

Heritability and Genetic Parameters Estimated for Fruits Size in F₁ Interspecific Apple Hybrids

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Abstract. Interspecific hybridization is a frequently used breeding method to create a wide genetic diversity useful to broaden selection base and creating new cultivars of apple. The aim of the present study was to determine the heritability of fruit size in F₁ apple hybrids belonging to interspecific apple pollenization between different cultivars and wild species (rustic apple crabs) like *Malus coronaria*, *M. floribunda*, *M. niedzwetzkyana*, *M. zumi*, and *M. prunifolia*. A wide variability of fruits sizes has been identified, in all families being identified a broad fruit size variability (e.g. CV = 47.1% in *M. zumi* × ‘Golden Delicious’, CV = 42.2% in *M. coronaria* × ‘Jonathan’ and ‘Rosu de Cluj’ × *M. prunifolia*). Between the five cyclic hybridizations, the mean of notes for fruit size was quite close, with limits ranging from 2.70, from hybridisation where the species *M. floribunda* and *M. prunifolia* participated as paternal testers, and 2.93, in the cyclic where specie *M. niedzwetzkyana* was hybridization as paternal tester (less than „3”, which express „small fruits”, by 1-9 scale). Even if the trait studied showed a strong genetic determinism (H^2 : 0.611-0.700), analysis of the heritability coefficients in narrow sense (h^2 between 0.074 when *M. prunifolia* was used as paternal tester and 0.154 when *M. floribunda* was used as paternal tester) revealed that not additive effects played the most important role, but non-additive effects, of dominance and epistatic. Genetic gain and expected selection response had similar values, which have shown that the chances of obtaining by selection a population of hybrids that have the desired fruit size are greater when in interspecific hybridization are involved as genitors *M. zumi* and *M. floribunda*, compared to *M. niedzwetzkyana* and *M. prunifolia*.

Keywords: hybridization, seedlings, species, heritability, genetic gain, selection response

INTRODUCTION

In apple breeding, interspecific hybridization is one of the best-known and used methods to challenge the artificial variability (Soltis and Soltis, 2009). Interspecific pollenization can create a wide genetic diversity useful to broaden selection base and creating new cultivars of apple (Morales and Traveset, 2008). The method is mainly used to obtain resistant cultivars to stress, as well as diseases and pest attack (Morales and Traveset, 2008). For this purpose, it uses different species of *Malus* (many of them used as ornamentals), e.g.: *M. baccata* and *M. prunifolia* for frost resistance; *M. floribunda*, *M. atrosanguinea*, *M. baccata*, *M. micromalus*, *M. prunifolia* etc. for apple scab resistance, *M. zumi*, *M. robusta*, *M. sargentii*, *M. baccata jackii* etc. for powdery mildew resistance etc. (Sestras, 2004).

In the interspecific crosses, apple crab species transmitted in addition to desirable traits of the wild parent, the undesirable ones, especially very small size and low fruit taste (Sestras, 2004). Since interspecific hybrids predominantly inherit such characters, they should be used in crosses and backcrossing with varieties valuable to correct undesirable characteristics of the rustic species. In the F₂-F_n generation, to avoid inbreeding and incompatibility phenomena another recurrent is introduced as genitors to improve fruit quality (as “backcross modified”) - Hough *et al.* (1953), Crosby *et al.* (1992), Janick *et al.* (1996). Based on such schemes well-known varieties were created such as ‘Prima’, ‘Priscilla’, ‘Sir Prize’, ‘Florina’, ‘Priam’ etc. To reduce the long process of obtaining new varieties, it is desirable that the selection for the next generation of interspecific populations assure identified genotypes with fruit with desirable characters, including fruits size.

Selection efficiency in offsprings produced by artificial pollenization is dependent on the ability of parental forms to submit the desired characters, respectively on their heritability (Sestras *et al.*, 2009, 2010). In this study, heritability of fruit size was determined in F₁ apple hybrids of interspecific hybridization, starting from the premise of polygenic type of heredity.

MATERIALS AND METHODS

Biological material used in the experience were represented by interspecific apple hybrids, which resulted from pollenization between different cultivars and wild species (rustic apple crabs) like *Malus coronaria*, *M. floribunda*, *M. niedzwetzkyana*, *M. zumi*, and *M. prunifolia*. The research was conducted at the Fruit Research Station Cluj, Romania, and the species being crossed with different cultivars or selections in the process of breeding.

There were examined 15 hybrid combinations in F₁, belonging to five cyclic hybridization schemes, of which, in two *Malus* species were used as mother genitors, and in three, as paternal genitors, but as tester in all combinations. Inside hybrid combinations (families), the number of hybrids was variable, from 31 to 142 descendants / combination (‘Cluj 218/2’ × *M. floribunda*, respectively ‘Reinette Baumann’ × *M. floribunda*). In the fields of hybrids, no pruning was performed on the trees, and no treatment was applied.

Fruit size was assessed by a scoring system adapted from UPOV rules for dessert apple and ornamental apple (UPOV 2003; 2005) and ISTIS (The State Institute for Variety Testing and Registration, Romania); comparisons and changes applied are presented in Table 1.

Experimental data processing was performed by statistical analysis of variance (ANOVA test, "t"), the data obtained being presented in summary tables, as mean values and the average hybrid combinations for each hybridization cyclic experiences. Genetic analysis of families involved the decomposition of variances in each hybrid cyclic siblings (half-sib), which are thought siblings having a common hereditary basis, derived from the common parent (mother or father). Calculation of broad sense heritability (H²) and narrow sense heritability (h²) was based on the variances of inter-families and intra-families of siblings, the proportion of common genes, namely 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 noted and computed by classical model, $H^2 = \sigma_G^2 / \sigma_P^2$, $h^2 = \sigma_{Ga}^2 / \sigma_P^2$ (Holland *et al.*, 2003; Piepho and Möhring, 2007). Heritability in narrow sense was used to predict the response (R) to selection, as $R = h^2 S$, where S was the differential selection (Falconer and Mackay, 1996).

Tab. 1

Assessment of fruit size in experiment comparing with UPOV for Fruit Varieties (2005) and Ornamental Apple (2003)

Note/Mark	UPOV – Apple Fruit Varieties (2005)	ISTIS – Fruit Varieties (g)	UPOV – Ornamental Apple (2003)	In present experience (scale 1-9), with following note/mark: 1, 3, 5, 7, 9
1	Very small ('Api Noir')	≤25	Very small (<i>Malus sargentii</i>)	<i>M. floribunda</i> , <i>M. zumi</i> , <i>M. prunifolia</i>
2	Very small to small ('Golden Harvey')	26-75	-	-
3	Small ('Akane', 'Miller's Seedling')	76-100	Small ('Profusion')	<i>Malus coronaria</i>
4	Small to medium ('Alkmene')	100-125	-	-
5	Medium ('Cox's Orange Pippin')	126-175	Medium ('John Downie')	<i>M. niedzwetzkyana</i>
6	Medium to large ('Gravensteiner')	176-200	-	-
7	Large ('Mutsu')	201-250	Large ('Wynema')	'Rosu de Cluj'
8	large to very large ('Bramley's Seedling')	250-275	-	-
9	very large ('Howgate Wonder')	>275	very large (<i>Niedzwetzkyana</i>)	All cultivars, except 'Rosu de Cluj'

The coefficient of variability/variation (CV%), coefficient of genetic variability (CGV%), Genetic Gain (GG or ΔG) and Expected Selection Response (R) were computed as follows:

$$CV\% = \sigma / \bar{x} \text{ and } CGV\% = \sigma_G / \bar{x}$$

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-notes for fruit size on F_1 hybrids).

$$\Delta G = k \times h^2 \times \sigma_p, \text{ or } \Delta G = i \times h^2 \times \sigma_p$$

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 combinations 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):

$$R = i \times \sigma_p^2 \times h^2, \text{ or } R = S \times h^2$$

where: σ_p^2 - phenotypic variance among families or populations represented by F_1 hybrids from each cyclic combinations of half-siblings.

RESULTS AND DISCUSSION

Of all the hybrid combinations (Tab. 2), the lowest average fruit size was recorded for offspring derived from crosses of 'Golden Delicious' \times *M. prunifolia* (2.30), followed by a combination of *M. zumi* \times 'Jonathan' (2.38). Hybrids with the highest fruit combinations were obtained in *M. zumi* \times 'Reinette Baumann' and 'Frumos de Voinesti' \times *M. floribunda* (in both hybrid families, the average of trait being 3.20).

Average notes (marks) in all hybrid combinations were much lower than expected average score between genitors with extreme notes, with an initial note “1” and “9” (e.g. *M. floribunda*, *M. zumi*, *M. prunifolia* × cultivars with large fruits), because for a typically quantitative trait, in this example the average had about “5” value).

Between the five cyclic hybridizations, the mean of notes for fruit size was quite close, with limits ranging from 2.70 (from hybridisation where the species *M. floribunda* and *M. prunifolia* participated as paternal testers) and 2.93 (in the cyclic where specie *M. niedzwetzkyana* was hybridization as paternal tester).

The results showed that *M. floribunda* and *M. prunifolia* species used as genitors transmitted to their progenies with sufficient fidelity the fruit size, and the hybrids belonging to them inherited the small fruit. *M. niedzwetzkyana* and possibly *M. coronaria*, both with larger fruits than most wild species, passed the fruit size of their descendants.

Tab. 2

Mean of notes for fruit size of F₁ interspecific hybrids and coefficient of variation (CV%) and significances of differences for each cyclic hybrid combination

Hybrid combination (with <i>Malus</i> specie used as tester ♀ or ♂)		Mean of rates $\bar{x} \pm s_x$	%	CV%	“t” value	Signif. of differ.
<i>M. zumi</i>	‘Golden Delicious’	3.00±0.63	104.9	47.1	0.2	-
	‘Jonathan’	2.38±0.24	83.2	40.3	-1.5	(o)
	‘Reinette Baumann’	3.20±0.36	111.9	35.5	0.8	-
Mean of experiment (Control)		2.86±0.20	100.0	41.0	-	-
<i>M. coronaria</i>	‘Jonathan’	3.00±0.52	104.2	42.2	0.2	-
	‘Reinette Baumann’	2.75±0.25	95.5	18.2	-0.3	-
Mean of experiment (Control)		2.88±0.31	100.0	30.2	-	-
‘Cluj 218/2’	<i>M. floribunda</i>	2.50±0.33	92.6	34.6	-0.5	-
‘Frumos de Voinești’		3.20±0.36	118.5	35.5	1.3	-
‘Reinette Baumann’		2.40±0.21	88.9	35.4	-1.1	-
Mean of experiment (Control)		2.70±0.17	100.0	35.2	-	-
‘Cluj 218/2’	<i>M. niedzwetzkyana</i>	3.10±0.09	105.8	11.8	1.3	-
‘Frumos de Voinești’		3.00±0.25	102.4	28.4	0.3	-
‘Reinette Baumann’		2.50±0.25	85.3	37.6	-1.6	(o)
‘Rosu de Cluj’		3.10±0.20	105.8	23.7	0.8	-
Mean of experiment (Control)		2.93±0.10	100.0	25.4	-	-
‘Golden Delicious’	<i>M. prunifolia</i>	2.30±0.28	85.2	42.0	-1.2	-
‘Reinette Baumann’		2.80±0.20	103.7	21.4	0.4	-
‘Rosu de Cluj’		3.00±0.32	111.1	42.2	0.8	-
Mean of experiment (Control)		2.70±0.17	100.0	35.2	-	-

*Scale 1-9, with following note: 1, 3, 5, 7, 9.

** Symbols for differences (“t” test): x, xx, xxx/o, oo, ooo; significant at P<0.05, 0.01 and 0.001 (positive, respectively negative).

Coefficients of variability were elevated in most hybrid combinations, illustrating that in all families were identified with a broad hybrid fruit size variability (e.g. CV = 47.1% in *M. zumi* × ‘Golden Delicious’, CV = 42.2% in *M. coronaria* × ‘Jonathan’ and ‘Rosu de Cluj’ × *M. prunifolia*). Therefore, the CV% values indicate virtually any combination that is possible to identify hybrid offspring with small size fruits (especially suitable for new ornamental forms) and large fruit (apple suitable for dessert, obvious where only fruit size is correlated with a good quality of fruit and trees, and other favourable traits).

To achieve new apple varieties by selection in interspecific hybrids is still extremely unlikely, because dominant transmission of rustic character by the wild parents (*Sestras et al.*,

2010), including small size fruit. Furthermore, genetic analysis and studies are difficult in apple. Because of the long terms commitment required by such programmes, quantitative genetic principles have not been applied extensively to apple breeding (Noiton and Shelbourne, 1992). Models used in the experience allowed the calculation of important genetic parameters (Tab. 3).

Tab. 3

Broad sense heritability (H^2), narrow-sense heritability (h^2) for fruit size and genetic gain calculated in F_1 interspecific hybrids

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG
Specie	Form		H^2	h^2		
<i>M. zumi</i>	♀	2.86	0.658	0.135	4.1	0.313
<i>M. coronaria</i>	♀	2.88	"F" was not statistically significant			
<i>M. floribunda</i>	♂	2.70	0.700	0.154	4.3	0.313
<i>M. niedzwetzkyana</i>	♂	2.93	0.681	0.104	1.5	0.163
<i>M. prunifolia</i>	♂	2.70	0.611	0.074	1.9	0.161

H^2 - heritability in broad sense; h^2 - heritability in narrow-sense; CGV% - coefficient of genetic variability; GG - genetic gain

According to available data, coefficients of heritability in the broad sense means a majority share of the genotype in manifestation of fruit size, but also a significant contribution to environmental factors, according to species participation as maternal or paternal tester.

Even if the trait studied showed a strong genetic determinism, analysis of the heritability coefficients in narrow sense revealed that not additive effects played the most important role, but non-additive effects. Thus, values obtained in the narrow sense heritability coefficients have range between 0.074 (when *M. prunifolia* was used as paternal tester) and 0.154 (when *M. floribunda* was used as paternal tester). The highest share of polygenic additive contribution to transmission and hybrid offspring determining fruit size occurred in the species *M. floribunda*. Nevertheless, even in this case, the additive effects were inferior compared to genetic effects of dominance and epistatic, which presented a stronger contribution in the manifestation of character.

In populations of interspecific hybrids, because of polygenic effects of dominance and epistasis genetic the effect of „small fruit” trait will express more loyalty, making possible to obtain populations in that more effective are selection in direction of ornamental forms with small fruit, like wild apple crabs. The difficulty of obtaining hybrid populations in which are identified seedlings with large fruits; suitable for dessert selection is probably exerted greater selection pressure over time for the character „large fruit”. However, by applying selection for this character can have a considerable genetic gain, but obviously different depending on the parental formula and genitors involved.

Genetic Gain and Expected Selection Response had similar values, which have shown that the chances of obtaining by selection a population of hybrids that have the desired fruit size are greater when in interspecific hybridization are involved as genitors *M. zumi* and *M. floribunda*, compared to *M. niedzwetzkyana* and *M. prunifolia*.

Although the cyclic hybridizations with *M. floribunda* and *M. zumi* achieve the same genetic gain, *M. zumi* specie provided a higher Expected Selection Response (0.352 vs. 0.308). This is clear from Figure 1, in the hybrid populations of *M. zumi* specie resulted the most spectacular Expected Selection Response for fruit size. Expected selection response (R) is widely used in plant breeding to compare populations and selection schemes (Bridges *et al.*, 1991), and the results obtained in the experience for this genetically parameter marked significant differences, even among *Malus* species with close fruit size.

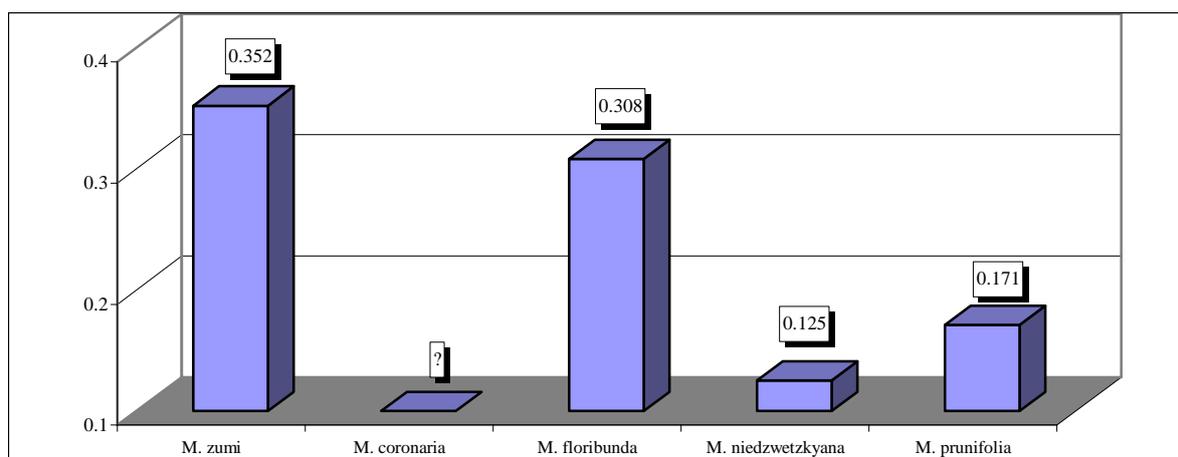


Fig. 1. Expected Selection Response for fruit size in F₁ hybrid populations obtained by interspecific hybridizations

Finally, it is interesting to note that if the coefficient of variability was high, the coefficient of genetic variability showed low or very low values (especially when testers were *M. niedzwetzkyana* and *M. prunifolia*).

In conclusion, the study shown that the interspecific hybrids have very low chances to becoming dessert cultivars, due to their small fruit size. Therefore, they should be included in programs to improve for future improvements through hybridization directed or „backcross modified”, 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 (Oraguzie, 2003; Oraguzie *et al.*, 2004; Sestras *et al.*, 2009, 2010) in order to obtain new genotypes for ornamental or dessert apple.

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