

Flower and fruit morphological characteristics of different crabapple genotypes of ornamental value

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Abstract

Crabapples (*Malus* spp.) are frequently used as ornamental trees. However, selections differ in decorative impact through blossoming and fruiting traits, growth habit, disease tolerance, ecological adaptability, and fruit litter, so that there can be other valuable benefits. The aim of the present investigation was to determine morphometric characteristics of flowers, fruits, and seeds within 15 different genotypes of crabapples studied at M.M. Grishko National Botanical Garden in Kyiv, Ukraine. Their main morphometric parameters were as follows: the highest number of flowers in the inflorescence was recorded within trees of 'Professor Sprenger' with 5.78 flowers, whereas the corolla was mostly decorative for 'Royalty' cultivar, with 5.7 petals; fruit weight varied from 1.49 g ('Adirondak') to 20.56 g ('Era'), length from 12.87 mm ('Van Ezeltine') to 25.11 mm ('Ola'), diameter from 12.0 mm ('Batterball') to 26.74 mm ('Rayka Rozeva'), fruits number 1.0 ('Era') to 5.5 ('Evereste'). The relationships between flowers, fruit and seed traits, accounting 16 traits and 15 cultivars investigated was highlighted by Pearson correlation and were clustered by unweighted pair group method with arithmetic mean (UPGMA). The variability observed in the apple crab genotypes offers the possibility to choose and use some valuable ornamental cultivars for the beautification of the landscape, as well as for possible future *Malus* breeding projects.

Keywords: crabapple; flower; fruit; *Malus*; morphometric traits; seeds

Introduction

Early apple utilisation by humans started with the gathering of wild apples, then proceeded to increasingly managed wild stands and ultimately the intentional cultivation of apple trees (Cornille *et al.*, 2019); trees were further cultivated at larger scales and even more, ornamental uses were also added to the advantages of the genus. Crabapples are the most widely cultivated small landscape tree in the northern United States and southern Canada (Brewer *et al.*, 1979; Guthery and Hasselkus, 1992; Draper *et al.*, 1996). Crabapples are worldwide popular because of their wide variety of growth habits and mature sizes, wildlife-attracting capabilities, and excellent flower display during the blossoming period in spring (Dirr, 1998; Roloff *et al.*, 2018). Hamilton (1986) surveyed members of the National Landscaping Association and found

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crabapple cultivars as the most popular flowering trees in 1956, 1970, 1976 and 1982. New cultivars are still established, with decorative flowers (Shen *et al.*, 2021), from white to red purple (Hu *et al.*, 2018), being highly appreciated and have great ornamental value because of their bright and colourful flowers, are useful pollinators (Campbell *et al.*, 1991) and valuable genetic pools for *Malus* breeding (Dan *et al.*, 2015).

The crabapple gets its name from English gardeners who divided apples into those that had a good taste and those that had a bitter or «crabbed» taste (den Boer, 1959). However, the major distinguishing feature between crabapples and dessert apples is the size of the fruit (Saracoglu and Altunta, 2021). Crabapple fruits are less than or equal to 5.1 cm (2 inches) in diameter, some having less than 2 cm. Fruits greater than 5.1 cm (2 inches) in diameter are considered commercial apples, with debatable limits. Thus, crabapple is the designation given to apple fruits that have a small diameter, wild or half-wild trees, which have high anti-adversity, adaptability, and close affinity with the main *Malus* cultivars.

Members of *Malus* genus are widespread and native throughout the Northern Hemisphere. Thousands of apple cultivars are grown worldwide to produce high-quality fruit for the fresh market, a range of beverages such as juice or cider, jelly, dried products, purees, etc. and other processed foods (Cornille *et al.*, 2019).

Recent studies concede tremendous progress in the reconstruction of apple history in regard with its origin, distribution, spread and hybridisation, given the increasing availability of genetic and phenotypic data, along with archeo-botanical information for both wild and cultivated *Malus* species. All these data have permitted the groundwork for a multidisciplinary re-evaluation of the biogeography of wild apples and the history of the cultivated species. There is still considerable debate surrounding the classification and nomenclature of crabapple (Way *et al.*, 1991; Coart *et al.*, 2003; Hussain *et al.*, 2021). For instance, a certain crabapple might be classified as a distinct species by some, while others might consider the selection a variety or hybrid (den Boer, 1959).

Because seed propagation is a method of sexual propagation, many seedlings do not resemble the parent plants due to genetic variability; however, seed propagation has led to the introduction of many new selections (Hartman *et al.*, 1990). Apple is also a model species for the study of the evolutionary processes and genomic basis underlying the domestication of clonally propagated perennial crops (Cornille *et al.*, 2019). Regional differences in appearance, seed propagation and the desire to introduce new species, hybrid, or cultivar, contributed to the misnaming or dual naming of many crabapple selections (Jefferson, 1970). Certain crabapples have been named and renamed several times with both common and botanically different names. For example, the Italian crabapple *Malus florentina* has been botanically reclassified for seven times (den Boer, 1959). Often, it is difficult, if not impossible, to trace the provenance of a particular cultivar since many species may have been involved in its development. Recent genetic analyses revealed a Central Asian origin for cultivated apple, together with an unexpectedly large secondary contribution from the European crabapple (Cornille *et al.*, 2014). Wild apple species display secure population structures and significant levels of introgression from domesticated apple and this may threaten their genetic integrity. Latter investigations have 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 role of crabapple genotypes is critical for several valuable traits (Cornille *et al.*, 2019; Volk *et al.*, 2021).

Rosy-bloom crabapples, so named because of their deep pink, rose or purple-rose petals, are either open-pollinated seedlings of the red vein crabapple – *Malus pumila* or crosses between *M. pumila* and the Siberian crabapple *M. baccata* (Fiala, 1994). The decorative value is given also by the number of flowers per tree, time of flowering, the persistence of flowers, the smell. Further, the fruit of crabapple can be considered ornamental or undesired. The value of crabapple fruit has been the subject of much controversy because of its litter potential, but, the ornamental value of the flowers and fruits is unquestioned (Green, 1996). Some selections, such as ‘Doubloons’ and ‘Hargozam’ have fruits that change from light green to lemon-yellow in summer, while they turn to lemon-gold after frost (Iles, 1999). Ecological factors, especially light conditions, may affect fruit colouration and biochemical compounds. The more or less coloured flesh (pulp) extracts of crabapple tissues have been associated with anti-oxidative activities, inhibition of fatty acid synthase, the flavonoids are present

in high quantity (Wang *et al.*, 2015), as well as volatile compounds (Zhao *et al.*, 2014). Crabapples (especially red crabapples) are recognised for their great potential as healthy food because are rich in phenolic compounds with high antioxidant and anti-proliferative activities to cancer cells (Han *et al.*, 2019).

Malus crabapple genotypes represent one of the most important germplasm resources in ornamental landscaping, food processing and nutritional products within the genus (Volk *et al.*, 2021; Yu *et al.*, 2021). Hence, the aim of the present study was to distinguish morphometric features of the crabapple collection established within M.M. Gryshko National Botanical Garden of Ukraine, investigate the most appropriate traits and thus prospect the cultivars that could be successfully grown and for wide stake use. The introduction population within the investigated phenotypes can contribute to successful results of directed selection work.

Materials and Methods

Location and biological material

The objects of the study were crabapple trees from the collection of the Department of Acclimatization of Fruit Plants of the National Botanical Garden (NBG) M.M. Grishko, National Academy of Sciences (NAS) of Ukraine. NBG is located on the South-Eastern outskirts of Kyiv on the Pechersk slopes of the low Kyiv hills in the Zverinets tract. The main type of soil on the territory of the NBG is dark gray podzol, which lies on loess and forest-bearing rocks and brown clays (the amount of humus is 0.5-2.0%).

Observations on the collection's forms of crabapples were performed during mass blossom and fruiting respectively, when the trees were eight years old. There were analysed and described phenotypic variation of floral organs in 15 crabapple genotypes used as ornamental trees, as follows: 'Adirondack', 'Butterball', 'White Jade', 'Van Eseltine', 'Golden Hornet', 'John Downie', 'Evereste', 'Era', 'King Beauty', *M. niedzwetzkyana*, 'Ola', 'Professor Sprenger', 'Rayka Rozeva', 'Royalty', 'Royalty Red'.

Morphometric characteristics

Pomological characteristics represented by reproductive traits of the investigated genotypes were analysed and statistically processed using 30 samples as mean of the trait and standard error of the mean (i.e., flowers, fruit, seed chamber, seeds, stalk etc.).

For a detailed analysis of the investigated genotypes, the following measurements were taken: a) Flower traits: number of flowers in the inflorescence, number of petals (corolla analysis), number of pistils within flowers (gynoecium analysis), number of stamina (androecium analysis), flower diameter (mm) and peduncle length (mm); b) Fruit traits: weight, expressed in g; height (mm); fruit width (mm); c) Seed traits: height and width (mm), seed chambers' height and width (g), number of seeds inside the seed chamber per fruit; d) Stem traits: stalk length and thickness (mm). All data used for further analysis were tested for normal distribution and were presented as mean per trait.

Statistical analyses

The data obtained on the physical and morphological characteristics recorded for flowers, fruit and seeds were processed as average values. These were presented in figures as mean traits and standard error of the mean (SEM), performed using Microsoft Excel Spreadsheet Software. Analysis of variance (ANOVA) was applied to the analyzed features and then the Duncan Multiple Test Range (Duncan MRT, $p < 0.05$) was used as a post-hoc test for the analysis of differences.

The correlations between the characteristics were analyzed by linear Pearson correlation ($p < 0.05$). The data were subjected to multivariate analysis, namely correspondence analysis (CA), both for the characters studied and for the genotypes. Clustering analyzes were performed by Paired group algorithm, UPGMA method, Gower similarity index. Calculations and graphical representations were performed with Past software (Hammer *et al.*, 2001).

Results and Discussion

There were significant differences among the investigated crabapple cultivars in regard with flower analysis. Flower traits are summarised in Figure 1, whereas it can be noted that there were several cultivars that stand out, being superior and statistically assured: the highest number of flowers in the inflorescence was recorded within trees of ‘Professor Sprenger’ with 5.78 flowers, closely followed by ‘White Jade’, ‘Van Eseltine’ and *M. niedzwetzkyana*. With significant negative data for the number of flowers were noted cultivars ‘Adirondack’ (4.32 flowers), ‘Ola’ and ‘Rayka Rozeva’. This trait is closely relevant in regard with the ornamental impact with the number of petals for each flower. So that, cultivars with flower abundance and more petals are of interest.

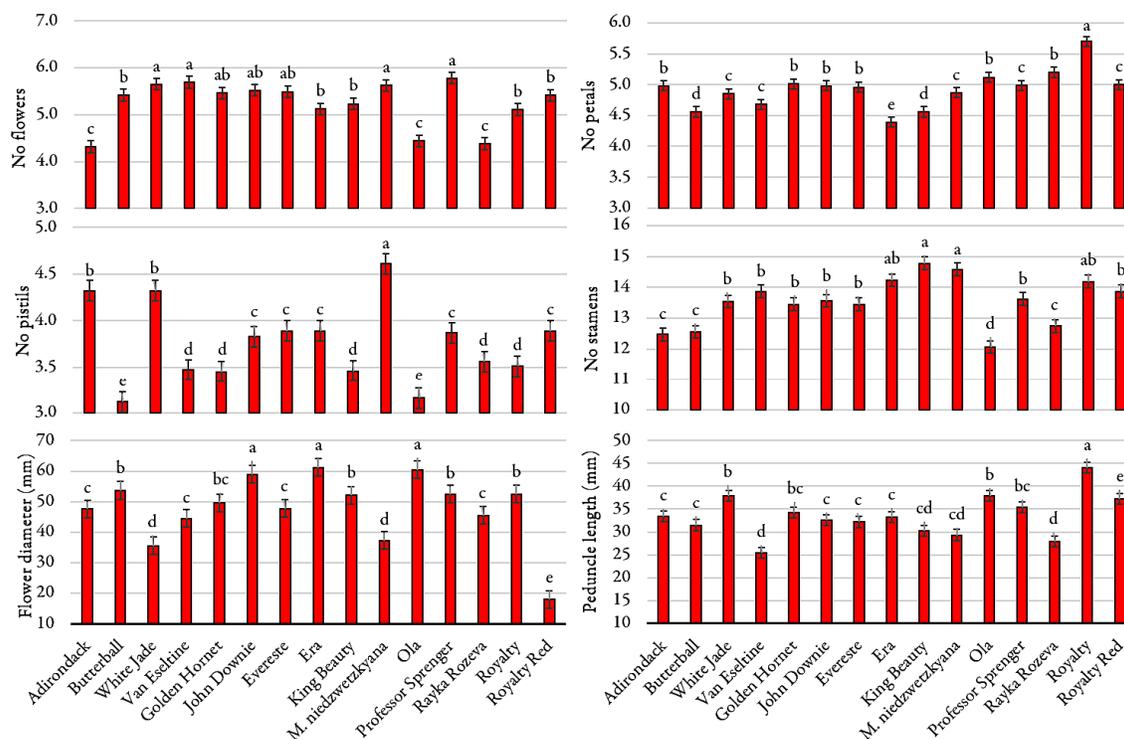


Figure 1. The main flower characteristics of the 15 cultivars of ornamental crabapples analyzed

Corolla was mostly decorative within ‘Royalty’ cultivar, with 5.7 petals, being statistically superior compared to the rest of the investigated phenotypes. The cultivars with a high value for the number of flowers had a typical pentamer corolla for Rosaceae, whereas the number of petals was close to 5. The smallest number of petals was identified for ‘Era’, statistically inferior, but the same cultivar was among the ones with the largest diameter of flowers, 61.23 mm respectively (Figure 1). It is interestingly to further investigate the fact that the cultivars with more flowers, had inferior data for the diameter of flowers and number of petals. *M. niedzwetzkyana* remarked for the reproductive flower organs, having superior data for pistil and stamens. Pistil is directly influencing the fruit and seeds, while stamens is important for the pollination (Volk *et al.*, 2015), whereas apple cultivars can be self-incompatible or self-pollinated (Kemp, 1996; Saito *et al.*, 2007).

The highest number of stamens was recorder for ‘King Beauty’ cultivar, followed by *M. niedzwetzkyana* and ‘Royalty’. Crabapple cultivars can be useful as germplasm resources and sometimes used for pollination, even though the distribution of pollen can be unbalanced among parental and progeny populations. Zhang *et al.* (2017) compared the pollen characteristics of 128 flowering crabapple germplasms (44 natural species and

84 varieties) and their results showed that no progeny had a degree of ornamentation greater than the maximal score of any of its parental genitor, which suggested that there is a high degree of consistency between individual evolution and population evolution. According to the morphometric parameters of the flowers, the longest peduncle was noted in crabapple 'Royalty' (44.09 mm), while the shortest was for cultivar 'Van Ezeltime' with 25.45 mm.

Based on the main characteristics of the fruits of the investigated genotypes (Figure 2), it can be accepted that they fall within the morphometric characteristics specific to crab apples (Saracoglu and Altuntas, 2021). The one cultivar that strike out was 'Era', with the largest fruits in terms of height, width and weight with 42.56 mm, 46.59 mm, and 20.56 g, respectively. To the opposite side, was 'Royalty' cultivar, which had the smallest fruit, with statistically assured data for height, width and weight, along with most of the investigated genotypes. Fruit shape index is an important indicator of marketability and attractiveness of apple fruits. According to the form index, varietal specificity was manifested. Fruit traits are genetically inherited in most part, with little influence by the technology of culture, thus future selection can use a vast variability within the studied genotypes.

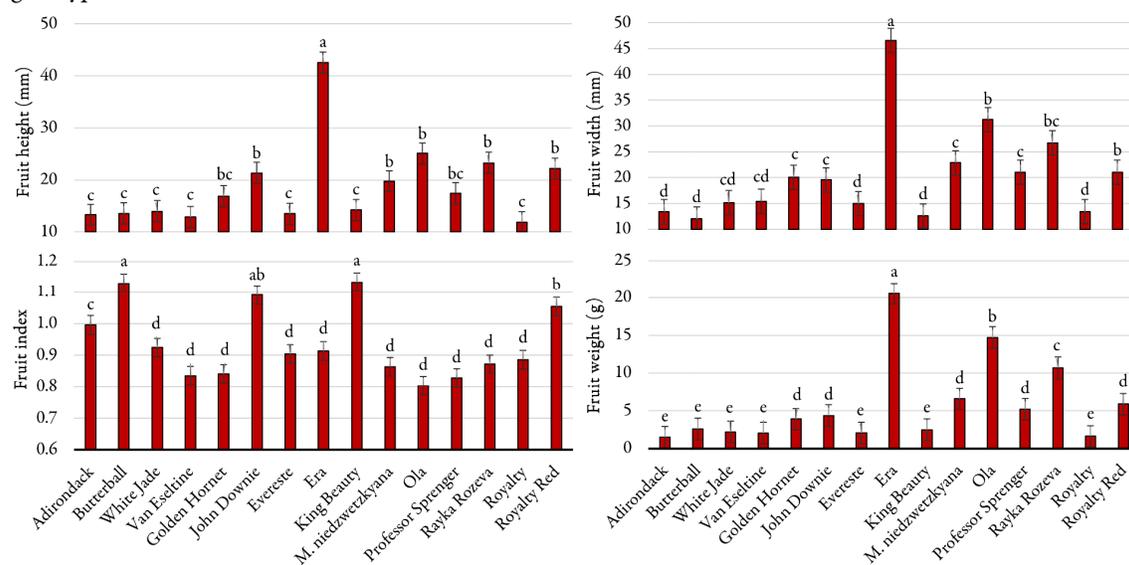


Figure 2. The main fruit characteristics of the 15 cultivars of ornamental crabapples analyzed

It will be interestingly to survey in the future the skin and the pulp of the crabapple fruit in the collection and the biochemical composition, as is already acknowledged that the products of colourful ornamental plants have great potential for human use (Wang *et al.*, 2015). Understanding the molecular mechanisms underlying fruit formation will be important, particularly for documenting the transcriptional regulatory networks of different coloured fruits and the differences in the accumulation and distribution of different compounds. This insight will contribute to the selection of plant germplasm resources for bioengineering and for exploiting the nutritional and medicinal value of decorative plants.

The stalk as part of the fruit attached to the stem often has specific features in ornamental apples compared to edible apples. Therefore, stalk length and thickness were recorded for the investigated *Malus* cultivars (Figure 3). The shortest stalk was noted within crabapple 'Era', having 16.89 mm, statistically inferior compared with the others, while the longest had 43.56 mm, within 'King Beauty'. Even so, the largest diameter was registered also for 'Era', followed by several cultivars with no significant differences, whereas a small thickness was registered for 'White Jade' and 'John Downie' cultivars. The variability of these traits was eloquent, denoting the possibility to select different genotypes for inducing the desired shape, from spur type to large spreading canopy for ornamental or breeding future *Malus* cultivars.

Specific seeds traits were also analysed. In order to promote generative reproduction of the valuable cultivars, is useful to investigate from start such aspects as well. In regard with the seeds chamber' dimensions and seed number, 'Era' genotype stands out, with superior values statistically assured for height and width (Figure 4), as well as seeds' weight (Figure 5). On the opposite side, 'King Beauty' and 'Adirondack' were noted to have small values, so that in the future propagation through seeds those genotypes should be treated with much attention. According to morphometric indicators, the largest number of seeds in one fruit was noted for 'Evereste' with 5.3 seeds, while the smallest value was 2.1 in 'King Beauty'. The smallest weight of seeds from one fruit had crabapple 'Adirondack', with only 9 mg (0.009 g), whereas the heaviest seeds were noted for 'Era' that summed up to 500 mg (0.5 g) (Figure 5).

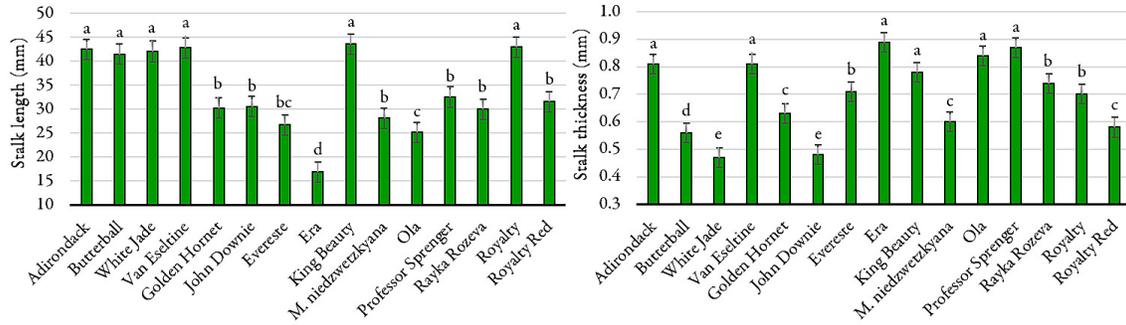


Figure 3. The main stalk characteristics of the 15 cultivars of ornamental crabapples analyzed

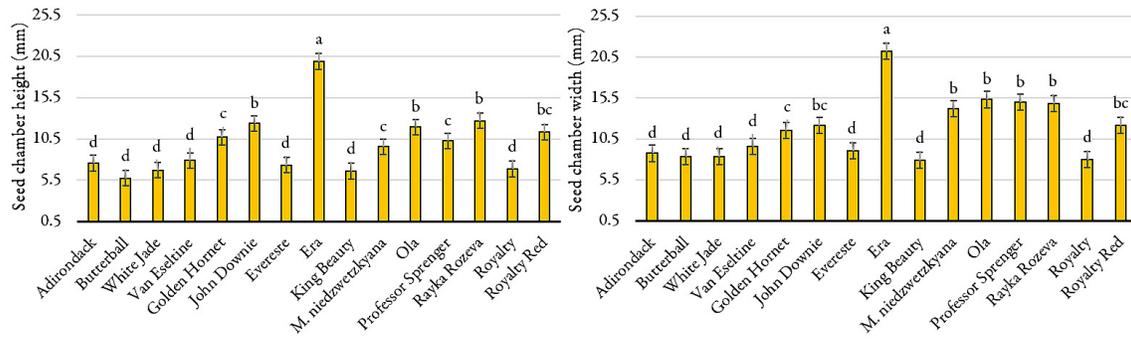


Figure 4. The main characteristics of the seed chamber of fruit in the 15 cultivars of ornamental crabapples analyzed

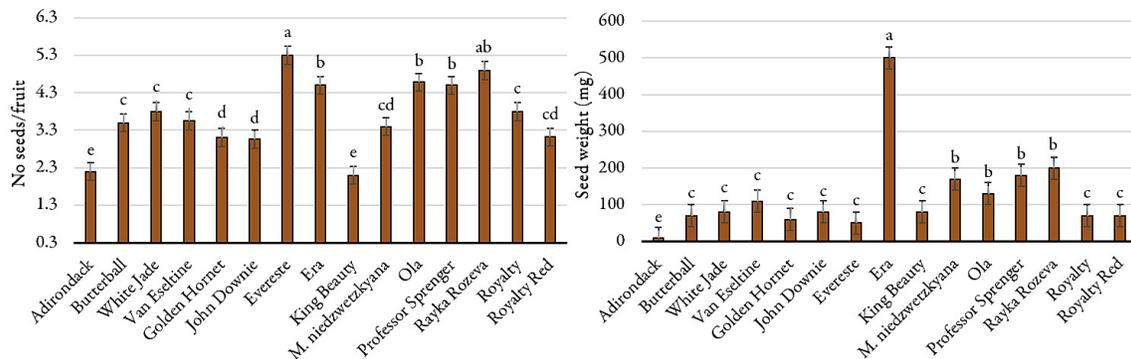


Figure 5. The main seeds characteristics of the 15 cultivars of ornamental crabapples analyzed

The gynoecium of *Malus* has been assumed to be imperfectly syncarpic, even though not accepted for all genotypes (Endress, 2001), whereby pollination of each stigmatic surface can result in fertilization within only one of the five carpels. Such aspects are essential for domestic species, being closely related with fruit quality (Sheffield *et al.*, 2005), as well as for crabapples and breeding programs. Researchers investigated the pollination, gynoecium structure and the pollen-tube pathway, fruiting and relationships of seed number and fruit weight and found variable carpel fusion (Ward *et al.*, 2001), thus different production and distribution of seeds.

The relationship between flowers and further fruit and seeds formation is a complex research keynote. It may refer to an economy in flower construction (Endress, 1994), thus the importance of crabapples genotypes with spectacular blossoming as a constant resource for valuable gene pool. Going further to the fact that *Malus* flowers attract a wide range of pollinators which are not specialized (Cambell *et al.*, 1991), so that the investigation of flower, fruit and seed traits as a starting point for the description of the genotypes in the NBG collection is justified.

The relationships between flower, fruit and seed traits investigated for the 15 *Malus* genotypes was highlighted by Pearson correlation (Figure 6). Positive correlations, which show a directional dependence, were displayed in blue colour and negative correlations in red colour. Colour intensity and the size of the circle are considered to be proportional to the correlation coefficients. The grey background boxes present in some cells illustrate the statistically assured values ($p < 0.05$).

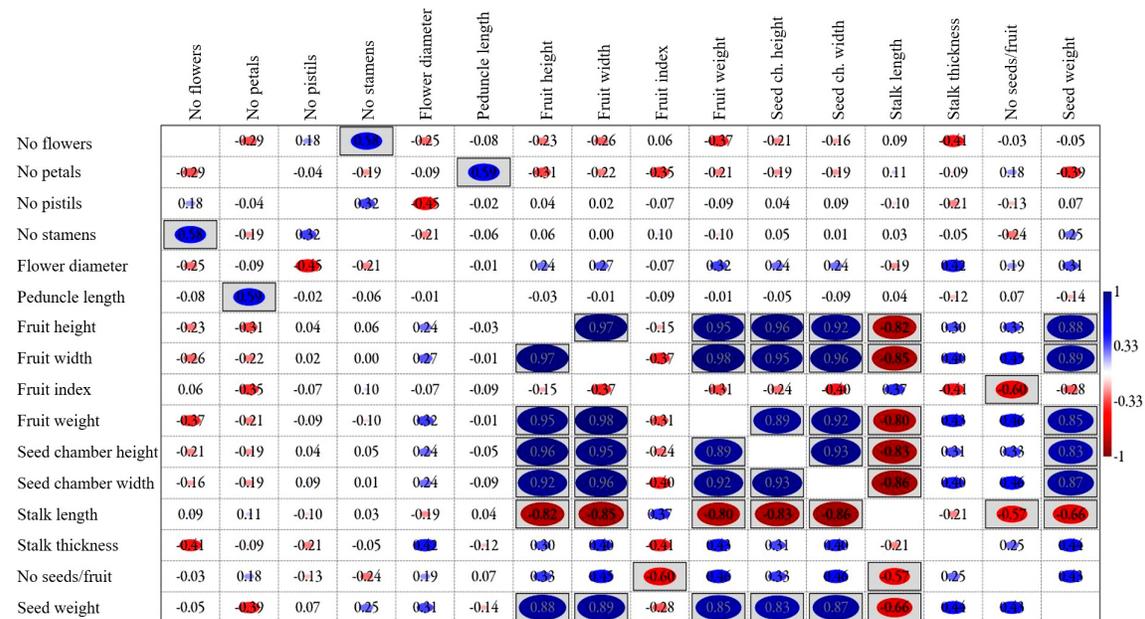


Figure 6. Phenotypic correlations between physico-morphological characteristics in 15 cultivars of ornamental crabapples analyzed (linear Pearson correlation; significant positive or negative correlations are enclosed in a grey box, $p < 0.05$)

Deriving out from the association between variables, respectively the investigated traits, it can be seen that fruit and seed traits were positively correlated and statistically assured, with numerous relationships established. The strength of the relationship for these traits was evidenced by positively statistically assured data among fruit dimensions and their weigh, dimensions of seed chamber respectively, where stronger relationship between the variables, closer to $r = 1$, can be seen. It is interestingly to further investigate flower traits and thus the ornamental impact, whereas the correlations hereby were not significant.

Multivariate method can be useful to elucidate and to represent the relationships between multiple traits (Figure 7) and several genotypes (Figure 8), such as in the current investigation. By identifying new variables as linear functions, it was found that the most distant *Malus* genotype was 'Adirondack', which was in negative correlation with 'Era'.

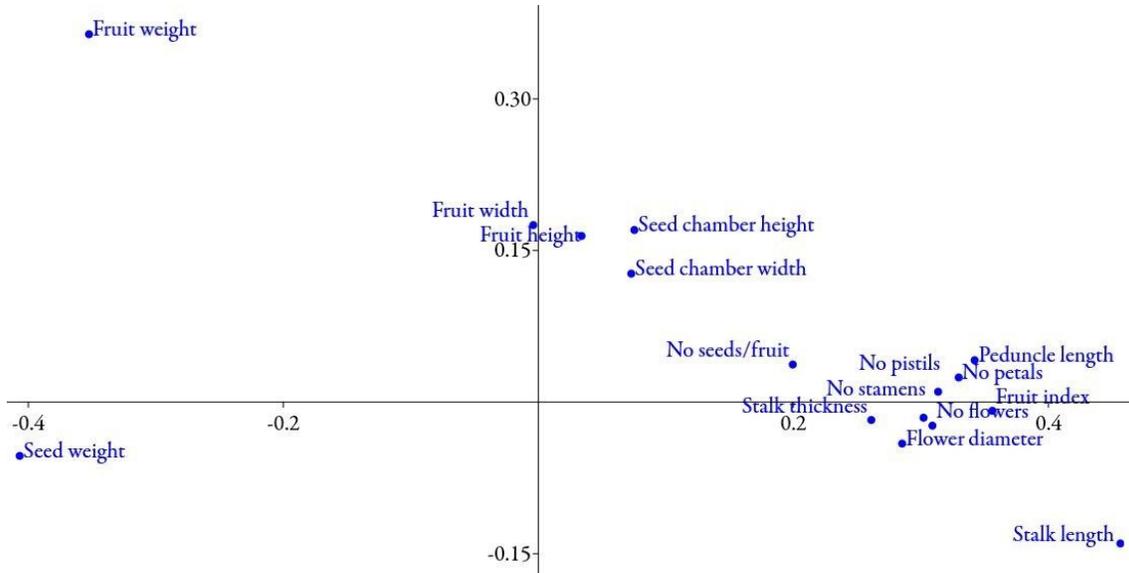


Figure 7. Multivariate analysis performed using correspondence analysis (CA) for the analysed physico-morphological traits, based on 15 ornamental crabapple cultivars
The first two axes explain 91.8% of the variation (of this total, axis 1 contributes 84.2% and axis 2 contributes 7.6%)

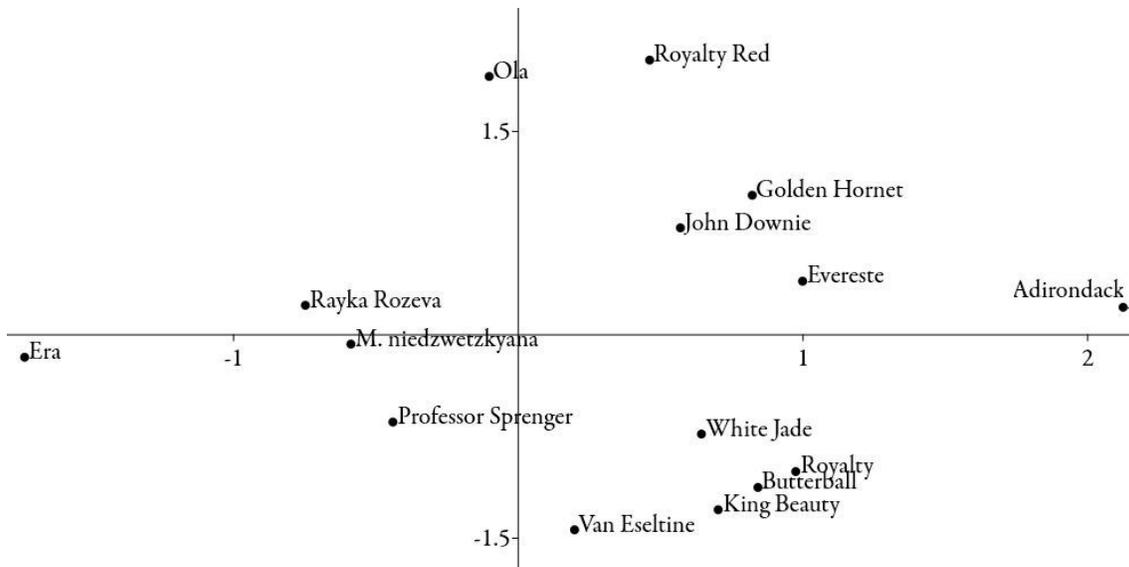


Figure 8. Multivariate analysis performed using correspondence analysis (CA) for the 15 ornamental crabapple cultivars, based on analysed physico-morphological traits

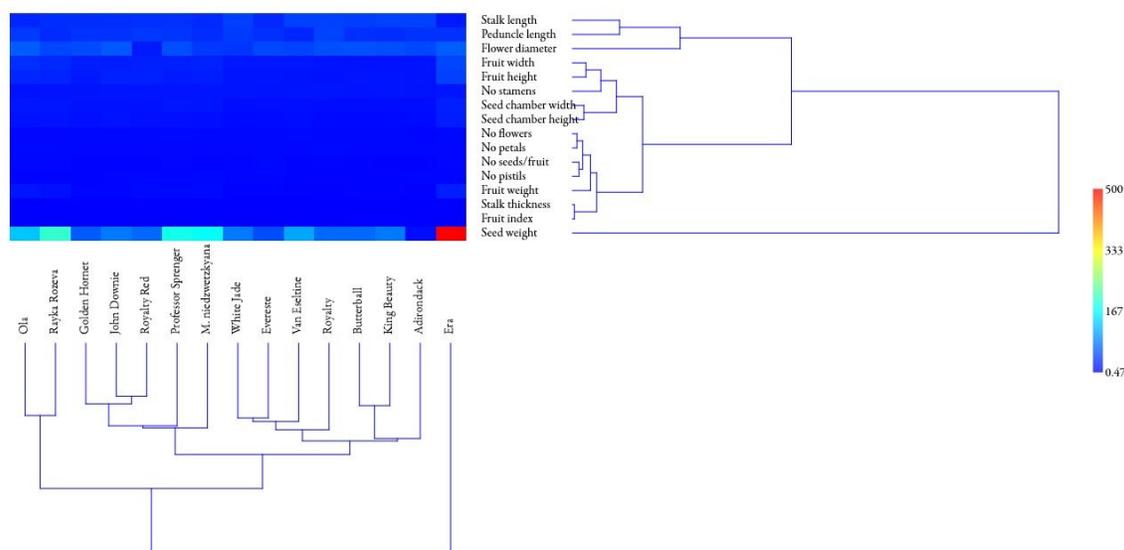


Figure 9. Clustering analyzes for 16 physico-morphological traits and 15 ornamental crab apple cultivars, performed by paired group algorithm – unweighted pair group method with arithmetic mean (UPGMA), Gower similarity index

In the case of the 16 analyzed morphometric characteristics and 15 crabapple cultivars, a distinct and obvious cluster was observed for 'Era' (Figure 9), sustained by the data registered within the study and the correlations pointed out. For the investigated traits, there are two major clusters, one referring to the seeds' weight and the other completed by the rest of criteria. The tree diagram confirmed the dispersed results, both by the pattern by which the clusters were formed and also by the similarity or distance levels of the clusters. Out of the many distinct subclusters, it worth mentioning the close grouping of the number of flowers and number of petals, respectively the number of pistils and the number of seeds per fruit.

The present study reveals both the differences in floral organs between ornamental apple cultivars and the relationships between flowers and reproductive traits given by the multivariate analysis. The genotypes analyzed showed different characteristics of floral and reproductive interest, which were well separated by the correspondence analysis (CA). The close correlations indicate links between essential characters for the decorative value of the ornamental apple, which could be useful in apple breeding and used as indirect selection indices. Multivariate analysis and Pearson correlations are working methods that have been well validated in crabapple genotypes (Yu *et al.*, 2021; Zhou *et al.*, 2021). The obtained results contribute to the knowledge of the taxonomic significance of the floral phenotypic variation at the level of some ornamental apple cultivars, completing similar information in the field (Zhou *et al.*, 2019, 2021). The phenotypic diversity of reproductive and decorative organs could better explore the genetic relationship between *Malus* genotypes, but also the manifestation of characteristics of ornamental interest in specific environmental conditions in which the evaluation was performed.

A thorough evaluation of these varieties tested for the first time under NBG conditions, could highlight all the features of ecological, landscape, ornamental interest. Many properties of crab apples are known in this regard. Their potential to capitalize on and beautify various urban areas, with different pedological and climatic conditions, to provide food sources for wild animals, wild birds, insects, etc. is well known (Fiala, 1994). Ornamental varieties are good pollinators and can be used to ensure foreign pollination in orchards, for commercial, edible apple varieties. Phenotypic and molecular assessment may also be of interest for the analysis of genetic diversity and phylogenetic relationships for use as parental forms in artificial hybridizations (Coart *et al.*, 2003; Sestras *et al.*, 2009). They can be a source of genes of great interest for inducing an extremely heterozygous structure in hybrid offspring, providing a good basis for the selection of new ornamental or edible

apple genotypes, fresh consumption or processing in various forms and use in food. It is accepted that selection process among natural mutations or progenies of collection populations of various cultivars (commercial, dessert or ornamental genotypes) use vegetative multiplication (cloning). Even more, referring mostly to the consecrated cultivars for *Malus* breeding the genetic base is narrowing (Dan *et al.*, 2015), so that crabapples could be valuable for manifesting favourable traits, both for commercial and ornamental apple cultivars. Breeding apple with genetic resistance to pests and diseases are also necessary for durable and environmentally friendly apple culture, along with the interest for production, fruit quality and the desire to beautify the surroundings. Crab apples offer a wide variability of response to the pathogens and pests attacks and are a valuable source of genes for apple breeding (Smith and Treaster, 1990; Spicer *et al.*, 1995; Sestras *et al.*, 2011; Volk *et al.*, 2015; Denoirjean *et al.*, 2021).

Unlike large-fruited dessert apples, small and even very small fruits (i.e., 1 cm in diameter) are preferred to ornamental apples, so that they do not produce dirt on the ground after ripening and falling and do not attract rodents, birds, and insects. Such fruits can have very beautiful colours, can remain on the tree long after the leaves fall and can significantly increase the ornamental appearance of the trees. Anyway, ornamental crabapple trees are generally valued for their landscape properties rather than their fruits (Volk *et al.*, 2021). Instead, the resistance to diseases and pests of trees is an objective pursued both at the apple for consumption and the ornamental one. Resistant or tolerant genotypes do not require so many phytosanitary treatments, are more beautiful, economical and contribute to a clean, healthy, ecological environment (Mitre *et al.*, 2010; Sestras *et al.*, 2011; Dan *et al.*, 2015).

Adequate response of the trees to the attack of the main diseases of the species, respectively resistance or tolerance (i.e., to apple scab – *Venturia inaequalis*, and powdery mildew – *Podosphaera leucotricha* etc.), or pests, obtaining fruits with special qualitative characteristics (i.e. taste, aroma), or food and nutritional properties of the fruits, or suitable for processing in the form of juices, jellies, jams, etc. are breeding objectives in which the potential of crab apples can still be exploited effectively (Sestras *et al.*, 2011; Wang *et al.*, 2018; Yu *et al.*, 2021; Muresan *et al.*, 2022). Probably a survey among NBG visitors during apple flowering using basic criteria for assessing characteristics of interest in ornamental varieties (e.g., after Romer, 2002: flower colour, fruit colour and persistence, environmental tolerance, growth habit - tree size and shape, disease resistance, food for wildlife etc.) would provide interesting and useful information both as a perception of respondents, as well as the direction of the future activity on conservation and ornamental apple breeding.

Conclusions

The study provided a good knowledge of the floral peculiarities of 15 crab apple genotypes, as well as the differences between them in the conditions of an area where the ornamental apple is not one of the most widespread and appreciated decorative tree species. The identification of genotypes with a high ornamental value under experimental conditions could be advantageous in order to extend them for the beautification of urban areas, or for use in new apple breeding works. Further surveys of the diversity of 'wild' and crabapple apple genotypes should facilitate the conservation of genetic resources *in situ* (e.g., conservation of genetically differentiated species and *Malus* cultivars) or *ex situ* (e.g., establishment of core collections of genetic resources, maximizing genetic diversity), along with dessert apple cultivars and ornamental ones.

Authors' Contributions

Conceptualization: IG and AFS; Data curation: IG, KV, GA; Formal analysis: IG and AFS; Investigation: IG, KV and GA; Methodology: IG, CD and AFS; Software: IG and AFS; Supervision: AFS; Writing - original draft: IG; Writing - review and editing: CD and AFS.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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