PROTECTION OF GENETIC RESOURCES OF POMOLOGICAL PLANTS AND SELECTION OF GENITORS WITH TRAITS VALUABLE FOR SUSTAINABLE FRUIT PRODUCTION

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MOLECULAR STUDIES ON THE VARIABILITY OF POLISH SEMI-WILD PEARS (Pyrus) USING AFLP

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ABSTRACT

In Poland, wild and semi-wild pear trees (*Pyrus pyraster* and *P. ×amphigenea*) and naturalized cultivated pear trees (*P. communis*) are an important element of the rural landscape. They are usually regarded as difficult to identify and taxonomically classify. Using AFLP to study the relationships among semi-wild pears in northeastern Poland revealed that most of the trees in this region belong to *Pyrus ×amphigenea*. Individual trees had various traits typical for *P. pyraster*, usually in combination with traits typical for *P. communis*. The hypothesis that the proportion of hybrid genes increases as one approaches regions of intensive fruit culture was not confirmed.

Key words: AFLP, genetic variability, *Pyrus ×amphigenea*, *Pyrus communis*, *Pyrus pyraster*

INTRODUCTION

Semi-wild pear trees grow along the edge of agricultural fields in Poland and other Central European countries. They are a beautiful, but vanishing element of the rural landscape. Taxonomic studies of semi-wild pears may also be an intriguing challenge for two reasons. First, the only pear species occurring naturally in Poland, Pyrus pyraster (L.)Burgsd., is very difficult to taxonomically define because of inadequate and conflicting early descriptions. Second, for many centuries P. pyraster has been growing in proximity to cultivated pears, representing dozens of cultivars formed by the hybridization of several heterogenic Pyrus species lumped under the name Pyrus communis L. (Kutzelnigg, 1995). This means that many individual plants identified as P. pyraster in fact represent various stages of hybridization between P. pyraster and *P. communis*. Precise identification requires very careful examination. Considering the long history of hybridization of the *Pyrus* taxa, the proportion of hybrid genes should increase as one moves from areas of extensive fruit production, such as northeastern Poland, toward areas of intensive fruit production, such as central Poland and the area around Warsaw.

The taxonomy of semi-wild pears is not at all simple. Polymorphic hybrids of *P. pyraster* and *P. communis* were first studied by Domin (1946), who included them in *Pyrus* ×amphigenea Domin ex Dostálek (Dostálek, 1989). Later, Dostálek, Hofmann (1993) and Wagner (1996) attempted to define the characteristic morphological features, especially leaf and fruit features, by which these taxa could be definitively distinguished from one another. The most important characters, which never should be considered separately, are: thin branchlets and thorns that persist beyond the juvenile phase of the tree; small leaves with round or oval blades not longer than 6 cm set on a petiole not longer than 6 cm; leaves, which if hairy, are never densely hairy or pubescent; small fruits without a visible reddish blush, less than 3 cm in diameter, and on a short petiole 2 mm in diameter and up to 4 cm long.

Marquardt (1999) states that *P. pyraster* is not a true taxon, but rather the shadowy idea of a taxon toward which students of the subject, using various subjective measures, have been asymptotically approaching ever since Burgsdorf's times. If such is the case, then the traditional morphological methods for analyzing variability in pears are no longer useful, and more powerful tools are needed. These new tools include molecular techniques and markers, which have proven useful in the identification of the cultivars and hybrids of various crops, including fruit trees (Wunsch and Hormaza, 2002). Molecular techniques have been used to elucidate the genetic relationships among *Pyrus* species and cultivars in Portugal, Asia and Europe (Monte-Corvo et al., 2000; Teng et al., 2002; Kimura et al., 2003).

The aims of this study were: 1) To assess the genetic variability of semi-wild pears in northeastern Poland; and 2) To check whether the proportion of hybrid genes does in fact increase toward areas of intensive fruit production.

MATERIAL AND METHODS

Tree sampling – In 2001 and 2002, twenty-one trees were systematically sampled to provide material for DNA extraction. The trees were spaced at twenty to thirty kilometer intervals along a transect from Warsaw eastwards toward Białowieża. The trees stood along the edges of fields or forests, or beside local and field roads. One tree stood in a cemetery (Tab. 1). The tree closest to Warsaw was in Sulejówek, and the tree closest to Białowieża was in Policzna. Every tree was sampled two or three times in different months so that there would be enough material to identify the tree down to the species level. Herbarium vouchers were deposited in the Herbarium of the Warsaw University Botanic Garden. In addition, eight samples of cultivated pear varieties were obtained from the Institute of Floriculture and Horticulture in Skierniewice, central Poland.

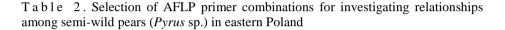
Table 1. Geographic position of the sampled *Pyrus* trees

Accession number	Latitude	Longitude	Short description of location
1	N 52°35.3'	E 23°26.4'	agricultural field
2	N 52°33.6'	E 23°17.6'	by the road
3	N 52°33.6'	E 23°17.4'	by the road
4	N 52°32.3'	E 23°10.7'	agricultural field
5	N 52°30.7'	E 23°07.6'	by the road
6	N 52°27.4'	E 23°01.9'	by the field road
7	N 52°27.4'	E 23°02.0'	by the field road
8	N 52°26.3'	E 22°54.6'	Agricultural field
9	N 52°23.5'	E 22°51.2'	by the road
10	N 52°23.8'	E 22°45.2	Agricultural field
11	N 52°24.3'	E 22°37.9'	Agricultural field
12	N 52°23.2'	E 22°25.9'	by the road
13	N 52°23.0'	E 22°24.9'	by the road
14	N 52°24.0'	E 22°15.1'	Cemetery
15	N 52°24.2'	E 22°06.1'	by the road
16	N 52°24.2'	E 22°03.5'	Agricultural field boarder
17	N 52°22.4'	E 21°56.6'	by the field road
18	N 52°22.0'	E 21°47.0'	by the field road
19	N 52°19.0'	E 21°36.9'	forest fringe
20	N 52°19.0'	E 21°36.9'	forest fringe
21	N 52°15.1'	E 21°16.6'	by the road

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DNA isolation and AFLP – Total DNA was extracted from herbarium specimens using the CTAB protocol (Murray and Thompson, 1980; Rogers and Bendich, 1985; Doyle and Doyle, 1990). Approximately 1 g of dry plant material was used for each extraction.

A standard kit was used for AFLP (Applied Biosystems 1997). Analysis of genetic differentiation was carried out in accordance with the protocol provided by the manufacturer. 64 primer pairs were applied, from which the 12 most polymorphic primers were chosen (Tab. 2). The data on variation in fragment length were generated by an automated AbiPrism sequencer and analyzed using Statistica PL. Various clustering methods and metrics were used to calculate distance trees, including UPGMA, the Ward method, and the Manhattan distance.



RESULTS AND DISCUSSION

We expected that the semi-wild pear trees would fall into two or three *Pyrus* taxa. However, most of the trees had a mixture of traits typical for *P. pyraster* and *P. communis*, and could therefore be described as *Pyrus* × *amphigenea*. For example, trees 16 and 19 had fruits similar to those of *P. pyraster* and leaves similar to those of *P. communis*. On the other hand, trees 3, 6, 10 and 15 had fruits similar to those of *P. communis* and leaves similar to those of *P. pyraster*.

The molecular data support this opinion. The objects representing cultivars tend to cluster in two or three branches, depending on the clustering method used (Fig. 1 and 2). Nevertheless, they are scattered among the wild trees.

The genotype of an individual semi-wild tree was usually not similar to the genotype of its closest neighbor. The only exception was trees 12 and 13 (Fig. 1 and 2). Furthermore, the genotype of an individual tree was more similar to the genotype of nearby cultivated pears than of nearby semi-wild pears. The semi-wild pears examined seem to mainly represent *Pyrus* × *amphigenea*.

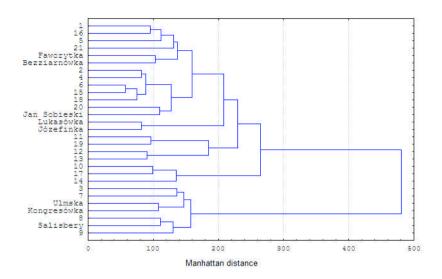


Figure 1. Distance tree of 21 accessions of semi-wild trees and eight cultivated varieties of *Pyrus*, calculated using AFLP data (Ward method/Manhattan distance). Accessions are numbered according to the transect route: the lowest number designates the most eastern tree; the highest – the tree closest to Warsaw

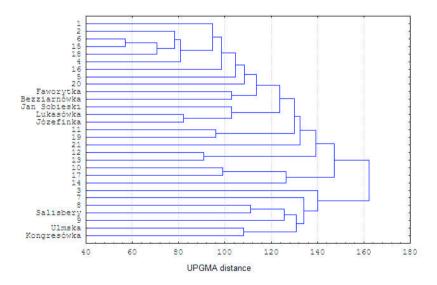


Figure 2. Distance tree, including 21 accessions of semi-wild trees and eight cultivated varieties of *Pyrus*, calculated using AFLP data (UPGMA method/Manhattan distance). Accessions are numbered according to the transect route: the lowest number designates the most eastern tree; the highest – the tree closest to Warsaw

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Although some of trees have several important traits typical for *P. pyraster*, none of the trees could be described as pure *P. pyraster*. Pure stands of *P. pyraster* probably grew in northeastern Poland sometime in the recent past, but the present study indicates that the population of *P. pyraster* may be strongly genetically eroded. If it is not the case already, *P. pyraster* will soon cease to exist as a pure taxon in Poland and will be completely mixed with *P. communis*. Many elements of the phenotype of *P. pyraster* have become incorporated into the phenotypes of the semi-wild pears which grow in the region. Preliminary research suggested that the proportion of *P. pyraster* genes in semi-wild pears should increase the further one goes to the northeast, but our results do not confirm this.

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MOLEKULARNE BADANIA RÓŻNORODNOŚCI POLSKICH ZDZICZAŁYCH GRUSZ (*Pyrus*) Z UŻYCIEM METODY AFLP

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STRESZCZENIE

Dzikie i dziczejące grusze (*Pyrus pyraster* i *P. ×amphigenea*) oraz naturalizowane odmiany gruszy uprawnej (*P. communis*) są ważnym elementem krajobrazu polskiej wsi. Drzewa te są zwykle uważane za trudne w oznaczaniu, a ich pozycja systematyczna jest niejasna. Zastosowanie metody AFLP do badania stosunków pokrewieństwa w obrębie grusz w północno-wschodniej Polsce ujawniło, że większość osobników w tym rejonie należy zaklasyfikować jako *P. ×amphigenea*. Poszczególne drzewa miały cechy typowe dla *P. pyraster*, jednak zwykle w połączeniu z cechami *P. communis*. Badania nie potwierdziły przypuszczenia, że udział genów mieszańcowych wzrasta w kierunku regionów intensywnej uprawy sadowniczej.

Słowa kluczowe: AFLP, różnorodność genetyczna, *Pyrus ×amphigenea, Pyrus communis, Pyrus pyraster*