Australian Journal of Crop Science AJCS
AJCS 5(6):748-755 (2011) ISSN:1835-2707

Possibilities to improve apple scab (*Venturia inaequalis* (Cke.) Wint.) and powdery mildew [*Podosphaera leucotricha* (Ell. et Everh.) Salm.] resistance on apple by increasing genetic diversity using potentials of wild species

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Abstract

Creating new apple cultivars largely depends on the availability of sufficient genetic diversity, while apple breeding has eroded in time the genetic base of domestic cultivars. The possibility to induce and exploit useful variability for selecting hybrids resistant to apple scab and powdery mildew attack was evaluated in interspecific descendants derived from five wild apple species (*Malus coronaria*, *M. floribunda*, *M. niedzwetzkyana*, *M. zumi* and *M. prunifolia*). A number of 1650 F_1 hybrids were analyzed, belonging to fifteen combinations, framed in five cyclic models of hybridization, in which crab apple species were used as maternal or paternal tester, each being crossed with two-four different apple cultivars (namely Cluj 218/2, Frumos de Voinesti, Reinette Baumann, Rosu de Cluj, Jonathan, and Golden Delicious). *M. coronaria* and *M. prunifolia* proved to have descendents with valuable response to scab attack (based on genetic gain and expected selection response), compared to *M. zumi* offsprings. The offsprings of *M. coronaria* offer a good background for an efficient selection of resistant individuals for powdery mildew. The additive effects proved inferior compared to genetic effects of dominance and epistasis in several analyzed crosses. Phenotypic correlations between responses to apple scab and powdery mildew attack on F_1 hybrids were identified in three out of the five cyclic combinations. The results reveal the potential of wild apple species as resources of genes for resistance to both scab and powdery mildew.

Keywords: expected selection response, heritability, hybridization, genetic gain, seedlings.
Abbreviations: PRI-Purdue, Rutgers and Illinois Universities cooperative apple breeding program, started in 1948; CGV-Coefficient of Genetic Variability; H^2-broad sense heritability; h^2-narrow sense heritability; σ_G^2-genotypic variance; σ_P^2-phenotypic variance; σ_{Ga}^2-additive variance; GG or ΔG-Genetic Gain; σ-square root of the total variance among families; σ_G-square root of the genotypic variance among families; $\bar{x}$-mean of trait; σ_P-square root of the phenotypic variance among families or populations represented by F_1 hybrids from each cyclic combinations of half-siblings; R-expected selection response.

Introduction

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., and powdery mildew, caused by *Podosphaera leucotricha* (Ell. et Everh.) Salm., are the main diseases that cause most damage in apple cultures (Janick *et al*., 1996; Dunemann *et al*., 2007; Holb, 2009; Mitre *et al*., 2010). The two fungal diseases can be controlled through treatments, but with considerable difficulties and costs, and with long-term consequences and negative effects both on the environment and on the quality of fruits (Berriea and Xua, 2003; Holb, 2009). In addition, depending on climatic conditions, the effectiveness of fungicide treatments is relative (Köller *et al*., 2004; Jamar *et al*., 2010; Holb, 2009). The creation of resistant cultivars to apple scab and powdery mildew attack is a one of the main goals in apple breeding programmes (Janick *et al*., 1996; Pitera, 2000, 2003; Bus *et al*., 2002, 2005; Sestras, 2004; Dunemann *et al*., 2007). Also, new resistant apple cultivars are necessary for organic growing and integrated fruit production, since many of the highly appreciated commercial cultivars are very susceptible to apple scab and powdery mildew attack (Sandskär and Gustafsson, 2004; Sestras, 2003a, 2003b; Mitre *et al*., 2010). Creating new apple cultivars largely depends on the availability of sufficient genetic diversity, while apple breeding has eroded in time the genetic base of domestic cultivars. The genetic diversity of apple trees is nowadays limited due to the previously use of domestic cultivars in breeding (Vanwynsberghe, 2006). In addition, apple cultivars used in modern commercial orchards are nearly all genetically based on several relatively disease susceptible ancestors like Golden Delicious, Cox Orange, Jonathan, McIntosh, Red Delicious and James Grieve (Bannier, 2011). One of the most popular methods, reckon to induce a wide genetic diversity, to enable a wider selection base and to create new resistant cultivars, is interspecific hybridization. Interspecific hybridization was previously used on different *Malus* species in order to induce resistance to apple scab (e.g. *M. floribunda*, *M. atrosanguinea*, *M. baccata*, *M. micromalus*, *M. prunifolia*, etc.) as well as to induce resistance to powdery mildew (e.g. *M. zumi*, *M. robusta*, *M. sargentii*, *M. baccata jackii* etc.) (Janick *et al*., 1996; Sestras, 2004). The morphological, biochemical and molecular variation within wild apple species indicates that the starting point in selection of domesticated apples could have come directly from wild apple, without the involvement of other species (Harris *et al*., 2002). The inheritance of resistance to apple scab and powdery mildew attack is twofold, monogenic and polygenic, as different genetic sources are being known and recommended as genitors in apple breeding programs (Visser *et al*., 1974; Janick *et al*., 1996). The use of *M. floribunda* specie in the creation of the first cultivar with genetic resistance to apple scab, on which PRI series has further been developed (based on *Vf* gene), is a relevant example in this sense (Crosby *et al*., 1992; Janick *et al*., 1996; Janick, 2002). The monogenic resistance has the advantage that it could be more easily incorporated in a cultivar compared to polygenic resistance, while its main disadvantage consists in the apparition of new pathogenic virulent strains of *Venturia inaequalis* (Cke) Wint. (strains with which it needs to fight). The polygenic resistance is considered more durable compared to the monogenic resistance due to the complexity of the involved mechanisms (Janick *et al*., 1996; Sestras, 2004). The efficiency of selection out of the offsprings obtained by artificial hybridization is dependent on the heritability of the valuable traits of genitors. Therefore, this study aimed to analyze the resistance heritability to apple scab and powdery mildew attack, starting from the premise that these features have a polygenic inheritance, respectively that the transmission and manifestation of the seedlings resistance are governed by polygenes. In addition, there was analysed the possibility to increase genetic diversity in apple using wild species as genitors, in order to broad genetic enrichment, necessary for selection of new cultivars resistant to apple scab and powdery mildew attack.

Materials and methods

Plant material

The response to apple scab and powdery mildew attack of 1650 F_1 hybrids was analysed at the Fruit Research Station Cluj-Napoca, Romania. The offsprings were obtained through crosses among *Malus* species and apple cultivars, belonging to fifteen hybrid combinations. The following wild species of *Malus* were used as male and/or female testers (Bojňanský and Fargašová, 2007; Sestras, 2004): *M. coronaria*, a North America specie, also known as sweet crab-apple or garland crab; *M. floribunda,* also known as Japanese flowering crab-apple, originated from Japan and East Asia, being very well known by apple breeders as monogenic source of resistance to apple scab (*Vf* gene); *M. niedzwetzkyana,* found in the fruit and nut forests of Central Asia, are considered rare apple trees; *M. zumi*, also known as O-zumi or Zumi Crabapple, is native to Europe and Asia; *M. prunifolia*, also known as pear-leaf or plum-leaf crab-apple or Chinese apple, ornamental tree native to China. The following cultivars were used as male and/or female testers (see Table 1): Cluj 218/2, Frumos de Voinesti, Golden Delicious, Jonathan, Reinette Baumann, and Rosu de Cluj. Among these, Jonathan and Rosu de Cluj are very susceptible to powdery mildew attack, while Golden Delicious and Jonathan are susceptible to apple scab (Sestras, 2003a, 2003b).

Hybridization procedure

The seedlings were framed in five cyclic models of hybridization, in which each specie used as maternal or paternal tester was crossed with minimum two (*M. coronaria* as maternal tester) and maximum four (*M. niedzwetzkyana* as paternal tester) cultivars, used as genitors. The number of progenies per combination varied from 31 (Cluj 218/2 × *M. floribunda*) up to 142 (Reinette Baumann × *M. floribunda*) (Table 1).

Fungi studied

Apple scab caused by the fungus *Venturia inaequalis* is the most important disease of apple worldwide (Bowen *et al*., 2011). This disease can cause extensive losses (70% or greater) where humid, cool weather occurs during the spring months. It can be seen frequently on leaves, petioles, blossoms, sepals, fruit, and pedicels. Powdery mildew occurs wherever apples are grown and may be a very serious disease, as many apple cultivars are susceptible to its attack (Sestras, 2003b). The fungus can affect leaves, buds, growing tips and fruit, and lead to leaf drop and dieback. Both apple scab and powdery mildew are threats to apple foliage and fruits every year. When severe attacks occurs over several years, the defoliation can seriously weaken trees, reducing the productivity, fruits qualities and survival capability of fruit trees, as well as the aesthetic value of ornamentals.

Diseases assessments

The response of hybrids to diseases attack was assessed on three years interval, on hybrids of seven to nine years old, in natural conditions of infection, without fungicide treatments. Each year, scab and powdery mildew incidence was assessed two times, in the first decade of July and August, based on scab attack on leaves and powdery mildew on shoots. A scale of 0 to 5 was used, following the standard diagram corresponding to an Attack Index or Attack Degree - AD%, whereby (Sestras, 2003a, 2003b): 0 = no attack (AD% = 0); 1 = very weak attack (AD%=0.1-1); 2 = weak attack (AD%=1.1-5.0); 3 = medium attack (AD%=5.1-15); 4 = strong attack (AD%=15.1-20); 5 = very powerful attack (AD%>20.1).

Statistical analysis

The statistical analysis of experiments data was carried out by applying the ANOVA test. The data were summarized as means and standard deviations for each cyclic hybridization. The genetic analysis of the families involved the decomposition of variances for each hybrid cyclic siblings (half-sib), which were grouped thought siblings having a common hereditary basis, derived from the common parent (mother or father). Calculation of the broad sense heritability (H^2) and of the narrow sense heritability (h^2) was based on the variances of inter-families and intra-families of siblings, the proportion of common genes, respectively the degree of relatedness, considering equal to 25%, or 1/4 (Falconer and Mackay, 1996; Souza *et al*., 2000; Gatti *et al*., 2005). Broad sense heritability and narrow-sense heritability were computed by the classical model, $H^2=\sigma_G^2/\sigma_P^2$, $h^2=\sigma_{Ga}^2/\sigma_P^2$,

Table 1. Hybrid combinations and number of F_1 hybrids

♀ \ ♂	Golden Delicious	Jonathan	Reinette Baumann	*Malus floribunda*	*Malus niedzwetzkyana*	*Malus prunifolia*
Malus zumi	139	122	76			
Malus coronaria		128	81			
Cluj 218/2				31	123	
Frumos de Voinesti				110	135	
Reinette Baumann				142	82	79
Rosu de Cluj					137	125
Golden Delicious						140

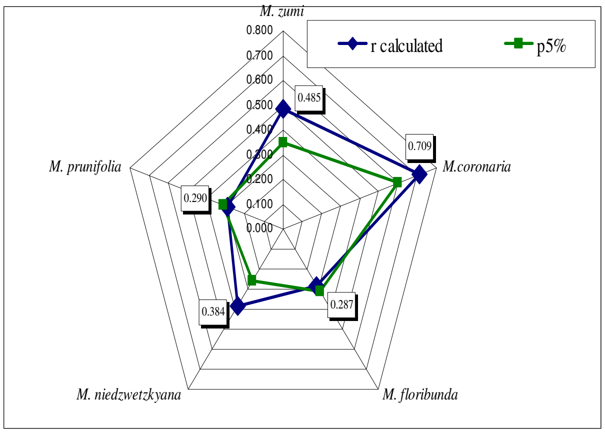

Fig 1. The phenotypic coefficient of correlation (r) between the response to apple scab and powdery mildew attack of F_1 interspecific hybrids, belonging to *Malus* species used as maternal and paternal genitors

where σ_G^2 is the genotypic variance; σ_P^2 is the phenotypic variance; σ_{Ga}^2 is the additive variance (Holland *et al*., 2003; Piepho and Möhring, 2007; Lu and Myers, 2011). Heritability in narrow sense was used to predict the response to selection (Falconer and Mackay, 1996) and to the expected selection response (R). The Coefficient of Genetic Variability (CGV%), Genetic Gain (GG or ΔG) and expected selection Response (R) were computed as follows:

$CGV\% = \sigma_G / \bar{x}$, where: σ = square root of the total variance among families; σ_G = square root of the genotypic variance among families; $\bar{x}$ = mean of trait (mean of marks for apple scab or powdery mildew response of F_1 hybrids).

$\Delta G = k \times h^2 \times \sigma_P$, or $\Delta G = i \times h^2 \times \sigma_P$, where: k or i = selection intensity (considered 2.06 for the top 5%); σ_P = square root of the phenotypic variance among families or populations represented by F_1 hybrids from each cyclic combination of half-siblings (Gatti *et al*., 2005). The expected selection response (R) was estimated considering the half-sib family selection method described by Nyquist (1991): $R = i \times \sigma_P^2 \times h^2$, where: σ_P^2 = phenotypic variance among families or populations represented by F_1 hybrids from each cyclic combination of half-siblings.

Results and Discussion

Response of F_1 hybrids to apple scab attack

The top-three hybrid combinations with the lowest average for apple scab attack derived from the following crosses: *M. coronaria* × Reinette Baumann, Frumos de Voinesti × *M. niedzwetzkyana* and *M. zumi* × Golden Delicious (Table 2). Top-three hybrids in regards of higher mean of rates for scab attack were: Cluj 218/2 × *M. floribunda* and Frumos de Voinesti × *M. floribunda* combination. Between the five cyclic hybridizations, the mean of marks for apple scab attack was quite dispersing, with limits ranging from 0.69 (*M. coronaria* used as maternal tester) to 3.09 (*M. floribunda* used as paternal tester). The results showed that *M. coronaria* used as mother genitor transmitted by crossing with two cultivars (Jonathan and Reinette Baumann) a valuable response to apple scab attack to their seedlings. *M. floribunda* specie used as father genitor transmitted to their progenies susceptibility to scab. *M. prunifolia*, *M. niedzwetzkyana* and *M. zumi* passed the intermediate response to scab to their descendants, compared with *M. coronaria* and *M. floribunda*. The obtained results when apple scab was investigated in F_1 offspring proved to be different in some cases compared to already published results. The most unexpected result consists in the high average obtained by the offsprings which have *M. floribunda* as parental genitor. Thus, the progenies of *M. floribunda* used in the analysed hybridisations had a substantially different reaction to apple scab attack, compared to the Clone 821, most frequently used as source for scab resistance, due to dominant *Vf* gene (Koller *et al*., 1994; Tartarini, 1996). The result does not rule out the possibility that *M. floribunda* specie used to hold the hereditary endowment major resistance gene *Vf,* and F_1 hybrids who have not inherited it have been particularly susceptible to scab so the result is a high average grade for the attack. Obviously, in combinations with *M. floribunda* specie, there is the possibility that some hybrids to have a good resistance to scab, due to monogenic resistance induced by the existence of *Vf* gene in their genome, or due to a combined strength given by the association of *Vf* gene with polygenes.The offsprings resulted from *M. niedzwetzkyana* and *M. prunifolia* species used as paternal testers and Rosu Cluj used as maternal genitor proved to be the ones with the highest mean of marks, and thus with a high rate of susceptibility to scab. The result is surprising, as Rosu de Cluj is known to have a certain tolerance to scab (Sestras, 2004; Quamme *et al*. 2003) due to polygenic resistance to apple scab attack. The descendents resulted from the combinations in which Golden Delicious, a cultivar susceptibility to scab (Sestras, 2004), participated as genitor, proved to have a small average of marks for scab attack: *M. zumi* × Golden Delicious (0.58 ± 0.18) and Golden Delicious × *M. prunifolia* (1.00 ± 0.25). Even if *Malus* species have the advantage of transmitting to their decedents the diseases resistance genes, this is unlikely to happen when interspecific crosses are used to create new cultivars. This could be explained by the dominant transmission of the rustic characters from the wild genitors (Sestras *et al*., 2010).

Furthermore, the genetic analysis is difficult to be carried out on apple since a long time is needed for reliable apple breeding programmes. For this reason, quantitative genetic principles have not been extensively applied in apple breeding (Noiton and Shelbourne, 1992). In addition, very few quantitative genetic studies have been conducted on apple, because the work to-date has been mainly devoted to field selection within progenies, rather than analysing well-

Table 2. Mean of marks* for the response of F_1 interspecific hybrids to apple scab attack and the significance of differences** for each cyclic hybrid combination

Hybrid combination (with tester as ♀ or ♂)		$* \bar{x} \pm s_{\bar{x}}$	%	Differ. (±d)	t-value	**p-value
M. zumi	Golden Delicious	0.58 ± 0.18	45.3	-0.70	-2.5	o(o)
	Jonathan	1.39 ± 0.33	108.6	0.11	0.3	-
	Reinette Baumann	1.87 ± 0.31	146.1	0.59	1.6	-
Mean of experiment (Control)		1.28 ± 0.21	100.0	-	-	-
M. coronaria	Jonathan	1.00 ± 0.19	144.9	0.31	1.27	-
	Reinette Baumann	0.38 ± 0.07	55.1	-0.31	-1.9	(o)
Mean of experiment (Control)		0.69 ± 0.15	100.0	-	-	-
Cluj 218/2	*M. floribunda*	3.52 ± 0.32	113.9	0.43	1.2	-
Frumos de Voinesti		3.23 ± 0.24	104.5	0.14	0.5	-
Reinette Baumann		2.51 ± 0.15	81.2	-0.58	-2.8	oo
Mean of experiment (Control)		3.09 ± 0.14	100.0	-	-	-
Cluj 218/2	*M. niedzwetzkyana*	1.13 ± 0.19	82.5	-0.25	-1.1	-
Frumos de Voinesti		0.53 ± 0.09	38.7	-0.84	-5.1	ooo
Reinette Baumann		1.74 ± 0.30	127.0	0.37	1.1	-
Rosu de Cluj		2.10 ± 0.29	153.3	0.72	2.23	x
Mean of experiment (Control)		1.37 ± 0.14	100.0	-	-	-
Golden Delicious	*M. prunifolia*	1.00 ± 0.25	63.7	-0.56	-1.8	(o)
Reinette Baumann		1.44 ± 0.34	91.7	-0.12	-0.3	-
Rosu de Cluj		2.25 ± 0.31	143.3	0.68	1.9	x
Mean of experiment (Control)		1.57 ± 0.20	100.0	-	-	-

* The response to apple scab attack of hybrids was grade by using the following scale: 0 = no attack; 1 = very weak attack; 2 = weak attack; 3 = medium attack; 4 = strong attack; 5 = very powerful attack. ** Symbols for differences (t-values): x, xx, xxx/o, oo, ooo; significant at $p < 0.05$, 0.01 , and 0.001 (positive, respectively negative);

$\bar{x}$ = mean; $s_{\bar{x}}$ = standard deviation relative to observed mean

Table 3. Heritability and other genetic parameters* for the response of F_1 interspecific hybrids to apple scab attack

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H^2	h^2			
M. zumi	♀	1.28	0.685	0.159	12.5	0.382	0.446
M. coronaria	♀	0.69	0.864	0.607	23.9	0.606	0.294
M. floribunda	♂	3.09	0.860	0.278	28.1	0.468	0.383
M. niedzwetzkyana	♂	1.37	0.887	0.222	18.4	0.478	0.499
M. prunifolia	♂	1.57	0.828	0.219	23.6	0.535	0.635

*H^2 = broad sense heritability; h^2 = narrow sense heritability;

CGV% = coefficient of genetic variability; GG = genetic gain; R = expected selection response

constructed mating designs (Durel *et al*., 1998). The present study allows us to calculate some important genetic parameters (Table 3). The broad sense heritability coefficient proved to have homogenous values among the investigated cyclic hybridizations, with values from 0.685 (*M. zumi* used as maternal tester) to 0.887 (*M. niedzwetzkyana* used as paternal tester). The results showed that all species used as genitors transmitted to their progenies, with satisfactory reliability, their response to apple scab attack. Coefficients of heritability in the broad sense means a majority response which shares the genotype effect in the manifestation of reactions of the seedlings to disease, but also a significant contribution of the environmental factors, according to species participation as maternal or paternal genitor. With one exception, the analysis of the heritability coefficients in narrow sense (h^2) revealed that the non-additive effects are more important than additive effects. The exception is represented by the cyclic combination of *M. coronaria* used as maternal tester. Therefore, except of the *M. coronaria* descendants, the narrow sense heritability coefficients varied from 0.159 (*M. zumi* used as maternal tester) to 0.278 (*M. floribunda* used as paternal tester). The highest share of polygenic additive contribution to transmission of seedlings resistance to the apple scab attack occurred at *M. coronaria*. This was the only case in the experiment in which the additive effects were superior compared to the genetic effects of dominance and epistasis, which presented a stronger contribution in the manifestation of the character in the other cyclic combinations. Coefficients of genetic variability proved to have high values in all hybrid cyclic combinations, showing the existence of hybrids with a broad response to apple scab attack, especially among the descendants of *M. floribunda* (28.1%). Therefore, the CGV values indicate that it is virtually possible to identify hybrid offsprings with a proper response to disease in any cyclic combination. The chances to obtain a population of hybrids with the desired response to apple scab proved to be higher when *M. coronaria* and *M. prunifolia* are used as genitors in interspecific hybridization, especially compared to *M. zumi* (this is sustained by the values of genetic gain). The highest value of the expected selection response was noticed among *M. prunifolia* descendants, offering a good background for an efficient selection for scab resistant individuals.

Table 4. Mean of marks* for the response of F_1 interspecific hybrids to powdery mildew attack and the significance of differences** for each cyclic hybrid combination

Hybrid combination (with tester as ♀ or ♂)		* $\bar{x} \pm s_{\bar{x}}$	%	Differ. (±d)	t-value	** p-value
M. zumi	Golden Delicious	1.00 ± 0.18	84.7	-0.17	-0.8	-
	Jonathan	1.06 ± 0.19	89.8	-0.12	-0.5	-
	Reinette Baumann	1.47 ± 0.26	124.6	0.29	1.0	-
Mean of experiment (Control)		1.18 ± 0.13	100.0	-	-	-
M. coronaria	Jonathan	1.28 ± 0.29	150.6	0.43	1.09	-
	Reinette Baumann	0.42 ± 0.42	49.4	-0.43	-0.87	-
Mean of experiment (Control)		0.85 ± 0.27	100.0	-	-	-
Cluj 218/2	*M. floribunda*	1.47 ± 0.41	108.9	0.12	0.3	-
Frumos de Voinesti		1.57 ± 0.47	116.3	0.22	0.4	-
Reinette Baumann		1.01 ± 0.14	74.8	-0.34	-1.5	-
Mean of experiment (Control)		1.35 ± 0.18	100.0	-	-	-
Cluj 218/2	*M. niedzwetzkyana*	0.77 ± 0.16	56.6	-0.59	-3.1	oo
Frumos de Voinesti		1.25 ± 0.26	91.9	-0.11	-0.4	-
Reinette Baumann		1.40 ± 0.19	102.9	0.04	0.2	-
Rosu de Cluj		2.02 ± 0.15	148.5	0.66	3.5	xxx
Mean of experiment (Control)		1.36 ± 0.11	100.0	-	-	-
Golden Delicious	*M. prunifolia*	1.81 ± 0.37	105.8	0.10	0.2	-
Reinette Baumann		1.30 ± 0.21	76.0	-0.41	-1.5	-
Rosu de Cluj		2.02 ± 0.23	118.1	0.31	1.1	-
Mean of experiment (Control)		1.71 ± 0.17	100.0	-	-	-

* The response to powdery mildew attack of hybrids was grade using the following scale: 0 = no attack; 1 = very weak attack; 2 = weak attack; 3 = medium attack; 4 = strong attack; 5 = very powerful attack; ** Symbols for differences (t-value): x, xx, xxx/o, oo, ooo; significant at $p < 0.05$, 0.01 and 0.001 , (positive, respectively negative); $\bar{x}$ = mean; $s_{\bar{x}}$ = standard deviation relative to observed mean

Table 5. Heritability and other genetic parameters* for the response of F_1 interspecific hybrids to powdery mildew attack

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H^2	h^2			
M. zumi	♀	1.18	0.521	0.018	1.4	0.028	0.021
M. coronaria	♀	0.85	0.754	0.424	29.5	0.737	0.622
M. floribunda	♂	1.35	0.515	0.012	2.5	0.025	0.025
M. niedzwetzkyana	♂	1.36	0.888	0.223	18.2	0.380	0.315
M. prunifolia	♂	1.71	0.607	0.071	5.6	0.148	0.150

*H^2 = broad sense heritability; h^2 = narrow sense heritability;
CGV% = coefficient of genetic variability; GG = genetic gain; R = expected selection response

Response of F_1 hybrids to powdery mildew attack

Compared with the average score recorded for the scab attack, inside the hybrid combinations, powdery mildew attack showed smaller oscillations, ranging from 0.42 to 2.02 (Table 4). The smallest average value of the marks, identifying the hybrids with the best response to the attack, was recorded in the same combination in which the average score for scab attack was the lowest, *M. coronaria* × Reinette Baumann respectively (see Table 4). The highest average grade was 2.02 and was recorded in two variants, both having Rosu de Cluj as maternal genitor, in combination with *M. niedzwetzkyana* and *M. prunifolia* used as testers. This sensitive response of the offsprings could be explained by the cultivars influences, Rosu de Cluj being known as sensitive to powdery mildew (Sestras *et al*., 2010). The mean of marks for powdery mildew attack was quite close, ranging from 0.85 to 1.71, among the five cyclic hybridizations. The lower limit was noted where the specie *M. coronaria* was used as maternal tester, in its cyclic combinations being registered the lowest value of marks for apple scab. Opposite, the *M. prunifolia* used as paternal tester gave birth to the most powerful attacked descendants (Table 4). The general combining ability, as well as the specific combining ability effects, proved to influence the transmission of the traits (Bus *et al*., 2005; Hampson *et al*., 2009; Soltanloo *et al*., 2010) as it was also noticed in cyclic hybridisations with *M. niedzwetzkyana* as paternal tester. Here, two combinations presented large differences as mean of marks for powdery mildew attack, strongly depending by the parental formula and therefore by the cultivars that participated in hybridization with the rustic specie. The F_1 hybrids belonging to Cluj 218/2 × *M. niedzwetzkyana* proved to have distinct significant negative differences (0.77) compared to the mean (1.36), while seedlings derived from Rosu de Cluj × *M. niedzwetzkyana* proved to have highly significant positive differences. *M. coronaria* has a very valuable polygenic background for assuring the resistance to *Podosphaera leucotricha* since the mean of marks correlates with broad sense heritability and narrow sense heritability (Table 5). Very low values of narrow sense heritability are surprising for several combinations, especially for those where *M. floribunda*, *M. zumi* and *M. prunifolia* (between 0.012 and 0.071) were used as testers. Moreover, also the genetic gain and expected selection response for the studied trait proved to be roughly insignificant in these cases. It does not mean that for these species, as for other wild ones, hybridizations could not be significant in the creation of new cultivars that carry economically important characteristics (Harris *et al*., 2002), including powdery mildew resistance. The results do not exclude the possibility that these species, when used as genitors, could produce descendents with favourable response (tolerant or even resistant) against powdery mildew attack, due to epistasis and dominance effects of polygene. There were identified phenotypic correlations statistically assured in three out of five cyclic combinations (Figure 1) when the responses to apple scab and powdery mildew attack of F_1 interspecific hybrids were investigated. The strongest correlation proved to be among the descendents of *M. coronaria* used as maternal tester ($r = 0.709$). The *M. coronaria* wild specie proved to be able to transmit to its offsprings both the resistance to apple scab and powdery mildew attacks. The response to scab and powdery mildew attack on seedlings belonging to *M. zumi* and *M. niedzwetzkyana* proved to be also assured at a significance of 0.5%. On seedlings belonging to *M. floribunda* and *M. prunifolia* the coefficient of correlations for the response to scab and powdery mildew attack had values close to the significance level. These results sustained the use of wild apple species as suitable resources of genes for both resistance to scab and powdery mildew attacks (Harris *et al*., 2002). Elite individuals resistant or tolerant to apple scab and powdery mildew attack and with other superior characteristics (including acceptable fruit size and valuable taste for dessert apple) have been identified among the F_1 offsprings, but the intensity of selection had relative small values (Sestras *et al*., 2010). Even if the seedlings had prevalent inherited rustic characteristics from the wild species, the elite individuals selected could significantly reduce the backcrossing procedure with regard to the use of the small-fruited wild *Malus* species that carry several undesirable agronomic traits.

Considerations regarding the use of interspecific hybridisations to increase genetic diversity for inducing resistance to diseases attack on apple

In conclusion, the large variability created through artificial interspecific hybridisations and the reckon values of genetic gain and expected selection response, showed that the chances to obtain a population of hybrids that have a valuable response to apple scab and powdery mildew attack are considerable. Interspecific hybridization also remains a useful method for increasing genetic diversity in apple, offering a good background for an efficient selection for resistant descendants to diseases. In some cyclic hybridization, genetic gain and expected selection response could be downright spectacular, like in offspring populations belonging to *M. coronaria* used as maternal genitor, regarding the response to powdery mildew attack. Even in such situations, the result depends on the specific combining ability of genitors, respectively on the parental formula, the inexistence of hybridisation barriers for interspecific crosses with *Malus* × *domestica*, ploidy etc. (Korban, 1986; Vanwynsberghe, 2006; Sestras *et al*., 2010). Whether that the genetic base of modern apple breeding programmes is too narrow to guarantee breeding progress in the future (Noiton and Alspach, 1996) or not (Vanwynsberghe, 2006), wild apple species remain of interest because they comprise a potential valuable genetic resource for the continuous improvement of the crops, respectively for sources of genes that control desirable traits in apple. According to available data on this particular case, coefficients of heritability in the broad sense means a majority shares the genotype in manifestation of resistance or susceptibility to apple scab and powdery mildew (Bus *et al*., 2002), but also a significant contribution of the environmental factors, according to the species participation as maternal or paternal tester. Even if the studied traits showed a strong genetic determinism, the analysis of the heritability coefficients in narrow sense revealed that not always the additive effects played the most important role, but non-additive effects. Depending on the parental formula, in some cases the additive effects were inferior compared to genetic effects of dominance and epistasis, which could present a stronger contribution to the manifestation of seedlings response to diseases attack. Finally, among populations of interspecific hybrids, because of the polygenic effects of dominance and epistasis that express themselves and the manifestation of other characters, e.g. small fruits, low quality of fruits, etc., the selection is generally more effective in the direction of ornamental forms with small fruit, like wild apple crabs, then dessert apple (Sestras *et al*., 2010). As such, interspecific hybrids have very low chances to becoming dessert cultivars, therefore, they should be included in programs for future improvements through direct hybridization or „modified backcross", according to classical models (Crosby *et al*., 1992; Janick, 2002). These hybrids can be used to achieve new generations of seedlings, needed to enrich the genetic future background for selections, or for recurrent selection as a new strategy, respectively for combining phenotypic selection with MAS selection (Stankiewicz et al., 2002; Oraguzie, 2003; Oraguzie *et al*., 2004; Sestras *et al*., 2009, 2010; Maric *et al*., 2010; Kumar *et al*., 2010; Bus *et al*., 2010), in order to obtain new genotypes for ornamental or dessert apple.

Acknowledgements

This study was financed by the National Council for Higher Education Research (CNCSIS), the Executive Unit for the Funding of Higher Education and Academic Research (UEFISCSU), Romania, project PCE-IDEI, no. 1105, code CNCSIS 1499.

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