parental forms and their F1 hybrids (21 and 22). L-100 bp ladder

Conclusions

Using Bulk Segregant Analysis there were obtained, for a few primers, markers linked in a coupling state with putative apple scab resistance genes, a necessary condition

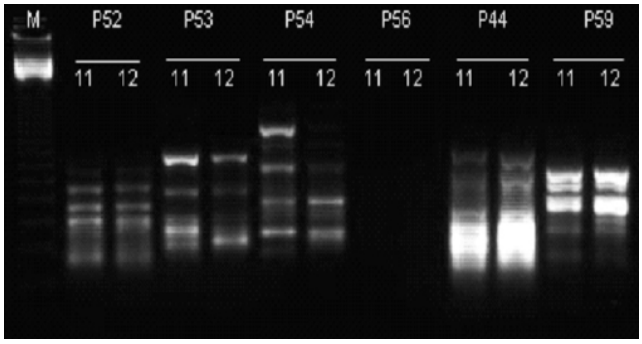

Fig. 7. RAPD amplification products obtained in F1 hybrids (11 - resistant to powdery mildew and 12 - sensitive to powdery mildew) originated 'Starkrimson' and 'Liberty' parental forms. L-100 bp ladder

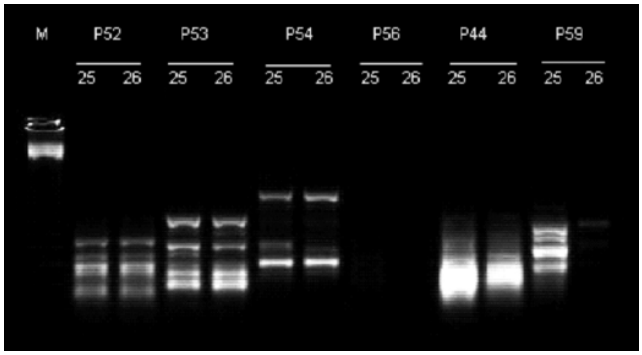

Fig. 8. RAPD amplification products obtained in F_1 hybrids (25 - standard and 26 - spur type) of 'Golden spur' and 'Florina' parental forms. L-100 bp ladder

for these markers to be successfully used in a Marker Assisted Selection (MAS) breeding program.

Tacking into account that by phenotypic selection there were obtained several resistant hybrids in combinations originated in parental forms lacking *Vf* allele, some of those markers could be linked with other genes for scab resistence.

Genetic association among the parental molecular polymorphism for scab resistance and the other two important traits (powdery mildew resistance and ideotype), was not conclusive.

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