

INVESTIGATION OF WILD SPECIES POTENTIAL TO INCREASE GENETIC DIVERSITY USEFUL FOR APPLE BREEDING

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The potential of testing new apple cultivars and the possibility to induce valuable traits is directly dependent on the availability of sufficient genetic diversity, while apple breeding has narrowed the genetic ground of commercial cultivars. Wild species were studied in regard to their influence upon progenies and their capacity to enlarge apple genetic diversity. The interspecific seedlings were framed in five biparental mating (paired crosses), in which *Malus* species were crossed with different cultivars, obtaining half-sib families. The number of F₁ progenies per combination varied from 31 (Cluj 218/2 × *M. floribunda*) up to 142 (Reinette Baumann × *M. floribunda*), with a total of 1650 hybrids F₁. The influences upon vigour and juvenile period and possible correlation among fruit size and taste were analyzed. Juvenile period varied from 6.00 (*M. zumi* × Jonathan) to 9.31 years (Cluj 218/2 × *M. floribunda*). Data based on correlation coefficient illustrated that the fructification year was not influenced by the vigour of trees. The highest value of correlation for fruit's size and taste was obtained among *M. coronaria* hybrids. This result might suggest that once the fruit are larger, there is a high chance the taste is also more appreciative and fruit quality for mouth feels increase. Depending on the parental formula, additive effects may be inferior compared to genetic effects of dominance and epistasis. Although *M. zumi* and *M. floribunda* achieved the same genetic gain (0.31), *M. zumi* had a higher expected selection response for fruit size. The difficulty of obtaining seedlings with tasty and large fruit when wild *Malus* species are used as genitors is resulting from the values of expected selection response data, but in the same time results confirm that wild *Malus* species are suitable resources for genetic variability, both for dessert and ornamental apple cultivars.

Key words: crabapple, expected selection response, genetic gain, genotype, heritability, hybridization, *Malus* wild species, seedlings

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INTRODUCTION

Most breeding programs use commercial cultivars as genitors, due to assured desirable complementary traits which they might transmit to offspring. Among progenies, individual phenotypes within full-sib families are selected and tested for release as new and improved cultivars, method also named recurrent mass selection (JANICK *et al.*, 1996; KUMAR, 2010). This can be complemented by general or specific combining ability selection, in order to increase the frequency of desirable alleles in the breeding population and thus, increase the probability to select a superior cultivar, essential for maximising on a long term the breeding gain (KING *et al.*, 2001; LIEBHARD *et al.*, 2003; VOLK *et al.*, 2015).

As only few cultivars are used as genitors, this caused a reduction of the effective population size (and therefore assuring fewer genes and reducing genetic diversity), while increasing the levels of inbreeding depression (NOITON and ALSPACH, 1996). Some of the inbreeding depression symptoms in apple are lacking vigour, decreasing genetic variance among families, and by this decreasing the genetic gain and increasing the uniformity within a cross, lengthening the juvenile period.

Even though *Malus* genus is very diverse, having more than 35 species (IGNATOV and BODISHEVSKAYA, 2011), or even up to 55 species (HARRIS *et al.*, 2002), domestic cultivars have a narrow genetic base. This is due to the common origin of most of the cultivars (*M. sierversii*, *M. sylvestris*, *M. pumila*), the selection process among natural mutations and the heterozygote feature maintained through vegetative multiplication (cloning). This represents a constant vulnerability to environmental changes and diseases (JANICK *et al.*, 1996). Breeding directions for apple largely focused on inducing resistance to main diseases (*Venturia inaequalis*, *Powdery mildew* etc.) and this contribute to a less wide genetic base for domestic cultivars (KUMAR *et al.*, 2014; SESTRAS *et al.*, 2010). By not studying more the wild apple species and referring mostly to the consecrated cultivars known for disease resistance and desirable traits for dessert apple, the genetic resources are narrow and the consequences may be destructive. Apple crop is vulnerable mainly because of the low number of cultivars that are in production, diseases, pests and climate stress, the longevity of orchards and the limited number of breeding programs (KHAN, 2012). An understanding of the diversity held in gene banks worldwide will allow a strategic determination of key *ex situ* populations that must be collected before important sources of wild diversity are lost (VOLK, 2014).

Under domestication, perennial plants are often clonally propagated, which, in addition to long juvenile phases, also decreases the number of sexual cycles separating domesticated individuals from their wild progenitors (MCKEY *et al.*, 2010; MILLER and GROSS, 2011) and therefore have slow rates of evolution (ZEDER *et al.*, 2006). Recent research (CORNILLE, 2014) has revealed a major role of hybridization of cultivated apple and has highlighted apple as an ideal model for unravelling adaptive diversification processes in perennial fruit crops. On the other hand, artificial selection caused cultivated populations to diverge morphologically and genetically from their wild progenitors (PICKERSGILL, 2007, 2009) and drive to a less broad variability within the genus.

Interspecific hybridisations arise several difficulties, such as artificial cross-pollination labour for obtaining seedlings, a large number of segregating descendants for assuring genes' recombination and chances for an efficient selection, which implies vast land plots and long time for field trials (IGNATOV and BODISHEVSKAYA, 2011), as well as expansive costs. Especially because the juvenile period is long in apple hybrids on own roots, varying from 3 to 8, or even more years (IGNATOV and

BODISHEVSKAYA, 2011), the breeding strategy is more difficult and deserve a lot of effort to evaluate segregating progenies regarding seedlings ensemble valuation.

If traits like vigour, architectural ideotypes of trees, response to stress factors, diseases attack etc. can be analysed during the juvenile period, other important features like fruit size and shape, fruit taste, acidity and sugars, firmness, productivity etc. are to be verified only after the beginning of fructification period, thus it is important to start within a short time (ACQUAAH, 2009; JEMRIĆ, 2013). Improved fruit quality is still a major objective (BAI, 2015; MOSTAFAVI, 2013), pursued in all apple breeding programs, but seedlings should be screened first for basic agronomic traits: disease and pest behaviour, abiotic stress resistance, ideotype, yield, and only the most promising lines to proceed for further testing (SESTRAS *et al.*, 2011; DAN *et al.*, 2015). Fruit quality is a complex trait that include besides the size, shape, color, texture, taste etc. also storage and shelf-life (JENKS and BEBELI, 2011).

It is known that juvenile period is influenced by many factors, including hereditary heritage (genotype), environmental conditions and cultural technologies, genotypes \times ecotypes interactions and others. Even though it can be shortened by grafting seedlings on faster flowering rootstocks, adventitious rooting (XIAO, 2014) and contemporary molecular techniques (ATHANASIADIS *et al.*, 2013; FOSTER, 2015; WARLOP, 2010; YAMAGISHI, 2013), for apple breeding and increasing variability within the genus, wild species are an important resource and their influence upon descendants needs to be better understood.

Genetic correlations provide data about the interactions between the environment and genotype, indicating in some cases the necessity of selection. Estimation of genetic parameters like heritability and genetic correlations are essential for making efficient decisions in choosing among breeding schemes (ORAGUZIE, 2001). Narrow sense heritability determines the response to selection based on individual phenotype or family mean for specific traits.

Being easily crossable with domestic apple, wild species are valuable donors for favourable traits, both for commercial and ornamental apple cultivars (BECKERMAN *et al.*, 2009; DAN, 2010; IGNATOV and BODISHEVSKAYA, 2011). By introducing less utilised wild apple species in breeding programs, a vast diversity can be exploited and the enlargement of the genetic pool can be assured.

The aim of the study was to investigate some aspects of breeding for important traits in apple, having several *Malus* species used as maternal or paternal genitor for F₁ hybrids, in order to evaluate their potential in regard with the genetic variability, heterozygosity, genetic gain and possible correlations among traits.

MATERIALS AND METHODS

Biological material

Several traits were analysed on 1650 F₁ interspecific hybrids planted on their own roots at the Fruit Research Station, Cluj-Napoca, Romania. The progenies were obtained through crosses among *Malus* species and apple cultivars (in parental generation, or P₀).

The following wild species of *Malus* were used as male and/or female testers: *M. coronaria*, a North American specie, also known as sweet crab-apple or garland crab; *M. zumi*, also known as O-zumi or Zumi crabapple, native to Europe and Asia; *M. floribunda*, also known as Japanese flowering crab-apple, originated from Japan and prevalent from Eastern Asia, being very well known as the monogenic source of resistance to apple scab (*Vf* gene); *M. niedzwetzkyana*, found in the forests of Central Asia, considered rare apple trees; *M. prunifolia*, also known as pear-leaf, plum-leaf crab-apple or Chinese apple, ornamental tree native to China.

The following cultivars were used as male and/or female testers: Cluj 218/2, Frumos de Voinești, Golden Delicious, Jonathan, Reinette Baumann and Roșu de Cluj.

Hybridization scheme

The F₁ apple seedlings were framed in five biparental mating of hybridization (or paired crosses, or cyclic models) adapted from North Carolina Design (ACQUAAH, 2009), in which the wild species, used as maternal or paternal genitor, was crossed with minimum two (*M. coronaria* as maternal tester) and maximum four (*M. niedzwetzkyana* as paternal tester) cultivars (Table 1). The number of progenies per combination ranged from 31 (Cluj 218/2 × *M. floribunda*) up to 142 (Reinette Baumann × *M. floribunda*), having a total of 1650 interspecific hybrids.

Table 1. Hybrid combinations and the number of F₁ hybrids

♀/♂	Golden Delicious	Jonathan	Reinette Baumann	<i>M. floribunda</i>	<i>M. niedzwetzkyana</i>	<i>M. prunifolia</i>
<i>Malus zumi</i>	139	122	76			
<i>Malus coronaria</i>		128	81			
Cluj 218/2				31	123	
Frumos de Voinești				110	135	
Reinette Baumann				142	82	79
Roșu de Cluj					137	125
Golden Delicious						140

Analysed traits

Hybrids' vigour was performed by evaluation marks, using the following grading scale for tree vigour (UPOV for Ornamental Apple, 2003): '3' = weak; '5' = medium; '7' = strong.

The year of first fructification was recorded when each hybrid (offspring seedling) produced fruit, used further for taste evaluation. Hybrids which did not bloom nor fructify until the tenth year after sowing were not taken into consideration for the current study.

Fruit size was assessed by compiling UPOV for Ornamental Apple (2003) and for Fruit Varieties (2005) scale (DAN *et al.*, 2010), using an interval of 1-9 marks, with four notes (grades): '1' = very small (by comparison with *M. floribunda*, *M. zumi*, *M. prunifolia* species, used as genitors); '3' = small (*M. coronaria*); '5' = medium (*M. niedzwetzkyana*); '7' = large (Roșu de Cluj cv.); '9' = very large (all cultivars used as genitors, except Roșu de Cluj).

A similar note system was used in order to evaluate fruit quality, but referring only to the taste (mouth feel) of fruit, analysed in a rapidly sensory evaluation test. The taste was assessed using a continuous scale from '1' to '9', where '1' represents extremely disliked and '9' represents extremely agreeable taste. On this scale, it could be considered that all *Malus* wild species were classified within the inferior classes ('1' or '2' score), while the cultivars used as genitors, situated on the other extreme end ('7', '8' or '9' score), having a pleasant taste, as expected.

Genetic parameters

The genetic analysis of the families involved the decomposition of variances for each of the F₁ hybrid siblings (half-sib), which were grouped thought siblings having a common hereditary basis, derived from the common genitor (mother or father) in P₀.

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; GATTI *et al.*, 2005; SOUZA *et al.*, 2000). Broad sense heritability and narrow-sense heritability were computed by the classical models:

$$H^2 = \sigma_G^2 / \sigma_P^2,$$

$$h^2 = \sigma_{G_a}^2 / \sigma_P^2,$$

where σ_G^2 is the genotypic variance; σ_P^2 is the phenotypic variance; $\sigma_{G_a}^2$ is the additive variance (HOLLAND *et al.*, 2003; PIEPHO and MÖHRING, 2007).

The Coefficient of Genetic Variability (CGV%), Genetic Gain (GG) and expected selection Response (R) were computed as the standard formulas:

$$CGV\% = \sigma_G / \bar{x},$$

where σ = square root of the total variance among families; σ_G = square root of the genotypic variance among families; \bar{x} = mean of marks/trait.

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

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 combination of half-siblings (GATTI *et al.*, 2005).

Heritability in narrow sense was used to predict the response to selection (FALCONER and MACKAY, 1996) and to the expected selection response (R). 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 combination of half-siblings.

Statistical analysis

The statistical analysis of experiment data was carried out by applying Duncan test, for more reliable data due to multiple pairwise comparisons. The data were summarized as means per trait and standard deviations for each hybrid combinations, respectively family of seedlings and cyclic hybridization. Excel (Microsoft Office) formulas were used for calculation.

RESULTS

The vigour for F_1 apple hybrids was under polygenic control, illustrated both by CV% and continuous range of expression of seedlings' growth. The range of variation was related to the expression of the character upon parents, as the progeny mean was quite related to the parental mean. The mean values ranged from 4.78 to 5.60 m between the cyclic hybrid combinations, and between 4.63 to 6.05 m among all hybrids (Table 2). It can be emphasised that Reinette Baumann induced a smaller vigour when used as paternal genitor, in combination both with *M. zumi* (4.93) and *M. coronaria* (4.63), having statistical assured differences. Also, when *Malus* species were used as paternal form and the cultivars as maternal genitor, the data showed significant differences, and mean values for vigour were higher; even so, Frumos de Voinești induced smaller vigour, both with *M. floribunda* and *M. niedzwetzkyana*. It can be concluded that a small vigour was obtained when cultivars Reinette Baumann as paternal genitor and Frumos de Voinești as mother genitor, were used, in combination with wild species *M. coronaria*, *M. zumi* and *M.*

floribunda, respectively. Exceptions may appear, caused probably by triploidy of some genitors (e.g. Reinette Baumann), which are vigorous, but when used as genitor (paternal in the current study), could decrease the vigour of the descendants; this can be explained on the basis of their aneuploidy and dominance or epistasis genetic effects (SESTRAS *et al.*, 2009).

Table 2. Vigour of trees (as mean of marks) and first year of fructification among F_1 interspecific hybrid combinations

Hybrid combination (with tester as ♀ or ♂)		Trees vigour - Mean*, error and CV%			First fruiting year - Mean*, error and CV%		
		\bar{x} **	S_x^-	CV%	\bar{x} **	S_x^-	CV%
<i>M. zumi</i>	Golden Delicious	5.60 ^{abcd}	0.43	17.2	7.60 ^{abc}	0.24	7.2
	Jonathan	5.13 ^{bcde}	0.14	11.1	6.00 ^c	0.41	27.2
	Reinette Baumann	4.93 ^{cde}	0.07	4.5	6.60 ^{bc}	0.58	27.8
<i>M. zumi</i> (as mother genitor)		5.22 ^B	0.11	10.9	6.73 ^C	0.30	20.8
<i>M. coronaria</i>	Jonathan	4.93 ^{cde}	0.66	32.9	7.33 ^{bc}	0.33	11.1
	Reinette Baumann	4.63 ^e	0.77	33.5	7.00 ^{bc}	0.41	11.7
<i>M. coronaria</i> (as mother genitor)		4.78 ^C	0.48	33.2	7.17 ^B	0.25	11.4
Cluj 218/2		5.40 ^{abcde}	0.26	12.6	9.31 ^a	0.37	10.4
Frumos de Voinești	<i>M. floribunda</i>	4.73 ^{de}	0.23	15.3	7.80 ^{ab}	0.39	15.8
	Reinette Baumann	5.66 ^{abc}	0.21	14.7	7.60 ^{abc}	0.16	8.6
<i>M. floribunda</i> (as father genitor)		5.26 ^B	0.15	14.2	8.24 ^A	0.20	11.6
Cluj 218/2		6.05 ^a	0.10	6.4	7.31 ^{bc}	0.25	13.9
Frumos de Voinești	<i>M. niedzwetzkyana</i>	5.38 ^{abcde}	0.15	9.9	7.25 ^{bc}	0.13	6.2
		5.53 ^{abcd}	0.19	12.8	7.01 ^{bc}	0.21	11.1
Roșu de Cluj		5.44 ^{abcde}	0.20	13.5	7.00 ^{bc}	0.10	5.6
<i>M. niedzwetzkyana</i> (as father genitor)		5.60 ^A	0.09	10.6	7.14 ^B	0.10	9.2
Golden Delicious		5.08 ^{bcde}	0.20	13.8	7.00 ^{bc}	0.28	13.6
Reinette Baumann	<i>M. prunifolia</i>	5.92 ^{ab}	0.16	8.2	6.78 ^{bc}	0.49	21.9
		5.39 ^{abcde}	0.18	13.5	6.81 ^{bc}	0.29	17.1
<i>M. prunifolia</i> (as father genitor)			0.12	11.8	6.86 ^B	0.19	17.5

* Hybrids' vigour was performed by evaluation marks, using the following grading scale for tree vigour (UPOV for Ornamental Apple, 2003): '3' = weak; '5' = medium; '7' = strong.

**In columns, means followed by the same letter are not different at $P \leq 0.05$ (Duncan's test), using small letters for hybrid combinations (SD5% vigour of trees: 0.90-1.11; SD5% first year of fructification: 1.73-1.81) and capital letters for means when using specie as tester (SD5% vigour of trees: 0.27-0.30; SD5% first year of fructification: 0.98-1.09).

Juvenile period varied from 6.00 years (*M. zumi* × Jonathan) to 9.31 years (Cluj 218/2 × *M. floribunda*) (Table 2). The means for the year of first fructification among hybrid combinations ranged from 6.73 to 8.24. The variability of the trait was high, as seen by the values of CV%, which reflects the extent of the variability of the juvenile period in relation to the mean obtained among hybrids. The earliest combination that had fruit was *M. zumi* × Jonathan (fruiting after 6 years), *M. zumi* being the specie that induced the shortest period among the studied populations when used as maternal genitor (6.73), with statistical assured differences, even though the variability coefficient for this hybrid combination was high (20.8%). After statistical data processing, it was noted that the variability of the juvenile period was lower (between 9.2% and 11.6%) when *Malus* species were used as a father genitor, except of *M. prunifolia*.

The interpretation of data regarding the size of F₁ hybrids' fruit (Table 3) emphasized that wild species strongly influenced this trait among hybrid seedlings. The means for all combinations were not distinctive, having no statistical assured differences, ranking fruit in 'very small'-'small' categories. The mean size of apples for the analysed hybrid combinations ranged from 2.7, when *M. prunifolia* and *M. floribunda* were paternal genitors, to 2.93 among *M. niedzwetzkyana* hybrids. Even though the variability coefficient was high among all combinations, the interval for all fruit size was between 2.3 (Golden Delicious × *M. prunifolia*) to 3.2 (*M. zumi* × Reinette Baumann and Frumos de Voinești × *M. floribunda*). *M. niedzwetzkyana* and possibly *M. coronaria*, both with larger fruit than most wild species, passed the fruit size to their descendants. As a more adequate genitor in this regard, even though the species pass on the rustic character, the statistical analysis emphasis *M. niedzwetzkyana*, but without statistically assured data. This illustrates once again that fruit size is a typical quantitative trait, with polygenic transmission.

Table 3. Fruit size and taste (as mean of marks) in F₁ interspecific hybrid combinations

Hybrid combination (with tester as ♀ or ♂)	Fruit size -			Fruit taste -			
	Mean*, error and CV%			Mean*, error and CV%			
	\bar{x} **	S_x	CV %	\bar{x} **	S_x	CV %	
<i>M. zumi</i>	Golden Delicious	3.00 ^a	0.63	47.1	1.90 ^d	0.19	22.0
	Jonathan	2.38 ^{bc}	0.24	40.3	1.91 ^d	0.09	19.7
	Reinette Baumann	3.20 ^a	0.36	35.5	2.30 ^d	0.33	44.9
<i>M. zumi</i> (as mother genitor)		2.86 ^A	0.20	41.0	2.04 ^C	0.12	28.9
<i>M. coronaria</i>	Jonathan	3.00 ^a	0.52	42.2	2.42 ^{cd}	0.49	49.7
	Reinette Baumann	2.75 ^{abc}	0.25	18.2	2.63 ^{bcd}	0.24	18.2
<i>M. coronaria</i> (as mother genitor)		2.88 ^A	0.31	30.2	2.52 ^{BC}	0.30	34.0
Cluj 218/2		2.50 ^{bc}	0.33	34.6	3.71 ^{abcd}	0.64	45.9
Frumos de Voinești	<i>M. floribunda</i>	3.20 ^a	0.36	35.5	3.60 ^{abcd}	0.62	54.3
	Reinette Baumann	2.40 ^{bc}	0.21	35.4	2.53 ^{bcd}	0.33	51.7
<i>M. floribunda</i> (as father genitor)		2.70 ^A	0.17	35.2	3.28 ^B	0.29	50.6
Cluj 218/2		3.10 ^a	0.09	11.8	5.13 ^a	0.49	38.3
Frumos de Voinești	<i>M. niedzwetzkyana</i>	3.00 ^a	0.25	28.4	4.33 ^{abc}	0.56	44.4
	Reinette Baumann	2.50 ^{bc}	0.25	37.6	3.39 ^{abcd}	0.46	50.8
Roșu de Cluj		3.10 ^a	0.20	23.7	4.39 ^{abc}	0.65	55.4
<i>M. niedzwetzkyana</i> (as father genitor)		2.93 ^A	0.10	25.4	4.31 ^A	0.28	47.2
Golden Delicious		2.30 ^c	0.28	42.0	4.42 ^{ab}	0.57	44.7
Reinette Baumann	<i>M. prunifolia</i>	2.80 ^{ab}	0.20	21.4	4.78 ^a	0.86	54.2
Roșu de Cluj		3.00 ^a	0.32	42.2	5.06 ^a	0.54	43.0
<i>M. prunifolia</i> (as father genitor)		2.70 ^A	0.17	35.2	4.75 ^A	0.36	47.3

* Fruit size was assessed by compiled UPOV for Ornamental Apple (2003) and for Fruit Varieties (2005) scale: '1' = very small; '3' = small; '5' = medium; '7' = large; '9' = very large. The taste was assessed using a continuous scale from '1' to '9', where '1' represents extremely disliked and '9' represent extremely liked.

**In the columns, means followed by the same letter are not different at $P \leq 0.05$ (Duncan's test), using small letters for hybrid combinations (SD5% fruit size: 0.48-0.57; SD5% fruit taste: 1.92-2.31) and capital letters for means when using specie as tester (SD5% fruit size: 0.27-0.30; SD5% fruit taste: 0.98-1.09).

Fruit taste registered an extent variability among the interspecific hybrids (Table 3), with the extreme values of 1.9 (*M. zumi* × Golden Delicious) and 5.13 (Cluj 218/2 × *M. niedzwetzkyana*), with statistical assured differences between all cyclic combinations, except of *M. niedzwetzkyana* and *M. prunifolia*, which also had the better taste of fruit, with means of 4.31 and

4.75, respectively. Small grades, reflecting an unpleasant mouth feel, were noted for *M. zumi* and *M. coronaria* (maternal genitors), followed by the descendants of *M. floribunda* (paternal genitor). The variability of fruit taste was vast, as CV% ranged from 18.2% to 55.4%, meaning a medium to high interpretation, sustaining the complexity of the trait and its genetic inheritance.

The correlation coefficient between the analysed traits, interspecific hybrids vigour and year of first fructification and fruit size and taste respectively, illustrated that *Malus* species used as maternal genitor come closer in a linear dependence, for p5%. In this regard, *M. zumi* had a positive correlation between vigour and juvenile period, while *M. coronaria* had the strongest positive correlation for fruit size and taste.

Correlation coefficients for F₁ interspecific hybrids vigour and the first year of fructification had small values, not being statistical assured (Fig. 1). The data illustrated that the fructification year was not influenced by the vigour of trees. Only *M. zumi* seedlings had the correlation coefficient close to statistical limit for p5% ($r' = +0.162$).

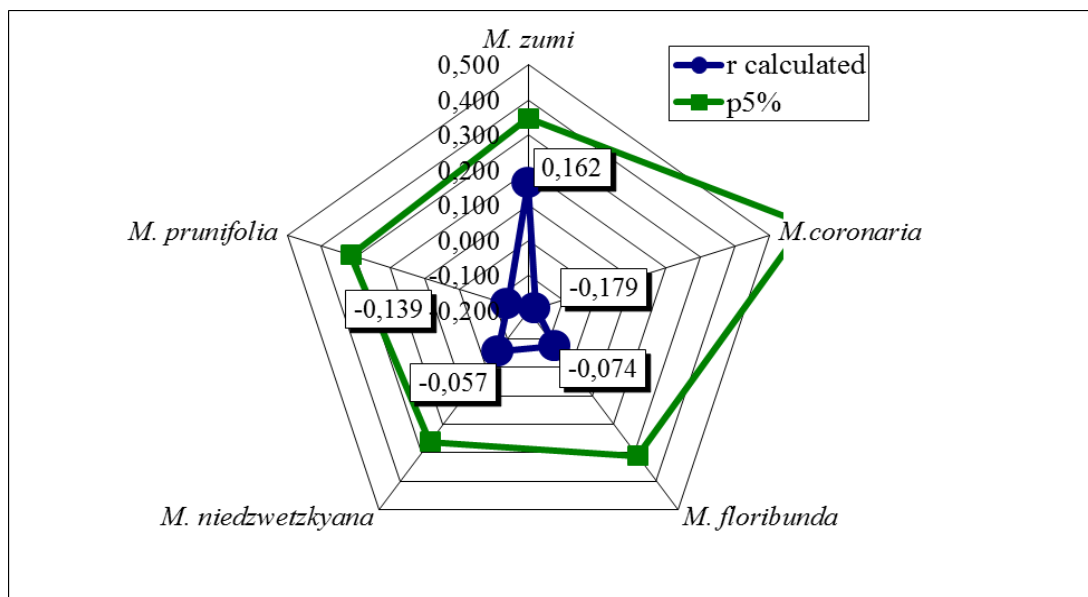


Fig. 1. Genotypic correlation (r) between the vigour of trees and the first year of fructification of F₁ interspecific hybrids, belonging to *Malus* species used as maternal and paternal genitors

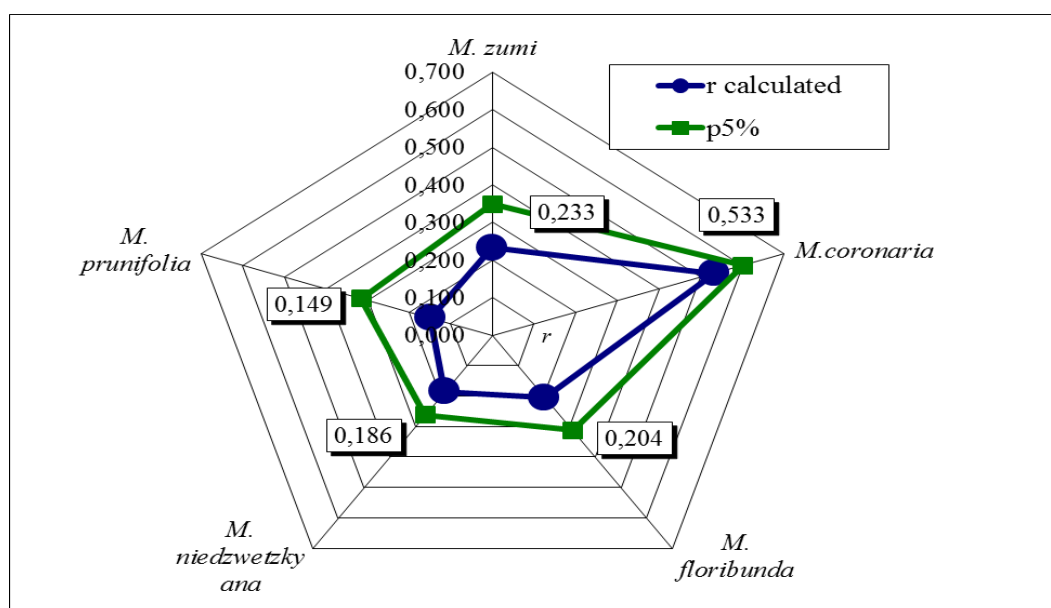


Fig. 2. Genotypic correlation (' r ') between fruit size and fruit taste of F_1 interspecific hybrids, belonging to *Malus* species used as maternal and paternal genitors

For the analysed F_1 interspecific hybrids, fruit size and taste were not correlated (Fig. 2). In spite of that, calculated values for ' r ' were close to the theoretical ones at p5%, especially for seedlings of *M. coronaria*. Data point out that regarding the origin of interspecific hybrids, once the size of fruit is improved, there is a high chance the taste is also more appreciative and the fruit quality for mouth feels becomes more valuable. This assumption is more likely to be possible with *M. coronaria* as genitor among the studied *Malus* species, which is in fact surprisingly as the hybrids from its combinations had fruit close the specific traits of the specie: flattened to spherical shape, 2.5-3 cm in diameter, crowned with calyx lobes and remnant of filaments, yellow green at maturity, fragrant and acid taste.

Heritability was estimated to determine the interactions between genotype and phenotype, as well as the stability of different traits expression across the environment. Also, genetic gain was pursued to emphasise possible improvements to future generations. Broad sense heritability denoted the fact that the vigour had a strong genetic determinism (Table 4). Environmental factors had a more significant contribution for tree height when *M. floribunda*, *M. prunifolia* and *M. niedzwetzkyana* are used as genitors, while narrow sense heritability revealed that non additive effects (dominance and epistasis) contributed mostly to the inheritance of tree vigour. In the case of *M. coronaria* data were not statistically significant. The highest share of polygenic additive contribution to transmitting vigour occurred for *M. floribunda* (0.264). Genetic gain and expected selection response for tree vigour had higher values (0.43 and 0.37, respectively) for the descendants of *M. floribunda*.

Table 4. Heritability and genetic parameters* for the vigour of F_1 interspecific hybrids

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H ²	h ²			
<i>M. zumi</i>	♀	5.22	0.700	0.171	0.9	0.21	0.12
<i>M. coronaria</i>	♀	4.78	0.082	(-0.696)	(-10.1)	(-2.17)	(-3.27)
<i>M. floribunda</i>	♂	5.26	0.819	0.246	3.9	0.43	0.37
<i>M. niedzwetzkyana</i>	♂	5.60	0.797	0.170	1.4	0.23	0.15
<i>M. prunifolia</i>	♂	5.46	0.803	0.202	2.1	0.30	0.22

*H² - broad sense heritability; h² - narrow sense heritability; CGV% - coefficient of genetic variability; GG - genetic gain; R - expected selection response

The length of juvenile period has a very complex genetic determinism, data confirmed also by the values of heritability coefficients calculated for the studied interspecific apple hybrids. The interpretation of the calculated parameters (Table 5) suggested that phenotypic variance plays a very significant role, although for *M. floribunda* and *M. zumi* broad sense heritability (0.898 and 0.663, respectively) showed genetic control of the trait.

Table 5. Heritability and genetic parameters* for the first year of fructification of F_1 interspecific hybrids

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H ²	h ²			
<i>M. zumi</i>	♀	6.73	0.663	0.140	3.9	0.48	0.78
<i>M. coronaria</i>	♀	7.17	0.286	(-0.357)	(-1.2)	(-0.58)	(-0.46)
<i>M. floribunda</i>	♂	8.24	0.898	0.307	7.8	0.71	0.80
<i>M. niedzwetzkyana</i>	♂	7.14	0.418	(-0.047)	(-0.1)	(-0.07)	(-0.05)
<i>M. prunifolia</i>	♂	6.86	0.105	(-0.263)	(-1.5)	(-0.63)	(-0.73)

*H² - broad sense heritability; h² - narrow sense heritability; CGV% - coefficient of genetic variability; GG - genetic gain; R - expected selection response

Genetic gain for fruit size proved that *M. zumi* and *M. floribunda* gave the most chances of obtaining hybrid offspring that have a more desirable fruit size (Table 6). Although *M. zumi* and *M. floribunda* achieved the same genetic gain (0.31), *M. zumi* provided a higher expected selection response (0.35), differentiating among the wild species used as genitors as the most promising one for a better fruit size.

The difficulty of obtaining seedlings with tasty fruit when wild *Malus* species are used as genitors is resulting from the values of expected selection response (Table 7), with no statistical data or with values that ranged between 0.04 and 0.74.

Table 6. Heritability and genetic parameters* for size of fruit of F_1 interspecific hybrids

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H ²	h ²			
<i>M. zumi</i>	♀	2.86	0.658	0.135	4.1	0.31	0.35
<i>M. coronaria</i>	♀	2.88	0.121	(-0.632)	(-6.8)	(-1.29)	(-1.28)
<i>M. floribunda</i>	♂	2.70	0.700	0.154	4.3	0.31	0.30
<i>M. niedzwetzkyana</i>	♂	2.93	0.681	0.104	1.5	0.16	0.12
<i>M. prunifolia</i>	♂	2.70	0.611	0.074	1.9	0.16	0.17

*H² - broad sense heritability; h² - narrow sense heritability; CGV% - coefficient of genetic variability; GG - genetic gain; R - expected selection response

Table 7. Heritability and genetic parameters* for the taste of fruit of F_1 interspecific hybrids

Genitor as parental tester		Mean of marks	Heritability		CGV%	GG	R
Specie	Form		H ²	h ²			
<i>M. zumi</i>	♀	2.04	0.544	0.038	0.5	0.05	0.04
<i>M. coronaria</i>	♀	2.52	0.095	(-0.674)	(-7.3)	(-1.31)	(-1.24)
<i>M. floribunda</i>	♂	3.28	0.666	0.128	7.6	0.44	0.73
<i>M. niedzwetzkyana</i>	♂	4.31	0.645	0.083	5.6	0.36	0.74
<i>M. prunifolia</i>	♂	4.75	0.225	(-0.183)	(-6.1)	(-0.82)	(-1.78)

*H² - broad sense heritability; h² - narrow sense heritability; CGV% - coefficient of genetic variability; GG - genetic gain; R - expected selection response

DISCUSSION

Most breeding programs use only a narrow range of the genetic pool (NOITON and ALSPACH, 1996) mostly based on crosses with well known cultivars like Golden Delicious, Red Delicious, Jonathan or Mc-Intosh (DUREL *et al.*, 1998). The main goals of conventional breeding programs are to increase fruit yield, upscale fruit quality and to achieve resistance to the main pests and diseases (VOLK, 2014). Contemporary understanding of improving fruit quality emphasise the potential of breeding programs and even more, the necessity of a vast genetic base. The potential contribution of wild relatives for crop improvement has long been recognized and forms an important component in breeding and conservation programs (DARWIN, 2009; KOVACH and MCCOUCH, 2008; PICKERSGILL, 2009). Ideally, a population is to be improved without its genetic variability being reduced, so that additional selection and progress can occur among future generations, where as the best individuals from each family are selected in a manner that can preserve the diversity within the population. In reality though, genetic erosion is a major concern.

Since the beginning of modern and intensive agriculture, breeding aimed to improve productivity and quality of yields, and now days the researchers also take into consideration the importance of genotypes whose cultivation could reduce the environmental impact (DIAMANTI and MEZETTI, 2011), towards new technologies for improved nutritional and nutraceutical values of fruit and enlargement of the genetic base (KUMAR, 2012). There is an essential need also for better adapted cultivars which can optimise organic apple production and low input systems (GILAD, 2012; WARLOP *et al.*, 2010).

It is important to note that the delineation of species within the *Malus* genus has been problematic. For apple, most of the cultivated varieties have common genitors (*M. sierversii*, *M. sylvestris*, *M. pumila*), therefore the use of wild species can bring valuable variability in successive progenies. On the basis of life cycle characteristics and the mode of reproduction, slow rates of evolution in perennial crops are expected (OLSEN and SCHAAL, 2007; PICKERSGILL, 2007; ZEDER *et al.*, 2006); however, numerous perennial crops exhibit substantial morphological and genetic divergence from their wild progenitors. Wild apple species display strong population structures and high levels of introgression from domesticated apple, and this may threaten their genetic integrity (CORNILLE, 2014). The recent research has revealed a major role of hybridization in the domestication of the cultivated apple and has highlighted the value of apple as an ideal model for unravelling adaptive diversification processes in perennial fruit crops.

The pedigree of many apple cultivars is completely or partially unknown, often reducing the efficiency of breeding schemes (SALVI, 2014). The research concluded that multi-generation pedigree reconstruction studies will find wide application in apple and other species thanks to the increasingly availability of high-coverage genome.

Perennial plants were deep-rooted considered as intractable systems for studying their evolution, due to long time, involving several generations, needed and low rates of selfing. However, new emerging technologies in conjunction with mature breeding collections and along with a steadily increasing body of literature, are now facilitating evolutionary analyses in perennial species, including apple (MILLER and GROSS, 2011) so that premises for obtaining valuable hybrids are very possible, especially if variability is vast enough and the possibility to study consecutive hybrid generations, that have wild *Malus* species as genitors, are analysed.

Means for vigour of the studied interspecific apple hybrids ranged from 4.63 (*M. coronaria* × Reinette Baumann) to 6.05 (Cluj 218/2 × *M. niedzwetzkyana*). A smaller vigour was obtained in combinations of Frumos de Voinești × *M. floribunda* (4.73) and *M. zumi* × Reinette Baumann (4.93), which also had the smallest coefficient of variability inner hybrid families (4.5%). Crossings between *M. coronaria* and Jonathan and respectively Reinette Baumann had the vastest vigour variability among the F₁ descendents. This is unanticipated, due to the frequent similarity seen in the seedlings of *M. coronaria*. Because of this uniformity among hybrids, it can be claimed that they are non authentic, and apomixes reproduction interfered. In a different approach, KRON and HUSBAND (2009) showed that interspecific pollination occurred at a high rate between wild *Malus coronaria* and domesticated apple, although no living hybrids survived in their study.

Out of the five *Malus* species, *M. coronaria*, *M. floribunda*, *M. niedzwetzkyana* and *M. prunifolia* had negative values for correlation coefficient ('r') calculated for vigour and fructification. This might attest that vigorous trees also have a long juvenile period.

Although several agrotechnical approaches, such as the common method of grafting juvenile twigs onto mature rootstocks, have been used to accelerate the phase transition in apple breeding, a period of at least 3-5 years is still required for apple seedlings to produce flowers (FISCHER, 1994; FLACHOWSKY *et al.*, 2009). After processing the statistical information, all hybrid combinations had assured statistical differences. Among these, one combination had inferior differences, while the rest got superior values, which actually means a tardy fructification. Therefore, the combination with the shortest juvenile period was *M. zumi* × Jonathan. Among cyclic hybrid combinations that implied *Malus* species as genitors in the current study, *M. zumi* and *M. prunifolia* formed seedlings with shorter juvenile period. Even so, no hybrid combination

fruited before 6 years of vegetation and based on the interpretation of the variability coefficient for this trait, the possibilities to get extreme values (with the focus for smaller ones, which implies an earlier fructification) is highly diminished.

HAJNAJARI *et al.* (2012) evaluated vegetative and productive features at apple seedlings, in order to select superior genitors, oriented also to produce new early-ripening cultivars. They obtained positive correlations among trees' height and other morphological traits such as branch distance, seedling stem and number of branches. Besides, heritability was found to be high among chlorophyll concentration and seedlings' height, in both half-sib and full-sib families. Heritability of half-sib progenies derived from the early-ripening genitors was higher than the ones of late-ripening. The relatively high heritability estimated for morphological traits, particularly in early-ripening cross combinations, suggested narrow-sense heritability as a criterion for early screening.

A short juvenile period is a desirable characteristic for apple growers and therefore breeding programs have to answer to this demand; many researchers are still studying possibilities to obtain early fructification cultivars and clarify the potential of gene flow (ACQUAAH, 2009; KUMAR, 2014; FISCHER, 1994; LANE *et al.*, 2003; KENIS *et al.*, 2008; YAMAGISHI *et al.* 2013).

The genus *Malus* includes both commercial apple and crabapple with the primary difference between them being the fruit traits; crabapple fruit have usually 1-4 cm, whereas commercial apples have fruit greater than 6 cm in diameter. Although cultivated apple (*Malus* × *domestica* Borkh.) arose primarily from *M. sieversii*, many commonly used crabapple species such as *M. prunifolia* (Willd) Borkh., *M. baccata* (L.) Borkh., *M. mandshurica* (Maxim) Kom. and *M. sieboldii* (Regel) Rehder may have hybridized with *M. sieversii* (LUBY, 2003). These potential progenitors to the commercial apple can be used in breeding programs and serve as a source of durable resistance to scab and other major diseases of *Malus*. The enlargement of genetic pool is considering also alternative approaches to introduce new quality and resistance traits, taking into consideration also the establishment of intergeneric hybrids for horticultural and agricultural crops (e.g. *Malus domestica* × *Pyrus communis*) (FISCHER, 2014).

M. floribunda (considered to be either a hybrid species, or derived from *M. sieboldii* × *M. baccata*), had mean fruit size of 3.2 (small fruit) whereas species characteristic is a diameter of 0.5-1 cm. *M. zumi* (hybrid species from *M. manshurica* and *M. sieboldii*) also had the mean fruit size of 3.2, while species characteristic fruit have 1-2.5 cm. *M. prunifolia*, even though is known as larger Chinese crab apple (JANICK *et al.*, 1996) and has the larger fruit size among the studied wild species, gave the smallest mean, of 2.7 (small fruit).

BROWN (2012) analysed fruit shape, size and time interval of ripening in progenies of the cultivated apple. By treating the data as an incomplete diallel cross and establishing midparental values, the researcher pointed out that the calculated main effects of each genitor allows predictions regarding the progeny means for untested combinations of parents.

Common features of domesticated woody perennials (trees) is dwarfism and larger fruit that are more variable in color, taste and other traits related to human preferences, that make apples "prettier, easier to eat and easier to transport" (rowfoodssos.com). The basis of taste is the balance between sweetness and acidity, and in this regard wild species are known to be sour, even bitter, acid, dry, unappetizing, and therefore are valuable in breeding programs for variability and genetic variation more that being genitors for dessert apple. In the current study, based on rapidly sensory evaluation test, it could be considered that all *Malus* wild species were classified within the inferior classes ('1' or '2' score), while the cultivars used as genitors, had a more agreeable taste, as expected.

The use of wild species in breeding for fruit quality improvement requires a good understanding of the gene flow, whereas studies are being unroll upon fruit quality given by composition (MA *et al.*, 2015). Wild *Malus* taxa were considered useful sources of genetic variation for apple breeding and their conclusion was that fruit acidity rather than sweetness is likely to have undergone selection during domestication. The researchers investigated the genetic relationship between cultivated and wild apple and the results showed that the wild relatives were closely related to cultivars. Therefore, the difference in sugar composition between wild and cultivated fruit is likely the result of the hitchhiking effect associated with artificial selection on fruit acidity during the process of apple domestication.

The generally low correlations obtained for the F₁ apple interspecies hybrids are in agreement with low values that ORAGUZIE (2001) come by, which suggested that the genotypes responded differently to the environment.

The similarity among some hybrids and the origin specie (e.g. *M. coronaria*, Fig. 2) might be due to 'non authentic hybrids', partenocarpic progenies. It is known that in the case of *M. coronaria* apomixis and unreduced gamete production have the potential to gene introgression which might be a barrier through hybridization (KRON and HUSBAND, 2009). Their results suggest that strong barriers to the establishment of hybrid progeny may follow relatively weak limits to hybrid seed production.

Heritability indicates whenever an individual phenotype can be used to predict the parental performance regarding interest traits. A low heritability indicates a relatively high importance of non additive genetic variance and/or environmental variance; it could also suggest the need for progeny testing or cloning for accurate selection. The other options would be to increase the heritability estimates by decreasing the environmental variance. In a broad sense, heritability can be defined as the ratio between the genotypic variance and the phenotypic variance. Hence, genes that correspond to visible traits have stronger influence than the environment on the trait expression.

CORNILLE (2015) stated that human activities and climate are key drivers of gene flow in wild temperate fruit trees and provide a practical basis for conservation, agroforestry and breeding programs for apples in Europe. In particular, the anthropization of landscapes and the cultivation of crops over extended areas have increased the opportunities for contact between cultivated plants and their wild relatives. However, in extreme environmental condition the environment can suppress the gene effect (SEGURA *et al.*, 2006). Therefore breeders can only select genetically inherited traits in order to achieve significant genetic improvement. Heritability definitions can be accurate only for specific population and environments (SOUZA *et al.*, 2000).

The genetic background of a population can affect the phenotypic expression of certain traits transmitted to the next generation. For instance if both parents are homozygous for a specific trait with different alleles, the individuals of the F₁ generation will all display heterozygous genotypes. Apple cultivars are known for their heterozygous genomes and therefore the results of heritability tests are relatively reliable (SEGURA *et al.*, 2006).

Heritability of fruit weight obtained from open-pollinated families hybrids by ALSPACH and ORAGUZIE (2002) were mostly consistent with those derived from parent-offspring regression using data of ORAGUZIE *et al.* (2001) and concurred with those of CURRIE *et al.* (2000) when crab apples were included in the study. DUREL *et al.* (1998) estimated the heritability for fruit size as 0.33, while ORAGUZIE *et al.* (2001) estimated for fruit weight a heritability range 0.51-0.61, for

crab apple. These differences may be partially related also with the chosen approach, therefore a bear comparison would not offer a clear conclusion.

Regardless the effect of methodology applied in the current study upon the values obtained for genetic parameters, data validate that by using wild species for new progenies, genetic base can gain variability and new hybrids enlarge *Malus* genetic pool. The vast heterozygote nature of the F₁ seedlings constitutes a key base, whereas the biological material - interspecific hybrids represent a valuable breeding material, for obtaining new dessert and ornamental cultivars.

CONCLUSIONS

The possibility to induce valuable traits is directly dependent on the availability of sufficient genetic diversity, while apple breeding has eroded in time the genetic base of domestic and commercial cultivars. The five *Malus* species crossed with several apple cultivars assured the variability within the hybrids, which represent a tremendous diversity of genetic background, regarding trees vigour, juvenile period, fruit characteristics and aesthetic traits. As F₁ interspecific hybrids have mostly small and not qualitative taste fruit, inherited as rustic traits from the wild genitor, it is improbable to become dessert cultivars. They might be used as gene pool for decorative apple, or included in breeding schemes that allow future improvements by contemporary methods and can bring new considerations of the gene flow through consecutive progenies. Nevertheless, the interspecific hybrids are to be used in future generations of seedlings and enlarge the genetic base for selection of consume and ornamental apple.

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ISTRAŽIVANJA POTENCIJALA DIVLJIH VRSTA U POVEĆANJU GENETIČKOG DIVERZITETA KORISNOG U OPLEMENJIVANJU JABUKACatalina DAN¹, Adriana F. SESTRAS^{1,*}, Calin BOZDOG^{1,2}, Radu E. SESTRAS¹¹ Univerzitet poľoprivrednih nauka i veterinarske medicine Cluj-Napoca, Romania² Stanica za istraživanje voćaka, Cluj-Napoca, Romania

Izvod

Vršena su ispitivanja uticaja divljih vrsta na potomstvo i njihov kapacitet u povećanju genetičkog diverziteta. Međuvrstni klijanci su ispitivani u pet parova ukrštanja kod kojih su *Malus* vrste ukrštane sa različitim kultivarima u čiju dobijanja *half-sib* familija. Broj F₁ potomstva po kombinaciji je varirao od 1 (Cluj 218/2 × *M. floribunda*) do 142 (Reinette Baumann × *M. floribunda*), sa ukupnim brojem od 1650 F₁ hibrida. Juvenilni period je varirao od 6.00 (*M. zumi* × Jonathan) do 9.31 godina (Cluj 218/2 × *M. floribunda*). U zavisnosti od roditeljske formule aditivni efekat gena može biti inferioran kada se vrši poređenje genetičkog efekta dominantnosti i epistaze. Iako su *M. zumi* i *M. floribunda* ostvarile istu genetičku dobit (0.31), *M. zumi* je imao viši očekivani odgovor na selekciju za veličinu ploda. Dobijeni rezultati potvrđuju da su divlje vrste pogodni izvori genetičke varijabilnosti kako za dezertne tako i za hortikulture vrste jabuka.

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