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Comparative Evaluation of *Pyrus* Species to Identify Possible Resources of Interest in Pear Breeding

Leontina I. Simionca Mărcășan [1], Rodica Pop [1,*], Peter A. Somsai [2], Ion Oltean [3], Sergiu Popa [4], Adriana F. Sestras [5,*], Mădălina Militaru [6], Mihai Botu [7] and Radu E. Sestras [1]

[1] Department of Horticulture and Landscape, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3–5 Manastur, 400372 Cluj-Napoca, Romania; leontina.marcasan@usamvcluj.ro (L.I.S.M.); rsestras@usamvcluj.ro (R.E.S.)
[2] Horticulture Research Station, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 5 Horticultorilor, 400457 Cluj-Napoca, Romania; petersomsai@yahoo.com
[3] Department of Plant Protection, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3–5 Manastur, 400372 Cluj-Napoca, Romania; ion.oltean@yahoo.com
[4] Department of Horticulture and Forestry, Technical University of Moldova, 42 Mircesti, 2049 Chisinau, Moldova; sergiu.popa@h.utm.md
[5] Department of Forestry, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3–5 Manastur, 400372 Cluj-Napoca, Romania
[6] Research Institute for Fruit Growing Pitesti, 402 Mărului, 117450 Mărăcineni, Romania; madamilitaru77@yahoo.com
[7] Department of Horticulture & Food Science, Faculty of Horticulture, University of Craiova, 200585 Craiova, Romania; mihai.botu@edu.ucv.ro
* Correspondence: rodica.pop@usamvcluj.ro (R.P.); adriana.sestras@usamvcluj.ro (A.F.S.)

Abstract: Pear is one of the most important fruit species grown in the temperate zones of the globe. Besides fruit production, pear species are highly valued in forestry and agroforestry systems; in landscaping, as ornamental features; as fruits of ecological value, and in other areas. The *Pyrus* species, obtained from a gene bank, were evaluated for the different morphological traits of the trees, leaves, flowers, and fruits, as well as their responses to attacks from principal diseases and pests. Phenotypic data were examined using correlation and multivariate analyses, and a dendrogram of morphological traits was completed via molecular investigations at the DNA level using the RAPD markers. The findings revealed the complexities of the phenotypic and genetic connections among *Pyrus* species, as well as the difficulty in establishing phylogenetic relationships among pear species. The findings also demonstrated that the wide variability between species with different geographical origins, and their multiple peculiarities of interest, represents a cornerstone as the source of genes of great utility for pear breeding or for utilizing trees for different edible crops and for silvocultural, landscape, or ecological purposes.

Keywords: diseases and pests; gene bank; genetic diversity; genetic relations; morphological diversity; phenotypic correlations; phenotypic traits; tree growth

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1. Introduction

Pear (*Pyrus* genre) is one of the oldest and most important economically fruit crops in the temperate zone [1] after apples (*Malus domestica* L.) and before peaches (*Prunus persica* L.) [2,3]. Besides being a significant global source of food, pears have multiple health benefits, including protection against cancer, type 2 diabetes, osteoporosis, inflammatory and acne disorders, skin infections, and so on [4–6]. They also contribute to the reduction in triglycerides and the detoxification of the body [7], the regulation of folic acid levels during pregnancy, and the prevention of congenital abnormalities in newborns [8]. The varied genetic traits of different *Pyrus* species make them useful for various purposes [9], and each part of the tree has multiple uses and medicinal properties [10–14].

Traditionally, people used the bark (rhytidome) and leaves of pears to heal wounds, a property attributed to arbutin [15]. Arbutin is also used in cosmetics, due to its skin-whitening property [16]. Pear wood is very durable, homogeneous, heavy, hard, elastic, light, and is easy to bend and to process [17]. It is one of the more expensive materials used to make high-quality woodwind instruments [16]. In addition, the species of the genus *Pyrus* can also be used for ornamental purposes, for example, in parks and various green spaces and landscapes [18–20]. Trees contribute to enhancing the landscape, eliminate monotony in flatness and color, mask city noise, lower air pollution, support a variety of living organisms, promote and maintain biodiversity, provide a variety of rest and relaxation possibilities, and lessen the negative effects on humans' psychological well-being [21–24].

The identification and description of *Pyrus* species were based for a long time on the traditional morphological characteristics of trees, leaves, flowers, and fruits [25], which in recent decades were supplemented with detailed molecular studies. The genus *Pyrus* comprises only woody plants, most commonly medium-sized trees, and only a few shrubby species [26]. The stem of the tree is straight and well-embedded in the ground. In general, the leaves are simple, arranged alternately, with a length between 2–12 cm and 3–5 cm wide, while petioles are stipulate and have whole or serrated limb edges [27]. Some species have glossy green leaves, whereas others are silvery and densely tomentose, and while most are deciduous, one or two Southeast Asian species show sempervirescent leaves [27]. The tree blooms in April–May, and the flowers are grouped in corymb-type inflorescences from 5 to 20 flowers [28]. The fruits are pomes that often have a pyriform shape and contain sclereids in the pulp. Fruits measure 1–4 cm in diameter in wild species and up to 18 cm long and 8 cm wide in some cultivated forms. The shape of the fruit varies from an elongated pyriform, in the case of European pear species (with a dense, consistent texture that is soft (butter/beurré pears) and juicy when ripe), to a round shape, in the case of Asian pear species, with porous, harder, and firm textures that do not change after harvest [27,29].

At least 22 known species of the genus *Pyrus* exist across the globe, and over 5000 different pear varieties have been described [30,31]. However, it is extremely probable that this number is much higher. In accordance with Hedrick et al. [26], more than 3000 distinct cultivars of the European pear (*P. communis*) were reported before 1921. It is obvious that since then, in over 100 years, modern breeding has produced numerous new cultivars. Excluding European pears, Teng [32] demonstrated that more than 3000 different cultivars of *P. ussuriensis*, *P. pyrifolia*, and *P. singkangensis* have been documented in China. These sources alone reflect a number of at least 6000 cultivars, roughly equally divided between European and Asian pears. The differences between the genotypes and phenotypes of European and Asian species are also reflected in the taste and other organoleptic characteristics of the cultivars and in consumer preferences for European and Asian varieties in Europe, America, Australia, and New Zealand and in Asia, respectively.

Even if there is a significant demand for these 'luxury' fruits, pear production is frequently influenced by the sensitivity of the cultivars to stress factors, especially attacks from diseases and pests [33]. These biotic stressors affect tree development, yield capacity, and fruit quality. Chemicals used to control diseases and pests are expensive and do not always have the desired efficiency. Furthermore, their effects and consequences are detrimental to the environment as well as human and animal health [34–37]. With an increased demand for ecological products in the fruit market and among consumers, pear breeding, similarly to other fruit or agricultural species, aims to develop and promote cultivars that are resistant or tolerant to stress factors [38,39].

Although there are thousands of pear varieties in the world today, and pear breeding is a traditional activity with notable results, many varieties have deficiencies, such as poor resistance to diseases and pests; fruiting alternation; poor fruit quality, including a reduced nutritional value or a low content of useful substances; sensitivity to handling and transport; poor fruit preservation, etc. [29,39]. Although the diversity of cultivars appears to be broad, only a small number of cultivars are widely distributed and cultivated on a large scale. As a result, it is estimated that only approximately ten cultivars comprise 90%

of the world's pear production [27]. In addition, many varieties have a common origin, deriving from common or related parents, which causes a narrowing of genetic variability among pear varieties and, at the same time, results in an increase in the degree of genetic vulnerability of the cultivated species [33].

At present, humanity is facing new challenges, such as global population growth (which has surpassed 8 billion people), climate change, soil erosion and desertification, aridity, salinization, and the appearance of new pathogenic and pest agents alongside an increase in their virulence and resistance to phytosanitary products used to protect orchards, etc. [40]. All of this contributes to growing concerns about the availability of human food resources, including fresh fruits and those necessary for industrialization, as well as compliance with the requirements of sustainable agriculture and the ecological environment [41].

In this regard, the availability of diverse pear varieties that are resistant to diseases and pests is essential for successful production. The identification of genes that provide resistance to disease and insect attacks is an important objective for breeding programs in order to enhance the genetic basis of cultivated pears. Such sources can also be represented by wild *Pyrus* species, although, when utilized in interspecific hybridizations with different varieties, they have the disadvantage of the extremely difficult and time-consuming recovery of the valuable recurrent parent's phenotype [29]. Another issue with species of spontaneous flora is the considerable decrease in the population sizes of wild *Pyrus* species because of the sixth mass extinction [42]. Consequently, the collection and preservation of *Pyrus* species in germplasm pools, as well as their assessment for possible use in pear breeding, are highly desirable goals. As a result, in the current study, certain wild pear species were tested for a set of phenotypic characteristics of interest related to the morphological peculiarities of their trees, leaves, flowers and fruits and their response to diseases and pest attacks, as well as molecular analysis to identify the genetic diversity among them.

2. Materials and Methods

2.1. Site Description and Biological Material

The pear genotypes were investigated at the Horticultural Research Station (HRS) of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca. Cluj-Napoca is located in northwest Romania. The average annual temperature in the area is 8.2 °C, and the total annual precipitation is 560 mm. The pear genotype plantation is located at an elevation of roughly 400 m on degraded chernozem, with suitable soil and general conditions particular to the Someș Mic Valley Corridor area [43].

The pear trees were grafted onto the *P. communis* seedlings as rootstock, known as 'franc', and planting was carried out at 4 m intervals between rows and 2 m intervals between trees in a row, resulting in a density of 833 trees/ha. A slender spindle planting system was adopted, with limited pruning at planting, to ensure that the trees develop the most natural crown possible, with persistent scaffold branches and slight renewal trimming. The experimental pear plantation was founded in 1992 as National Pear Collection and included 365 genotypes, the majority of which were European cultivars, but wild species of also different origins were also extant, including Asian species. The biological material used in the study, represented by species or hybrid forms of *Pyrus*, is presented in Table 1. The study of the phenotypic traits and the response of trees to diseases and pests was carried out during the period of 2018–2019. The experimental circumstances were the same for all genotypes, and each species was represented by three trees. Due to HRS's financial constraints, no tree maintenance or pruning was performed during the evaluation period, and phytosanitary treatments were reduced to a minimum of 3–4 treatments with specific fungicides and insecticides per year.

Table 1. The biological material used in the study, represented by species or different hybrids of *Pyrus* [1] from the Romanian National Germplasm Collection of HRS [2].

No.	Species	Origin or Geographical Distribution—References or Sources
1	*P. betulaefolia*	North and Central China, Laos Southern Manchuria [19,25]. Syn. *P. betulifolia*, respectively, *Sorbus betulaefolia*? (https://powo.science.kew.org/, accessed on 10 March 2023).
2	*P. canescens*	Interspecific hybrid between *P. nivalis* × *P. salicifolia* [9]; https://www.genesys-pgr.org/t/Pyrus, http://www.worldfloraonline.org/ (accessed on 10 March 2023).
3	*P. caucasica*	Wild form of *P. communis*. The common varieties of pear are probably complex hybrids with var. *pyraster* and var. *caucasica*, and *P. nivalis* [25].
4	*P. communis*	Southeast and Western Europe, Turkey. *P. communis* sensu lato (common pear) is the ancestor of most of the cultivated pears in Europe, America, Australia, and New Zealand [19,25,44,45].
5	*P. cordata*	France, Spain, Turkey, and possibly south-west England [19,25], as well as Portugal [9]. Considered to be merely a form of *P. communis* [46], a possible relict from a fairly early stage in the evolution of *Pyrus* species [25].
6	*P. drovara*	Accession of unknown provenance from the HRS and RIFG germplasm collection (https://www.genesys-pgr.org/t/Pyrus, accessed on 10 March 2023).
7	*P. eleagrifolia*	Southeast Europe, Turkey, Crimea [19,25]. It often appears under the name *P. elaeagrifolia*.
8	*P. korshinskyi*	Afghanistan, Kyrgyzstan, Tajikistan, Uzbekistan. Critically endangered—threatened by overgrazing and overharvesting [19].
9	*P. lindlezi*	Accession of unknown provenance from the HRS and RIFG germplasm collection (https://www.genesys-pgr.org/t/Pyrus, accessed on 10 March 2023).
10	*P.* ×*malifolia*	Accession of unknown provenance from the HRS and RIFG germplasm collection (https://www.genesys-pgr.org/t/Pyrus, accessed on 10 March 2023).
11	*P. persica*	Syn. *Pyrus spinosa* (https://powo.science.kew.org/, accessed on 10 March 2023).
12	*P. ussuriensis*	Northern China, Manchuria, Korea, Siberia. One of the edible pears of which various forms were cultivated along with *P. pyrifolia* (also known as *P. serotina*) early in Chinese history [19,25].
13	*P. variolosa*	Syn. *P. pashia*? [19]; https://powo.science.kew.org/, https://www.genesys-pgr.org/t/Pyrus (accessed on 10 March 2023).
14	×*Pyronia veitkii*	Artificial hybrid of *Pyrus* and *Cydonia oblonga* [19].
15	×*Sorbopyrus*	A triploid selection developed in the early 1800s from a cross between *Sorbus* and *Pyrus* [9].
16	*P. eleagrifolia* × Curé	Accession from the HRS collection whose provenance is unknown (according to the name, it is an interspecific hybrid, but this is debatable as Curé is a triploid cultivar)
17	*P. longipes*	Origin: Algeria [25]. Syn. *P. cossonii* is one of the three North African pear species [27]. Considered a relict species, close to *P. communis* [47].
18	*P. luxemburgiana*	A clone of ×*Pyronia veitkii* (var. *luxemburgiana*?) [19].
19	*P. nivalis*	Western, central, and southern Europe [25]; south–central Europe, Ukraine, France [19]. *P. nivalis* Jacq., called snow pear, is considered a subspecies of *P. communis* [29].
20	*P. pyraster*	A subspecies of *P. communis* [29]. The trees grow wild throughout Europe and were domesticated as early as 300 BC, being the ancestral form of the European pear [45]. In some European countries, it is threatened; efforts are being made to maintain genetic resources through in situ and ex situ conservation [19].

[1] Species numbered 1–15 were used in phenotypic and molecular analyses. Species numbered 16–20 did not form fruit in the study years, and their phenotypic characteristics are not shown in the results but are included in the molecular analysis. [2] The pear germplasm collection in Romania is preserved at Horticultural Research Station (HRS) Cluj-Napoca and the Research Institute for Fruit Growing (RIFG) Pitesti, Mărăcineni.

2.2. Measuring and Assessing Morphological Traits of Trees, Leaves, Flowers, and Fruits

The measurement of the trees was carried out in the autumn, at the end of the vegetation period. The heights of the trees were measured from the ground level to the top of the crown and were expressed in meters (m). The heights of the trunks were measured from the ground level to the first main branch and were expressed in meters (m). The diameters of the trunks were measured with a caliper, in the direction of the rows of trees, at a height 30 cm from the ground, and were expressed in centimeters (cm). The diameters of the crowns were measured as projections at ground level, in the direction of the rows of trees and perpendicular to them, calculated as averages and expressed in meters (m). The number of branches (scaffold branches) on each tree were also determined via counting. The measurements of the trees included in the results are from 2018, and the measurements of the leaves, flowers, and fruits are the mean of the two years of study.

Leaf characteristics were measured in late August and flowers were measured at full opening, generally in the second or third decade of April. For each element, 100 leaves and 100 flowers per tree were analyzed from different areas of the crown. The main characteristics of the fruits (peduncle length, fruit length, fruit diameter, fruit weight, and fruit firmness) were analyzed at full ripeness. For the genotypes with more abundant fruiting, the determinations were made with respect to at least ten fruits taken at random, and for those with few fruits, assessments were performed using as many fruits as were found on the tree. Fruit firmness (kgf/cm^2) was measured using the HPE III Fff penetrometer (Bareiss Qualitest HPE-IIFFF, Germany). Results for the specified phenotypic characteristics are reported for 15 *Pyrus* species, as some did not produce relevant flowers and fruits in the study years.

2.3. Assessing the Response to Attacks by Principal Diseases and Pests

The most common diseases and pests were examined in the field under infection or natural infestation conditions (Figure A1). A traditional method for assessing diseases and pests using a pictorial key was adapted to rate the severity of attacks on a scale of 1 to 9 [48,49]. The assessment was carried out according to the pathogens or harmful insects identified in the plantation, for example fire blight (*Erwinia amylovora*) on shoots; pear scab and septoria on leaves; psyllids, according to the amount of leaf surface covered by nymphs; leaf miner larvae, depending on the amount of surface covered by mine galls, etc. [33,50].

The assessments were performed over a period of two years during repeated visits to the plantation, and in the summer months of June, July, and August, the evaluations were performed every two weeks. Scores were awarded in increments, depending on the intensity of the attack, along with the severity and the incidence, from a score of '1' to a maximum score of '9′ for an extremely strong attack: 1 = no attack; 2 = sporadic traces, first symptoms; 3 = the affected surface, damage, injuries, lesions, or galls, etc., comprising up to 5% of the surface of the analyzed organ/organs; 4 = affected surface comprising 5–15%; 5 = affected surface comprising 15–25%; 6 = affected surface comprising 25–40%; 7 = affected surface comprising 40–50%; 8 = affected surface comprising 50–75%; 9 = affected surface comprising 75–100%.

2.4. Genetic Diversity Analysis

Fresh leaves were used for DNA extraction and molecular analysis. The method used followed the procedure of Teng et al. [51], in regard to DNA extraction, purification protocol, and DNA amplification. The majority of primers used in RAPD analysis had been selected and used previously [51–53]. Table 2 displays an inventory of the primers used in our RAPD analysis together with their sequences. The dendrogram obtained encompasses a total of 20 *Pyrus* species from the collection, specifically the fifteen species studied for phenotypic characteristics, including fruit attributes, as well as the five species that did not bear fruit during the study.

Table 2. Primers used in the RAPD analysis and their sequences.

No	Primer	Sequence (5′–3′)
1	OPA20	GTTGCGATCC
2	OPAL20	AGGAGTCGGA
3	OPAB11	GTGCGCAATG
4	AB11	GTGCGCAATG
5	OPA01	CAGGCCCTTC
6	OPB10	CTGCTGGGAC
7	OPA17	GACCGCTTGT
8	OPB08	GTCCACACGG
9	OPB18	CCACAGCAGT
10	OPC–14	TGCGTGCTTG
11	OPC14	TGCGTGCTTG

2.5. Statistical Analysis

Recorded data for tree, leaf, flower, and fruit phenotypic traits were processed as mean values from three trees for each *Pyrus* species (Figure A2). After checking the data for normality and applying an ANOVA test, when the null hypothesis was rejected, Duncan's post hoc test (with an alpha of 0.05) was applied in order to illustrate differences between species. For the trees' responses to biotic stressors, descriptive data, and results of scores were presented without statistical processing, due to the assessment under natural conditions of infections or infestations and the estimation method used. Multivariate analysis was performed using PAST software [54], the pear species dendrogram was based on Nei and Li's (1979) [55] similarity coefficients, and UPGMA was used as the clustering algorithm. To perform the PCA analysis and obtain the UPGMA dendrogram, the mean values of all phenotypic traits were scaled to between 0 and 1 in order to ensure a better and objective distinction between the morphological traits and genotypes represented by the *Pyrus* species.

3. Results

The differences in tree vigor among the 15 pear genotypes were significant, with large variability between *Pyrus* species. The heights of the trees were between 2.38–3.36 m, with significant differences between species (Table 3). The highest increases in tree height were recorded for *P. persica*, *P. variolosa*, *P.* ×*malifolia*, *P. betulaefolia*, and *P. lindlezi*, and the smallest increases were seen in ×*Sorbopyrus*. Trunk height varied between 0.47 m (*P. korshinskyi*) and 0.81 m (*P. lindlezi*). *P. variolosa*, *P. lindlezi*, *P. eleagrifolia*, and *P. persica* stood out due to the large diameters of their tree trunks. On the other hand, ×*Sorbopyrus* and *P. drovara* had the lowest values. The values of differing genotypes with extreme trunk diameters varied between 9.55 cm (*P. drovara*) and 18.77 cm (*P. variolosa*). The diameters of the crowns ranged between 1.53 m (*P. cordata*) and 2.90 m (*P. eleagrifolia*), and the number of branches per tree ranged between 3.67 (×*Sorbopyrus*) and 7.33 (*P. ussuriensis*). Trees of the species *P. caucasica*, *P. canensis*, ×*Pyronia veitkii*, *P. betulaefolia*, and *P. variolosa* displayed a high branching tendency.

Table 3. The main characteristics of the trees of 15 species of *Pyrus* [1].

No.	Species	Tree Habit (1–6 UPOV) [2]	Height of Trees (m)	Trunk Height (m)	Trunk Diameter (cm)	Crown Diameter (m)	No Branches Per Tree
1	*P. betulaefolia*	4	3.15 ^{a}	0.72 ^{a,b}	12.50 ^{e}	2.62 ^{b}	6.00 ^{b,c}
2	*P. canescens*	4	2.72 ^{b}	0.79 ^{a}	11.92 ^{e}	2.00 ^{c}	6.33 ^{b}
3	*P. caucasica*	4	3.07 ^{a}	0.61 ^{b}	12.47 ^{e}	2.60 ^{b}	6.67 ^{b}
4	*P. communis*	4	2.53 ^{b}	0.77 ^{a,b}	11.99 ^{e}	1.92 ^{c}	5.67 ^{c}
5	*P. cordata*	3	2.72 ^{b}	0.61 ^{b}	10.54 ^{f}	1.53 ^{d}	4.67 ^{d,e}
6	*P. drovara*	3	2.63 ^{b}	0.76 ^{a,b}	9.55 ^{g}	1.83 ^{c}	4.33 ^{e}
7	*P. eleagrifolia*	4	2.92 ^{a,b}	0.54 ^{c}	15.88 ^{b}	2.92 ^{a}	5.67 ^{c}
8	*P. korshinskyi*	4	2.97 ^{a,b}	0.47 ^{d}	13.46 ^{d}	2.22 ^{c}	4.33 ^{e}
9	*P. lindlezi*	3	3.13 ^{a}	0.81 ^{a}	16.42 ^{b}	2.53 ^{b}	5.00 ^{d}
10	*P.* ×*malifolia*	3	3.27 ^{a}	0.56 ^{c}	14.63 ^{c}	2.42 ^{b,c}	5.00 ^{d}
11	*P. persica*	4	3.36 ^{a}	0.56 ^{c}	15.34 ^{b,c}	2.06 ^{c}	5.67 ^{c}
12	*P. ussuriensis*	5	2.94 ^{a,b}	0.63 ^{d}	10.54 ^{f}	2.22 ^{c}	7.33 ^{a}
13	*P. variolosa*	4	3.30 ^{a}	0.56 ^{c}	18.77 ^{a}	2.13 ^{c}	6.00 ^{c}
14	×*Pyronia veitkii*	4	2.93 ^{a,b}	0.64 ^{b}	11.30 ^{e,f}	2.40 ^{b,c}	6.33 ^{b}
15	×*Sorbopyrus*	5	2.38 ^{c}	0.54 ^{c}	9.98 ^{g}	2.28 ^{c}	3.67 ^{f}

[1] Different letters between species in each column indicate statistically significant differences for the investigated traits at a significance level of $p < 0.05$ (Duncan's test). [2] The scores for tree habit were awarded according to the UPOV [56] scale (1–6) as follows: 1—fastigiate; 2—upright; 3—semi-upright; 4—spreading; 5—drooping; and 6—weeping.

The growth and branching of *Pyrus* trees was included in one of the architectural types or habitus classified by UPOV [56] and ECPGR CPVO-UPOV [57] (Figure 1). None of the species had a fastigiate, upright, or weeping tree habitus. The largest proportion of the species had spreading architectural (60.0%), semi-upright (26.7%), and drooping (13.3) growth, according to UPOV. According to the CPVO framework, in which there is no semi-upright ideotype, this form in the previous category was considered to be closer to the spreading habit.

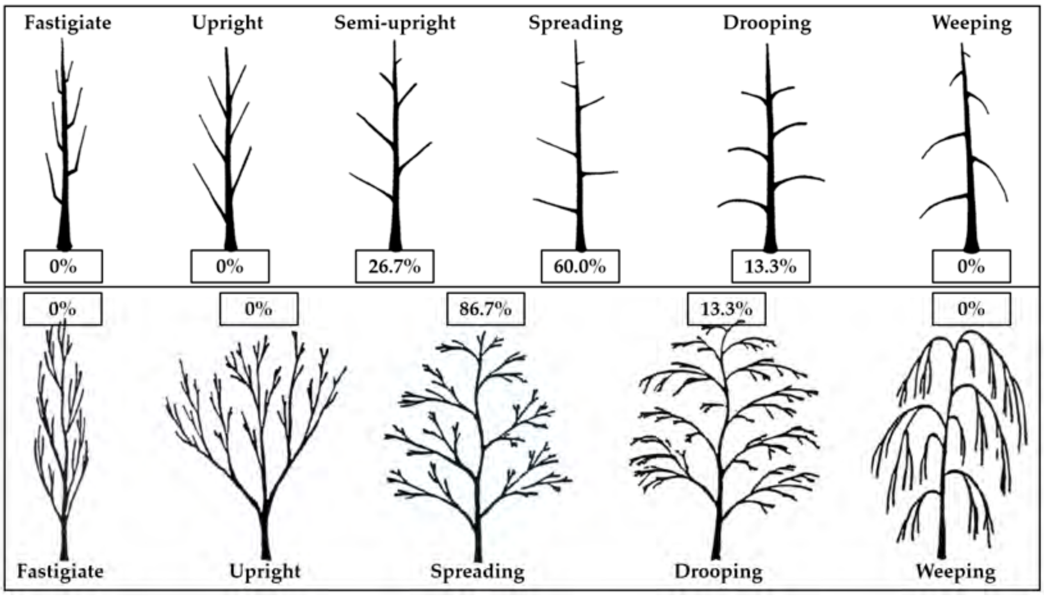

Figure 1. Percentage of tree habits (tree growth, habitus, or architectural 'ideotypes') of the 15 *Pyrus* species, according to the classifications of UPOV 2000 (**top**) and ECPGR CPVO-UPOV (**bottom**).

Significant differences in leaf characteristics were identified amongst *Pyrus* species (Table 4). Leaves with long blades were recorded in *P.* ×*malifolia*, *P. eleagrifolia*, *P. lindlezi*, *P. korshinski*, and *P. persica*, and leaves with very short blades were seen in *P. cordata*, *P. ussuriensis*, ×*Sorbopyrus*, and *P. betulaefolia*. In the trial with the 15 species of *Pyrus*, leaf lengths ranged between 4.57 and 11.14 cm. Differences in leaf width between species were significant, ranging from 1.92 to 6.67 cm. *P. lindlezi* and *P.* ×*malifolia* had the widest leaf blades, and in contrast, ×*Sorbopyrus*, *P. canensis*, *P. caucasica*, *P. ussuriensis*, *P. cordata*, *P. eleagrifolia*, and *P. betulaefolia* had the narrowest leaf blades. ×*Sorbopyrus* was categorized as having leaves with the shortest petioles (1.73 cm). On the other hand, *P. persica* (5.43 cm) and *P. lindlezi* (5.37 cm) were recorded as having the longest petioles.

The main flower attributes revealed notable variations amongst the *Pyrus* species (Table 5). The largest flowers were reported in *P. lindlezi*; in this species, all floral elements (corolla diameter, petal length, and petal width) displayed the largest dimensions. Thus, *P. lindlezi* had flowers with the largest average corolla size (54.20 mm). Another characteristic of this species was the almost spherical shape of the flowers; in fact, the petal length was equal to the petal width (17 mm). The species *P. betulaefolia*, *P. caucasica*, and *P. drovara* also had flowers with large corollas, while the smallest flowers were recorded in *P. korshinski* but also in *P. cordata* and ×*Pyronia veitkii*. Petal length ranged between 11.66 and 17.00 (mm), and petal width ranged between 8.33 and 17.00 (mm). The minimum values for petal length were recorded in ×*Sorbopyrus*, and the smallest values for petal width in ×*Pyronia veitkii*.

Table 4. The main characteristics of the leaves of 15 species of *Pyrus* [1].

No	Species	Leaf Length (cm)	Leaf Width (cm)	Petiole Length (cm)
1	*P. betulaefolia*	4.57 [g]	4.03 [d]	4.53 [b]
2	*P. canescens*	6.10 [e]	2.52 [f]	3.50 [c]
3	*P. caucasica*	6.23 [e]	3.30 [e]	3.58 [c]
4	*P. communis*	6.23 [e]	5.20 [b]	4.17 [b,c]
5	*P. cordata*	5.38 [f]	3.50 [e]	3.14 [d]
6	*P. drovara*	7.51 [d]	4.17 [d]	3.78 [c]
7	*P. eleagrifolia*	10.67 [a]	3.57 [e]	3.48 [c]
8	*P. korshinskyi*	9.78 [b]	5.43 [b]	2.11 [e]
9	*P. lindlezi*	10.00 [b]	6.67 [a]	5.37 [a]
10	*P.* ×*malifolia*	11.14 [a]	6.20 [a]	4.74 [b]
11	*P. persica*	8.98 [c]	4.83 [c]	5.43 [a]
12	*P. ussuriensis*	5.18 [f]	3.52 [e]	3.41 [c]
13	*P. variolosa*	7.83 [d]	5.21 [b]	3.57 [c]
14	×*Pyronia veitkii*	6.17 [e]	5.83 [a]	4.67 [b]
15	×*Sorbopyrus*	4.78 [g]	1.92 [g]	1.73 [f]

[1] Different letters between species in each column indicate statistically significant differences for the investigated traits at a significance level of $p < 0.05$ (Duncan's test).

Table 5. The main characteristics of the flowers of 15 species of *Pyrus* [1].

No	Species	Corolla Diameter (mm)	Petal Length (mm)	Petal Width (mm)
1	*P. betulaefolia*	39.58 [b]	15.34 [a,b]	12.68 [b,c]
2	*P. canescens*	34.34 [c]	14.20 [c]	11.00 [c]
3	*P. caucasica*	38.00 [b]	16.34 [a]	12.34 [c]
4	*P. communis*	34.68 [c]	11.67 [d]	11.68 [c]
5	*P. cordata*	30.10 [d]	13.67 [c]	12.20 [c]
6	*P. drovara*	38.00 [b]	16.34 [a]	12.10 [c]
7	*P. eleagrifolia*	34.34 [c]	14.68 [b]	11.68 [c]
8	*P. korshinskyi*	28.33 [d]	11.66 [d]	14.20 [b]
9	*P. lindlezi*	54.20 [a]	17.00 [a]	17.00 [a]
10	*P.* ×*malifolia*	34.68 [c]	15.00 [b]	12.10 [c]
11	*P. persica*	31.34 [c,d]	14.68 [b]	12.20 [c]
12	*P. ussuriensis*	34.68 [c]	14.66 [b]	11.68 [c]
13	*P. variolosa*	35.34 [c]	13.68 [c]	12.00 [c]
14	×*Pyronia veitkii*	30.34 [d]	13.00 [c,d]	8.33 [d]
15	×*Sorbopyrus*	35.32 [c]	11.65 [d]	11.00 [c]

[1] Different letters between species in each column indicate statistically significant differences for the investigated traits at a significance level of $p < 0.05$ (Duncan's test).

Fruit attributes varied greatly among the 15 *Pyrus* species, starting with the length of the peduncle (Table 6). Thus, the length of the peduncle was between 4.10 and 1.43, the lowest values being recorded in *P. drovara* and the highest values in *P. korshinskyi*. The length of the fruits ranged between 1.73 cm (*P. korshinskyi*) and 6.33 cm (*P. variolosa*), and the diameter of the fruits ranged between 0.55 cm (*P.* ×*malifolia*) and 5.53 (*P. caucasica*). The weight of the fruits varied greatly, with the lowest weight and extremely small fruits being seen in *P. korshinskyi* (2.70 g). The highest average fruit weight was recorded for *P. caucasica* (91.00 g), followed by *P. variolosa* (78.70 g).

Most of the statistical correlations recorded between the phenotypic characteristics analyzed were generally predictable (Table 7). The closest links were identified between the main particularities of fruit size, in particular, fruit length, diameter, and weight (all positive, at the significance levels of 1% or 0.1%). Instead, the three attributes of fruit size were negatively correlated with fruit firmness.

Table 6. The main characteristics of the fruits of 15 species of *Pyrus* [1].

No.	Species	Peduncle Length (cm)	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Weight (g)	Fruit Firmness (kgf/cm^2)
1	*P. betulaefolia*	3.20 ^{b}	1.93 ^{g}	2.43 ^{d}	6.23 ^{i}	96.0 ^{a}
2	*P. canescens*	1.97 $^{d.e}$	2.68 ^{e}	0.75 ^{f}	11.94 ^{h}	90.0 ^{b}
3	*P. caucasica*	2.37 ^{d}	5.20 ^{b}	5.53 ^{a}	91.00 ^{a}	81.7 ^{d}
4	*P. communis*	1.87 ^{e}	3.33 ^{d}	0.87 ^{f}	11.99 ^{h}	83.7 ^{d}
5	*P. cordata*	2.23 ^{d}	2.77 ^{e}	3.57 ^{b}	19.13 ^{f}	94.0 ^{a}
6	*P. drovara*	1.43 ^{f}	4.20 ^{c}	3.53 ^{b}	28.13 ^{e}	83.0 ^{d}
7	*P. eleagrifolia*	1.53 ^{f}	2.13 ^{g}	2.47 ^{d}	7.30 ^{i}	93.3 ^{a}
8	*P. korshinskyi*	4.10 ^{a}	1.73 ^{h}	1.57 ^{e}	2.70 ^{j}	94.7 ^{a}
9	*P. lindlezi*	2.30 ^{d}	3.47 ^{d}	3.13 ^{b}	49.23 ^{d}	84.1 ^{d}
10	*P. ×malifolia*	2.23 ^{d}	3.37 ^{d}	0.55 ^{f}	16.23 ^{g}	94.0 ^{a}
11	*P. persica*	2.23 ^{d}	2.63 ^{f}	3.37 ^{b}	58.23 ^{c}	82.3 ^{d}
12	*P. ussuriensis*	2.20 ^{d}	2.88 ^{e}	0.73 ^{f}	9.56 ^{h}	87.7 ^{c}
13	*P. variolosa*	2.83 ^{c}	6.33 ^{a}	5.23 ^{a}	78.70 ^{b}	80.2 ^{d}
14	*×Pyronia veitkii*	3.23 ^{b}	2.73 ^{e}	2.63 ^{c}	8.48 ^{i}	95.0 ^{a}
15	*×Sorbopyrus*	2.10 ^{d}	3.10 ^{d,e}	2.77 ^{c}	14.97 ^{g}	86.3 ^{c}

[1] Different letters between species in each column indicate statistically significant differences for the investigated trait at a significance level of $p < 0.05$ (Duncan's test).

Table 7. Phenotypic correlations between the pairs of traits, calculated from the mean values of each species of *Pyrus* [1].

	Trunk Height	Trunk Diameter	Crown Diameter	Branches/Tree	Leaf Length	Leaf Width	Petiole Length	Corolla Diameter	Petal Length	Petal Width	Peduncle Length	Fruit Length	Fruit Diameter	Fruit Weight	Fruit Firmness
Tree height	–0.25	0.73	0.38	0.37	0.49	0.55	0.59	0.15	0.46	0.30	0.37	0.18	0.24	0.47	–0.02
Trunk height		–0.22	–0.18	0.19	–0.28	0.06	0.44	0.60	0.40	0.17	–0.36	0.06	–0.15	–0.05	–0.21
Trunk diameter			0.39	0.12	0.67	0.52	0.38	0.27	0.20	0.41	0.16	0.30	0.26	0.47	–0.21
Crown diameter				0.26	0.33	0.11	0.17	0.35	0.30	0.12	0.15	–0.11	0.03	0.04	0.20
Branches/tree					–0.25	–0.03	0.35	–0.02	0.26	–0.29	0.03	0.14	–0.03	0.20	–0.08
Leaf length						0.60	0.30	0.14	0.23	0.44	–0.02	–0.02	–0.07	0.11	0.02
Leaf width							0.62	0.24	0.12	0.36	0.36	0.08	–0.06	0.11	0.02
Petiole length								0.41	0.56	0.15	–0.12	0.04	0.01	0.25	–0.12
Corolla diameter									0.65	0.66	–0.23	0.26	0.19	0.31	–0.35
Petal length										0.41	–0.33	0.26	0.32	0.44	–0.23
Petal width											0.10	0.02	0.11	0.25	–0.20
Peduncle length												–0.19	0.03	–0.07	0.40
Fruit length													0.65	0.80	–0.74
Fruit diameter														0.81	–0.50
Fruit weight															–0.76

[1] Significant correlations at the level of $p <$ 0.05; 0.01; 0.001 (two-tailed).

Tree height was positively correlated with trunk diameter but also with leaf width and petiole length (the last two links being less predictable). Less anticipated were the positive correlations identified between trunk diameter and leaf length and leaf width or those between petiole length and petal length.

Principal component analysis (PCA) revealed a close relationship between several species, i.e., *P. communis*, *P. cordata*, *P. canescens*, and *P. ussuriensis* (Figure 2). They were placed at close distances from each other, but in a quadrant (Q3) located in opposition to *P. ×malifolia*, *P. persica*, and, especially, *P. lindlezi* (Q1). In the two opposite quadrants, the most distant position was between *P. lindlezi* and *×Sorbopyrus*. A large distance reflecting the different phenotypic characteristics between the two species was recorded between *P. korshinskyi* and *P. caucasica*, also located in opposite quadrants (Q2 and Q4). *P. eleagrifolia* and *P. betulaefolia* appear to be quite close phenotypically, both placed in quadrant Q4.

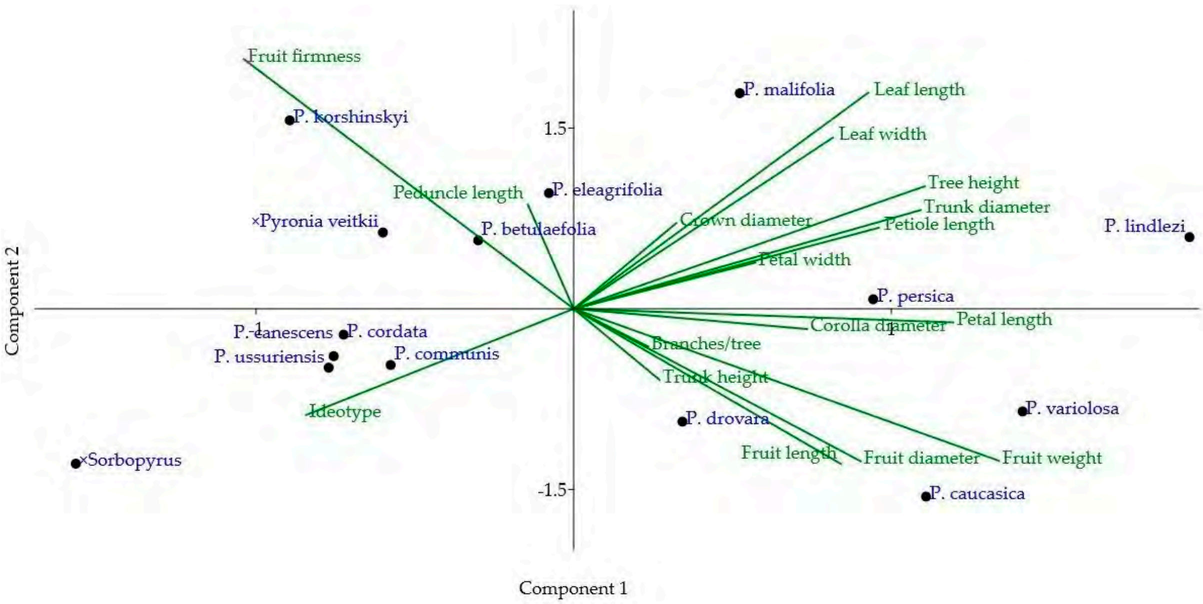

Figure 2. Principal component analysis (PCA) of the 15 *Pyrus* species and 17 phenotypic characteristics of the trees, leaves, flowers, and fruits; mean values of all traits were scored between 0 and 1.

Most phenotypic traits were arranged in quadrants Q1 and Q2. Some tree (e.g., tree height, trunk diameter, or crown diameter) or leaf (leaf length or leaf width) characteristics placed in Q1 are in opposition to the architectural ideotype of trees, the only characteristic placed in Q3. Fruit firmness, located in Q4, is in opposition and far from fruit size characteristics (fruit length, fruit diameter, and fruit weight), placed in Q2.

Multivariate analysis, using a hierarchical clustering-paired group UPGMA (unweighted pair group method with arithmetic mean) with a Euclidean similarity index, was carried out with mean values of all phenotypic parameters, scored between 0 and 1, highlighting interesting relationships regarding both the interactions between *Pyrus* species (column dendrogram) and the approach or distance of the characteristics of trees, leaves, flowers, and fruits (row dendrogram) and their heatmaps (Figure 3).

In the column dendrogram of species, two main branches can be differentiated, namely the one on the left, containing a single species (*P. lindlezi*), clearly separated from the others, and the cluster on the right, which contains fourteen species divided into two subclusters. The group on the right includes a branch with two subbranches, one represented by *P. caucasica* and the other by the pair formed of *P. persica* and *P. variolosa*. On the opposite side, the subcluster on the left results in more subordinate relationships, with the exception of one species (*P. korshinskyi*), which is categorically different from the others. On the remaining tree, another clear differentiation compared to most of the other species is represented by ×*Sorbopyrus*. The greatest phenotypic proximity is recorded between *P. canescens* and *P. communis*, both located in a common subcluster with *P. ussuriensis*. In pairs, but located in different subclusters, ×*Pyronia veitkii* and *P. betulaefolia* and *P. eleagrifolia* and *P.* ×*malifolia*, as well as *P. cordata* and *P. drovara* are located.

In the row dendrogram of the phenotypic traits, there are two subclusters, but one is represented only by one fruit attribute, clearly differentiated from the rest: fruit firmness. The other subcluster includes all other traits, grouped into two subclusters, each with different branches. The closest relationships are highlighted by the dendrogram between the following pairs of characteristics: fruit weight and fruit length; petal width and corolla diameter; leaf length and trunk diameter; and petiole length and leaf width. Tree ideotype (habitus) forms a pair with branches/tree arranged in a common subcluster under whose pruning line the majority of the nodes are found that correspond to the branching positions of the other traits grouped in pairs.

Both dendrograms highlight the clusters and cells and their corresponding links in the heatmap, particularly prominent being the bright red cells at the intersection of the vertical alignment represented by the *Pyrus* species with the horizontal alignment represented by the analyzed phenotypic traits.

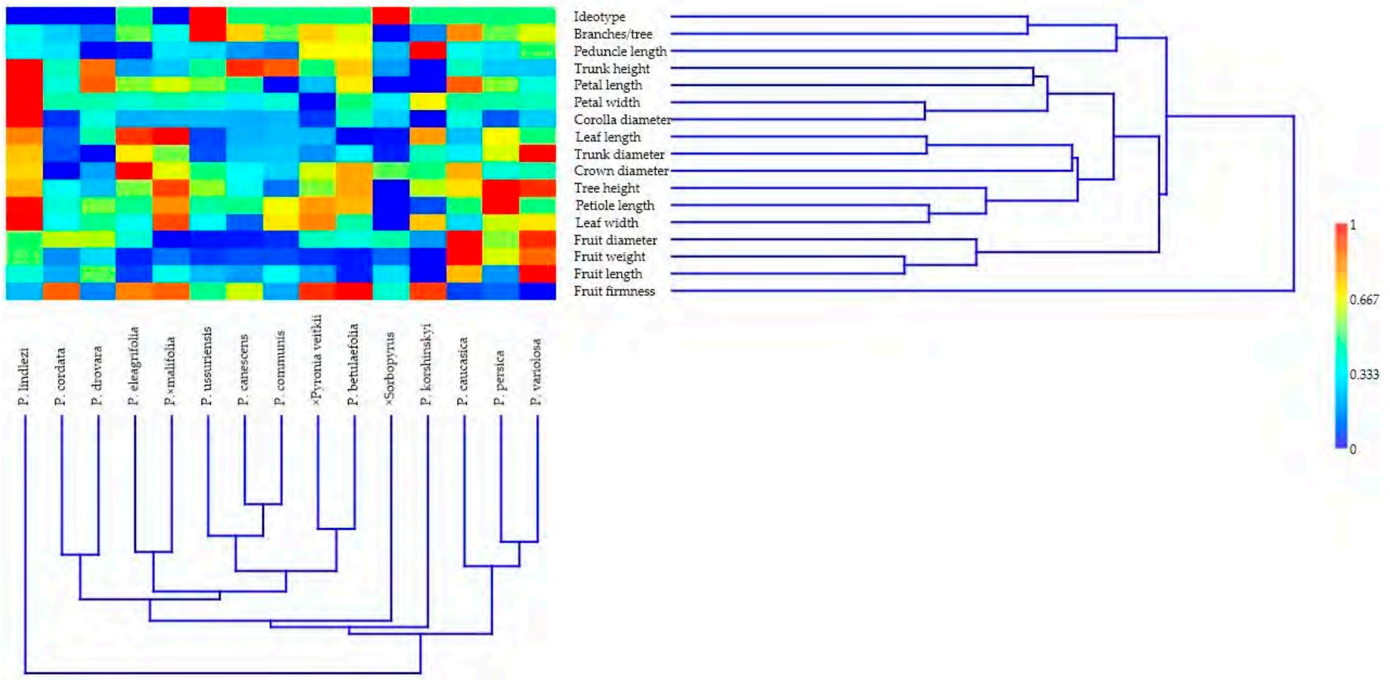

Figure 3. Hierarchical clustering—as a paired group UPGMA (unweighted pair group method with arithmetic mean)—similarity index (Euclidean) of the 15 *Pyrus* species and 17 phenotypic characteristics of the trees, leaves, flowers, and fruits; mean values of all traits were scored between 0 and 1.

During the two years of studies, the main diseases examined in the field under natural circumstances of occurrence and spread were *E. amylovora*, *V. pyrina* Aderh., *S. pyricola* Sacc., *Monilinia fructigena* Honey, and *Gymnosporangium sabinae* (Dicks.) Oerst. The most common pests were *Psylla* spp., *Phyllonorycter corylifoliella* Hubner, *Phyllonorycter blancardella* Fabricius, *Stigmella malella* Stainton, *Leucoptera scitella* Zeller, *Neurotoma flaviventris* Retzius, *Aphis pomi* De Geer, and *Eriophyes pyri* Pgst. (Figure 4). A greater general sensitivity to diseases was observed in *P. caucasica* and *P. drovara*, and the greatest sensitivity to pest attack was seen in ×*Sorbopyrus*. On the other hand, *P.* ×*malifolia*, *P. korshinskyi*, and ×*Pyronia veitkii* were recorded as having the lowest cumulative sensitivity scores for diseases, and *P. drovara*, *P. ussuriensis*, and *P. betulaefolia* had the lowest for pests.

Twenty species of *Pyrus* were analyzed using RAPD primers, and the results showed reliable and reproducible polymorphic bands (Figure 5). The estimation of genetic relatedness among the 20 *Pyrus* species using random amplified polymorphic DNA (RAPD) polymorphism is shown in the dendrogram in Figure 6.

The two main clusters were differentiated: each divided into two further subclusters with different branches, up to the level of the closest pairs of species. The closest proximity appears between ×*Pyronia veitkii* and ×*Sorbopyrus*, which are in the same subcluster with *P. ussuriensis* but at a pruning line located above all the subclusters where the rest of the similar branching nodes of subclusters fall. The species *P. eleagrifolia* and *P. pollveria*, located in the other main cluster, also appear genetically very close. Next to their pair is *P. eleagrifolia* × *Curé* in a common subcluster. In the same subcluster, *P. cordata* and *P. lindlezi* are found in pairs, as well as *P. communis* and *P. betulaefolia* (the latter is more distant in terms of genetic closeness). Other relatively closely related pairs are *P. caucasica* and *P. drovara* as well as *P. longipes* and *P. nivalis*. Additionally, in pairs, but at a greater genetic distance, are *P. variolosa* and *P. pyraster* as well as *P.* ×*malifolia* and *P. luxemburgiana*.

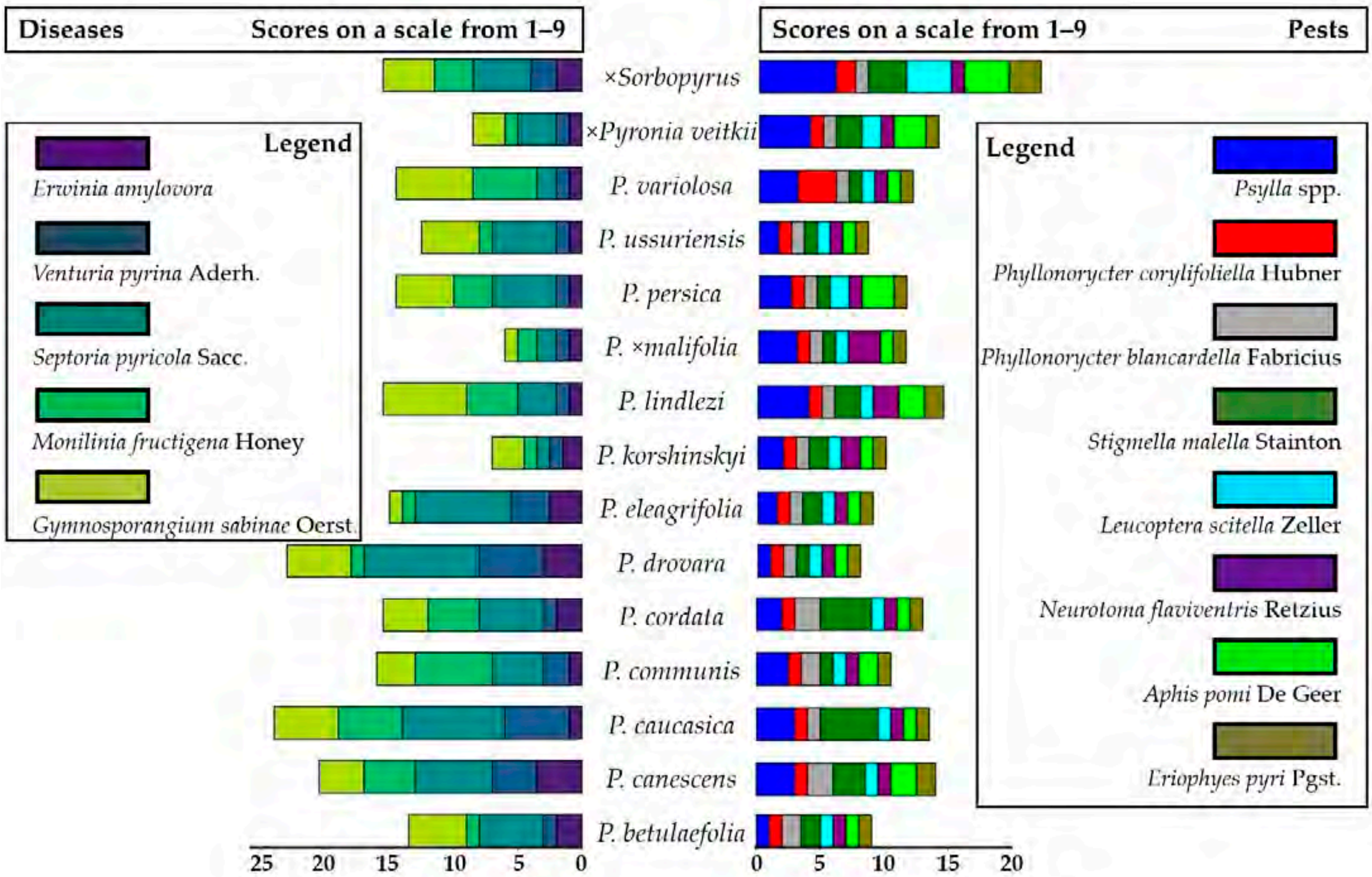

Figure 4. Evaluation of the main diseases and pests that were identified in the trial with 15 species of *Pyrus* over the two years of the experiment (rating scale with scores from 1 to 9; 1—no attack; 9—extremely strong attack). On the horizontal bars, each segment of a specific color represents the score on the 1–9 scale given to a specific disease or pest.

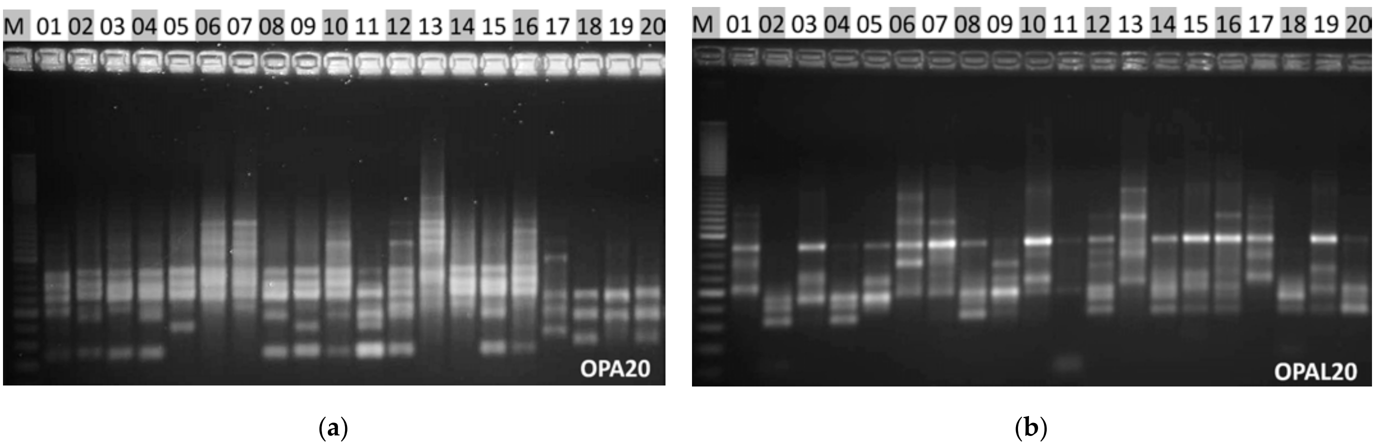

Figure 5. Example of RAPD amplification and polymorphism revealed using the arbitrary primers OPA 20 (**a**) and OPAL 20 (**b**), through the DNA of 20 species of *Pyrus* (in Figure 6). On the left side, the lane ladder was used as a size marker (M).

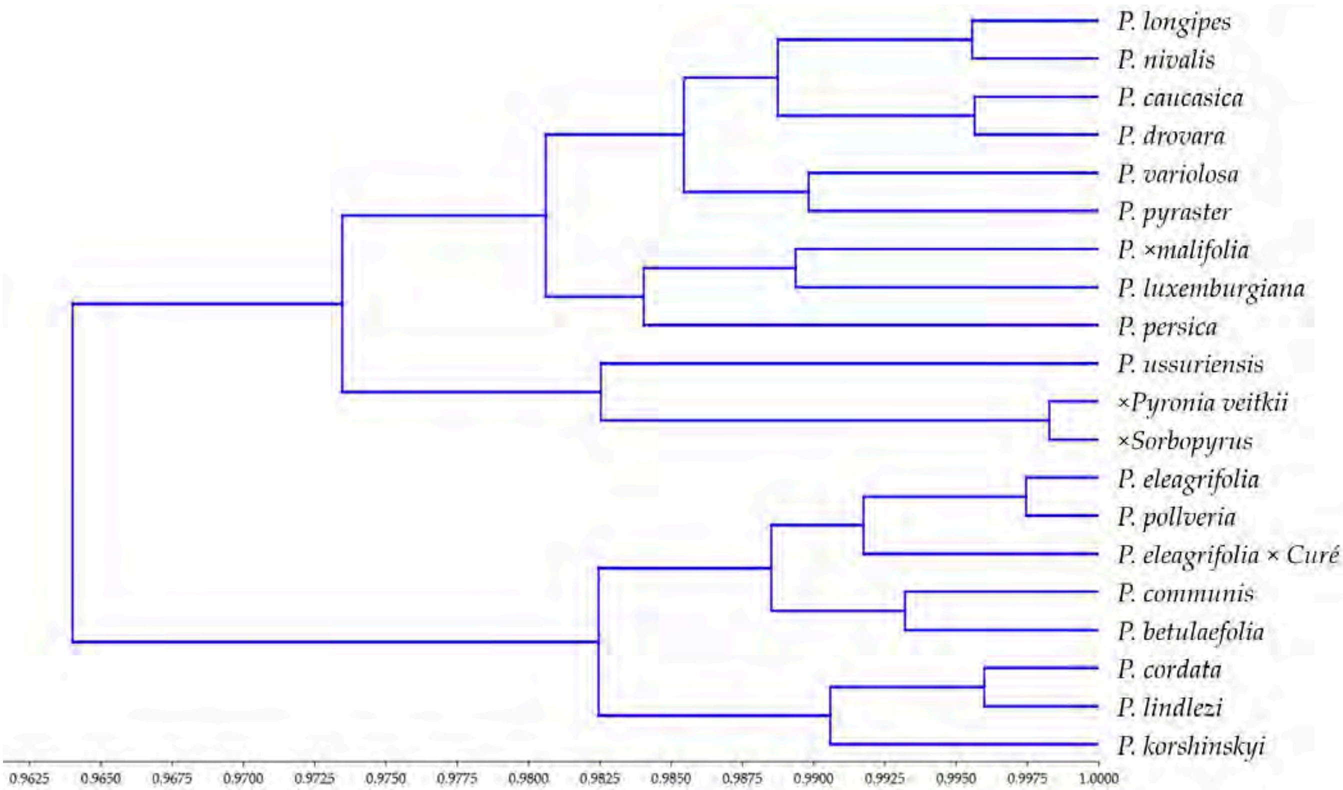

Figure 6. Dendrogram of genetic relatedness among the *Pyrus* species using random amplified polymorphic DNA (RAPD) polymorphism.

4. Discussion

Wild *Pyrus* species are particularly important biological materials in terms of forestry and ecological and ornamental resources, and they provide invaluable genetic resources for cultivated pears and pear breeding, as well as rootstocks [58]. Cold resistance, drought resistance, disease resistance, barren tolerance, saline–alkaline tolerance, and high adaptability characterize the trees of spontaneous forms. The diversity in the habitus and architectural growth of trees are of interest in the field of landscaping, as their flowers, leaves, and fruits are rather attractive and can have a specific decorative value [59,60]. The pear fruit is high in fruit acids, vitamins, carbohydrates, and minerals that are beneficial to human health. The fruits can be fermented into wine or used in the making of different products, such as dried and preserved pears. They have significant therapeutic value and can be utilized to bring down a fever, hydrate the lungs, soothe coughing, and eliminate phlegm. Pear timber is highly valued for its numerous uses, and pear trees and fruit contribute to ecological diversity, and sustain biodiversity and a wide range of life forms (birds, rodents, insects, microorganisms, etc.). *Pyrus* species are a significant wild resource with high exploitation potential due to their beneficial characteristics to both humans and the environment.

Our results revealed a wide heterogeneity in the majority of the studied characteristics in the *Pyrus* species. Differences across species were obvious in terms of the elements of growth and vigor of the trees. Some species accumulate more biomass than others; therefore, the characteristics and quality of pear wood for breeding purposes should receive more attention. The pear is considered among the most commonly used dual-purpose fruit trees, along with walnut, cherry, and apple, which produce fruits but also litter, fuel wood, and timber [61]. Pear is regarded as a high-value tree in agroforestry systems, planted in cropland, pasture, or riparian buffer strips [62], where the word "high value" refers to the cultivation of fruit trees, such as apple, cherry, olive, orange, and various nuts [63]. In France, both the *P. communis* and *Sorbus* species are widely utilized in silvopastoral and, to a lesser extent, silvoarable systems [63], and pear trees showed remarkable early growth [61]. Pear tree growth is important in agroforestry plots because it allows for shorter rotations, which has a positive psychological influence on landlords and farmers. Rapid early growth can also aid in the pruning of a tree to obtain desirable log shapes [61]. In orchards, pear pruning wood waste is frequently reincorporated into the soil to mitigate nutrient losses caused by fruit harvesting [64]. In addition, given their numerous positive ecological and human-related beneficial properties, all pear species are considered "noble hardwood species" [65].

Based on our results, *P. lindlezi*, *P. betulaefolia*, and *P. canescens* (recorded as having high tree vigor) could probably represent a valuable addition to wood production and to the future breeding of pear in order to improve its capacity to accumulate woody mass. These findings are presumably the outcome of biological versatility, adaptation, or a favorable responses within the ecological conditions under assessment. Obviously, *P. communis* ssp. *Pyraster* can be added to these, being well known for its spontaneous forms that are well-adapted to the study area's ecological conditions, as well as being one of the oldest cultivated species. Its use for fruits and for other purposes, such as firewood, construction, furniture, and tools, can be traced back to the Neolithic period in the Balkans [66–68]. In future research, the intrinsic quality of the wood, the fast growth rate of the trees, the suitability of the trees and wood for special purposes, etc., should also be analyzed and monitored.

Significant variations in the basic characteristics of leaves and flowers were observed among *Pyrus* species. Aside from their crucial roles for the plants themselves, leaves and flowers are also important for species identification. This aspect is important because the information regarding the number of existing *Pyrus* species is quite contradictory compared to other species. Referencing nine different bibliographic sources, Vidaković et al. [65] affirm that the estimated number of pear species ranges from between 20 to 80. Often there is confusion regaring their identification due to different names used for the same species (synonyms), erroneous classifications, or interspecific hybrids that phenotypically express the intermediate characteristics of their parents, complicating their identification and classification. For example, it is estimated that there are eight distinct European pear species, counting interspecific hybrid taxa that developed spontaneously [19]. However, for the same species, there can be multiple different names. For the European wild pear (*P. communis* subsp. *pyraster*) alone, Terpó [20] specifies six different subspecies, varieties, and forms, which are currently considered synonyms [69]. The broad phenotypic variability of European wild pear leaves is also reflected by the different values reported by different authors for leaf length (between 2–8 cm), leaf width (between 1.5–5 cm), and petiole length (between 1.5–7 cm) [69]. Different botanical synonyms for pear species, varieties, and forms can be confusing, but narrow-shaped leaves are thought to be the result of the mesomorphic pear species' adaption to xerophytic environments [65].

Among the analyzed species, flowers of the largest size were found in *P. lindlezi*, and *P. caucasica*, *P. variolosa*, and *P. persica* had the largest fruits. Other species such as *P. betulaefolia*, *P. drovara*, and *P. caucasica* have been recorded as having large petals and corollas, also resulting in their decorative potential or as pollinators for pear orchard cultivars. Because *P. communis* cultivars are self-incompatible, interfertile cultivars or appropriate spontaneous species as pollinators are required in orchards [70]. Different species have been recommended as potential pollinators, such as *P. amygdaliformis*, *P. longipes*, *P. nivalis*, *P. salicifolia*, *P. betulaefolia*, and *P. syriaca*. In addition, a *P. betulaefolia* selection was recommended as a pollinator for major cultivars, such as Williams, Conférence, and Doyenné du Comice, due to their prolific flowering and regular and extended blooming season [71]. A mixture of two or three different pollinizers (i.e., ornamental *Pyrus* accessions) should be considered for commercial orchards in order to assure the maximum overlap of the flowering periods of the principal cultivars each year [72].

Some selections of wild species of pears have a recognized ornamental value, because in addition to the rapid growth of the trees, the varied range of shapes and sizes, rusticity, low demands on the soil, or ability to thrive in different ecological conditions, they have attractive foliage, with glossy green leaves, flowers, and fruits that are particularly decorative [73,74]. These trees lend themselves very well to landscaping, and they offer an abundance of white flowers in the spring and spectacular colors from spring to late autumn. The trees have ideal characteristics for screening and providing the desired shade in hot summers. Among the known cultivars of ornamental pears, there are some with a habitus suitable for placement in narrow spaces, such as between buildings and apartment blocks, accessways, and alleys [74]. Apart from certain well-known ornamental cultivars from *P. calleryana*, valuable ornamental forms have also been obtained from other species [19], including from those included in the present study, e.g., *P. ussuriensis*, *P. nivalis*, etc. Other characteristics of interest from the other species studied can be identified and used for various purposes. However, the invasive potential of some species or their hybrids (interspecific or even intraspecific) introduced into new areas must be monitored through future research. *P. calleryana*, a self-incompatible Chinese species that is widely planted as an ornamental tree in the United States, is now escaping cultivation and occurring in disturbed environments, where it has the potential to establish dense thickets and migrate into natural and managed lands where these trees could cause complex and varied harmful ecological effects [75,76].

In Romania, the native wild pear (*P. pyraster*) has been recognized since ancient times as a tree that is not demanding in regard to climate and soil, is heat-loving, prefers sunny places, and is resistant to frost and drought. These trees were used for valuable wood or to protect animals from the heat of summer and the cold of spring or autumn by providing shade or acting as shelters. Even when the fruits were small and astringent, they were widely used in domestic consumption or processing to obtain a traditional pear distillate ('palinka' or 'tzuika') [77]. The pear, like the plum and the apple, has long cultural and historical traditions in southeastern European countries, with many cultivars created over the years and throughout the most recent period of modern breeding. Despite the fact that pear breeding in Romania produced good edible cultivars [78–80], ornamental pears have yet to be created in this region.

Similarly to cultivated pears, the wild species of *Pyrus* are attacked by a large number of pathogenic agents and harmful insects. No major issues occurred during the years in which the research was conducted, but previous attacks of fire blight and psyllids resulted in significant losses, including the disappearance of some genotypes from the germplasm collection [50,81,82]. New research can reveal the sources of useful genes in different wild accessions, which could be used to improve edible or ornamental pears. Resistance to diseases, especially to *E. amylovora*, or to pests, especially to *Psylla* sp., remains of great interest. In addition, some disadvantages of the ornamental pear (sensitivity to certain environmental factors, better suitability for specific uses, fruits that attract too many insects or rodents, fruits that require frequent cleaning, etc.) could be taken into account by using appropriate genetic resources in future breeding programs. Our results highlight the differences between *Pyrus* genotypes, and the large variability of responses to the dominant diseases and pests provides the opportunity to choose useful parental forms for new breeding programs.

The use of correlations and multivariate analyses to the explore specific features of interest is extremely useful and provides particularly interesting and relevant information for pear breeding. According to Zarei et al. [59], accessions from the same geographical area have higher phenotypic closeness than those from different regions. In our investigation, we discovered a high level of morphological variability, not just across species from various geographic origins but also among accessions from the same areas. The cluster analysis separated the species into two major groupings, each with a mix of species origins.

The morphological diversity was also highlighted by the molecular aspects, using RAPD markers. Random amplified polymorphic DNA (RAPD) has been frequently used in fruit tree genetic investigations, including into pear, due to their ease of application, low requirement for genomic DNA, and low cost, as compared to other molecular marker techniques [19,83]. RAPD markers have been successfully utilized to assess genetic variation and relationships among various pear accessions (species or cultivars), and genotype grouping by RAPD largely corresponded with morphological classification [51,52,84]. Monte-Corvo et al. [53] discovered that AFLP markers were five times more efficient than RAPD in detecting polymorphism per response. Although some minor discrepancies between the dendrograms produced by AFLP and RAPD were identified, both approaches provided equivalent results [53]. Our dendrograms did not reveal the correspondence of the phenotypic relationships with the molecular aspects but confirmed the difficulties in assessing the phylogenetic relationships and categorizing the *Pyrus* genotypes referred to by Rehder [46], Rubtsov [47], Challice and Westwood [25], and others [85–89]. Data interpretation becomes more difficult if some synonyms (homonyms, duplications, errors, or controversial classifications) are used: for example, *P. variolosa* is synonymous with *P. pashia* (the wild Himalayan pear); *P. longipes* with *P. cordata*; *P. pollveria* with ×*Sorbopyrus auricularis* (a hybrid *Sorbus aria* × *P. communis*), etc. In addition, ×*Sorbopyrus* is a hybrid of *Sorbus* and *Pyrus*, and ×*Pyronia veitchii* is a hybrid of *Pyrus* and *Cydonia* [90]. Finally, citing Robertson et al. [91] and Browicz [92], Uğurlu Aydın and Dönmez [93] demonstrate that the genus *Pyrus* L. contains a number of species between 41 and 73, and *P. communis* is closer to *P. caucasica* and *P. nivalis*, rather than *P. pyraster* (interpreting the results of Zheng et al. [94]). However, it is accepted that for future pear-breeding projects, species or genotypes from differing geographical areas with high levels of genetic variation can serve as useful genetic resources [88,95,96]. Similar to apples, this could mitigate the threat posed to pear cultivars by a reduction in genetic diversity [40,97–99]. Regardless of the techniques employed to assess genetic diversity, new research indicates that the genotypes of pears are gravely threatened by overuse, habitat loss, and environmental changes, all of which will have a negative impact on breeding programs in the future. Thus, it is necessary to conserve pear cultivars and their wild relatives both within and outside their natural habitat (in situ and ex situ, respectively) [42,100,101].

Unfortunately, research organizations have been experiencing increasing financial difficulties as well as losses of land, biological material, and human resources in recent decades [40,102]. Urgent measures are needed to prevent the extinction of pears by collecting, conserving, evaluating, and propagating local or old cultivars, as well as their wild relatives [33,103–105]. A lack of financing and support for the collection and conservation of the genetic resources of cultivated species can have severe consequences for ensuring humankind's food supply and sustainable agriculture in the future. In the future, gene pools could be of immeasurable importance, providing the biological potential for new cultivars. In an academic city such as Cluj-Napoca, the pear germplasm is also culturally and educationally significant, and it is an excellent source of learning and knowledge for university students and school children. The comparison of wild species of pears with valued cultivars, the fruits of which can be found in markets, is the best example for a young person to learn how cultivated species evolve from wild ones, namely through the work and effort of anonymous people from ancient times. For young and developing minds, the image of trees and fruits of wild species as well as cultivars, such as Williams (Bartlett), Abate Fetel, Conference, Beurré Bosc, etc., as well as their history told in the field by people dedicated to science, remains memorable.

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Appendix A

(**a**) (**b**) (**c**) (**d**)

Figure A1. Identification of the symptoms, the assessment of occurrence, and the spread of different pathogens: (**a**,**b**) are the typical symptoms of fire blight (*E. amylovora*) on the shoots, specifically 'shepherd's crook'; (**c**) pear scab (*Venturia pyrina*) and (**d**) septoria (*Septoria pyricola*) on the leaves.

Figure A2. Tree, foliage, and fruit peculiarities in various *Pyrus* species in the collection: (**a**) *P. cordata*; (**b**) *P. eleagrifolia*; (**c**) leaf detail and scab symptoms; and (**d**) the general appearance of the three trees belonging to *P. variolosa*.

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