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PHENOLOGICAL, MORPHOLOGICAL AND GENETIC VARIABILITY OF 15 CLONES OF ROOTSTOCKS FOR APPLE

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Abstract. In many countries, apart from creative cultivation, clone selection is also carried out to get the most valuable rootstocks for apple trees. In years 2008–2010, in the Department of Dendrology and Nursery Production of University of Life Sciences in Poznań, an experiment, which is described below, was conducted. Its aim was to estimate a degree of morphological, phenological and genetic variability of 15 clones originating from crossing A2 and B9 rootstocks. Observations that were carried out confirmed variability of the above mentioned features of clones. The differences were found in morphology of shoots, leaves, buds and in terms of the beginning of vegetation. Analysis of the results made it possible to separate four clones with desired feature of the reduction of the vigour of growth. The clones were marked as number 5, 11, 12 and 13, and they can turn out to be valuable rootstocks for apple trees. With help of PCR-RAPD method genetic variability of the clones number 2 and 3 was shown, as well.

Key words: new clones, rootstocks for apple, variability

INTRODUCTION

In the process of cultivation of vegetative rootstocks one can find individual variability. Its reason can be different methods of production, spontaneous mutations, presence of viruses and new methods of obtaining e.g. radiation [Przybyła 1988, Zagaja et al. 1988, Przybyła and Zagaja 1989, Przybyła et al. 2009]. Also, within the range of clones of one rootstock morphological differences can occur depending on ontogenetic stage of development [Maćkowiak 1995, Kolasiński 1996, Barritt et al. 2004]. These factors resulted in diversity in vigour of growth and productivity of apple trees [Engel 1986, Oosten 1986, Hrotko et al. 1997, Jadczuk 1997, Bielicki et al. 1999, Engel 1999, Masseron and Simard 2002, Czynczyk et al. 2003, Webster 1997, 1999, Barritt et al. 2004, Piestrzeniewicz et al. 2009] as well as in vigour of growth of maiden apple trees

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in a nursery [Stachowiak and Świerczyński 2011]. Sometimes, however, such differences can not be observed [Słowiński and Sadowski 2002] or the difference may result from the presence of viruses in nursery material [Poniedziałek et al. 2001].

In many countries, apart from creative cultivation, a clone selection is also carried out to get the most valuable rootstocks for apple trees [Engel 1977, Masseron and Roche 1993, Webster and Hollands 1999, Johnson et al. 2001, Robinson 2001, Webster and Tobutt 2001, Robinson and Hoying 2004, Robinson et al. 2004]. Also in Poland scientists [Zagaja et al. 1988, Jakubowski 1993, 1999, Jakubowski and Zagaja 2000, Czynczyk and Jakubowski 2007] conduct various works to obtain new rootstocks for apple trees. The effects of such works in the Institute of Pomology and Floriculture in Skierniewice, Poland are, among others, rootstocks: P1, P2, P14, P16, P22, 'Polan', P60, P62, P63, P64, P66, P67. Also, in former Agricultural University, and present Poznań University of Life Sciences, Poland, for many years (1995–1999) different hybridization and selection works (1998–2003) as well as propagation (2004–2008) works have been conducted to produce new rootstocks for apple trees. The estimation of the efficiency and quality of stoolings obtained by hybridization of rootstocks A2 and B9 was presented by Stachowiak and Świerczyński [2009]. Their usefulness for the production of maiden apple trees in a nursery was shown by Stachowiak and Świerczyński [2011] too.

The aim of the present experiment was to determine morphological, phenological and genetic variability of 15 clones originating from crossing A2 and B9 rootstocks.

MATERIALS AND METHODS

Studies on variability of morphological and phenological features of clone rootstocks were carried out in the Department of Dendrology and Nursery Production of University of Life Sciences in Poznań, in years 2008–2010. 15 clones of rootstocks coming from crossing A2 and B9 rootstocks constituted research vegetal material. These clones were considered in earlier experiments carried out in the mother field and in the nursery [Stachowiak and Świerczyński 2009, 2011]. They were chosen from tens of tests obtained as results of crossing due to the ease of their propagation. Wybrano je spośród kilkudziesięciu pojedynków otrzymanych w wyniku krzyżowań ze względu na łatwość w ich rozmnażaniu. Each clone was represented by 9 mother plants growing in three different places in the mother field.

Phenological studies concerned dates of the beginning and end of vegetation period, as well as the start and finish of the development of leaves and their fall.

Morphological studies covered determination of colour of leaves in the vegetation period, shape of leaves, nervation, tip and edge of the leaf plate, length and width of the leaf plate (mm), length of the petiole (mm), length of stipule (mm), area of the leaf plate (after scanning it was calculated with SKWER program and given in cm^2), length of the internodes on long shoots (mm), length of buds [mm], shape of buds, position of buds against shoots and their very position on the shoot, presence of trachea and their distribution, and finally, colour of the bud and kind of its surface.

Colour of the buds and leaves was determined in the period of leaves development on the basis of colour catalogue [R.H.S. 1966]. Measurement of buds concerned only those growing on the main shoot. For this reason three, the most typical buds from each mother plant, were taken. Leaves were chosen randomly in number of four from each plant. Measurement of internodes length was carried out on main shoots. Similarly to buds, also three the most typical shoots were taken.

Statistical analysis of the results was carried out by variance analysis using Duncan's test for means separation at $p \leq 0.05$. The results presented in tables are mean values from years 2008–2010.

Studies on DNA of the clones using PCR-RAPD were conducted in the Department of Biochemistry and Biotechnology of the University of Life Sciences in Poznan. Young leaves of clones collected at the end of April 2009 constituted the material for DNA studies. The method that was used relied on amplification of DNA with the use of RAPD markers. The amount of DNA needed for one reaction was 10–25 ng. The stage of starters' binding in RAPD reaction was conducted at relatively low temperature (first 20 cycles 35°C, next 20 cycles 38°C) to ensure stable elongation of short starters. A commercial set of starters of Operon Company was used (series OPL and UBC). Reactions (volume 20 µl) were conducted on ice with use of ready 2 × PCR Mix of Fermentas Company.

Products of DNA amplification were separated on 1,5% agar gel in the presence of ethidium bromide for about 2 hours at the voltage of 70 V. Each reaction was repeated twice for its authentication. Results were evaluated and visualized by pictures taken in UV light, (transluminator). They are presented in the result section.

RESULTS AND DISCUSION

Phenological studies. Individual clones differed in terms of the beginning of their vegetation. The earliest term of the beginning of opening leaf buds took part for clone number 1 (between 13 and 15 April). Clones number 2, 6, 9, 10 and 11 started their vegetation from 19 till 21 April, and clones number 12, 13, 14 and 15 between 24–27 April. In case of other clones the beginning of the vegetation period was observed between 29 April and 3 May. Late beginning of vegetation can suggest smaller danger of freezing during delayed spring frost.

Beginning of half-opening of leaf plates was observed between 18 and 21 April for clone no 1, between 25 and 27 April for clones number 2, 6, 9 10 and 11 and between 30 April and 4 May for clones number 12, 13, 14 and 15. For the rest of clones half-opening of leaves took part between 13 and 18 May.

On the basis of carried observations no significant differences were found in dates of the end of vegetation of individual hybrids. The beginning of fall of leaves was observed in the first decade of November, and the end of their fall in the third decade of November.

Morphological studies. All buds growing on mother plants had common morphological features such as: shape, position against a shoot and a very position on a shoot. The buds were hairy, dull-edged, adherent and placed torsionally on shoots.

Table 1. The length of buds, internodes and number of thorns of 15 apple rootstock clones
Tabela 1. Długość pąków, międzywęźli i liczba cierni 15 klonów podkładek jabłoni

Number of clone Numer klonu	Average – Średnia		
	length of buds długość pąków (mm)	length of internods długość międzywęźli (mm)	number of thorns liczba cierni
1	5.3 a*	28.7 g	1.8 ab
2	4.9 a	23.2 de	2.3 abc
3	4.0 a	18.9 ab	2.0 abc
4	4.6 a	19.0 ab	1.3 ab
5	3.1 a	18.2 a	1.5 ab
6	5.0 a	23.1 de	1.0 ab
7	5.1 a	22.6 d	1.3 ab
8	4.5 a	24.7 e	0.3 a
9	5.6 a	22.8 d	5.7 c
10	3.6 a	19.8 ab	4.3 bc
11	5.3 a	20.5 bc	1.3 ab
12	4.7 a	20.5 bc	0.3 a
13	4.4 a	20.1 bc	1.0 ab
14	5.2 a	21.8 cd	3.0 abc
15	6.1 a	26.7 f	2.4 abc

*Means followed by the same letters in the column are not significantly different at the level of $\alpha = 0.05$
*Średnie oznaczone tymi samymi literami w kolumnie nie różnią się między sobą istotnie przy $\alpha = 0,05$

Table 2. The length and width and area of the leaf plate of 15 apple rootstock clones
Tabela 2. Długość i szerokość oraz powierzchnia blaszki liściowej 15 klonów podkładek jabłoni

Number of clone Numer klonu	Average – Średnia		
	length długość (mm)	width szerokość (mm)	area of leaf plate powierzchnia blaszki liściowej (cm)
1	68.1 d*	44.1 b	80.3 e
2	61.4 cd	36.1 ab	75.4 de
3	60.6 cd	37.4 ab	80.6 e
4	61.3 cd	40.0 ab	63.8 bcde
5	55.5 abc	33.4 ab	48.2 abc
6	57.2 a-d	36.7 ab	66.8 cde
7	57.7 bcd	38.7 ab	76.3 e
8	52.6 abc	34.8 ab	51.4 abc
9	54.0 abc	39.5 ab	63.8 bcde
10	53.2 abc	33.3 ab	66.1 cde
11	45.0 a	26.1 a	38.9 a
12	47.1 ab	30.7 ab	45.0 ab
13	51.0 abc	30.0 ab	38.0 a
14	53.2 abc	33.1 ab	56.6 abcd
15	55.3 abc	35.2 ab	61.2 bcde

*For explanation, see table 1
*Wyjaśnienie, patrz tabela 1

Measurement of lengths of buds that were growing on the main shoot was carried out as well. Results were presented in table 1. Taking into account mean values of lengths of buds it was found that buds of clone number 15 were almost twice as long as buds of clone number 5. However, no significant differences in values for individual clones were observed.

Individual clones differed significantly in lengths of internodes. The smallest mean values of this parameter were noticed for clones number 3, 4, 5 and 10, and the highest for clone number 1 (tab. 1). Because of the shortest internodes these clones can be seen as potential rootstocks with the smallest vigour of growth, and of clone number 1 as the one with the strongest vigour of growth.

The most thorny shoots were created by clones number 9 and 10 (tab. 1). Big thornily restricts practical application of such rootstocks.

Clones had singular leaves with full plates and feathery ribbing. Most of leaf plates had the same features. They had elliptical shape, with a double serrate edge, sharp top and rounded base.

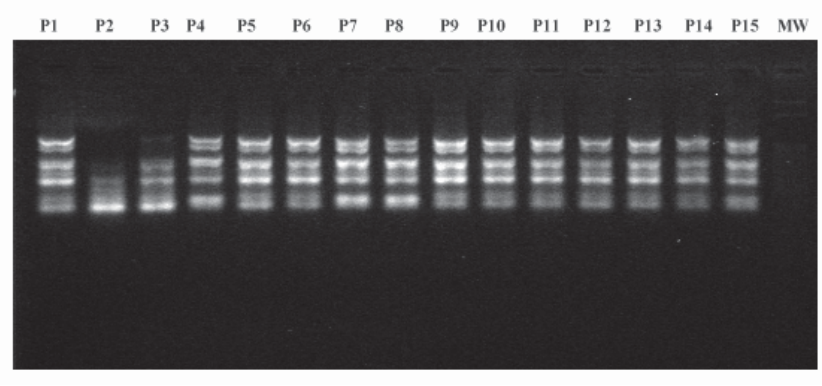
Leaves coming from clone number 5 and 7 had plates with oval shape. Tops of leaves from clones number 2 and 7 were pointed and curved. On their bottom side leaves, as well as petiole and shoots were quite mossy.

On the basis of mean values of lengths and widths it was found that leaves of clone no 1 were the biggest and of clone number 11 the smallest (tab. 2). The size of leaves can determine the vigour of growth, which in case of clone number 1 corresponds with the biggest length of internodes.

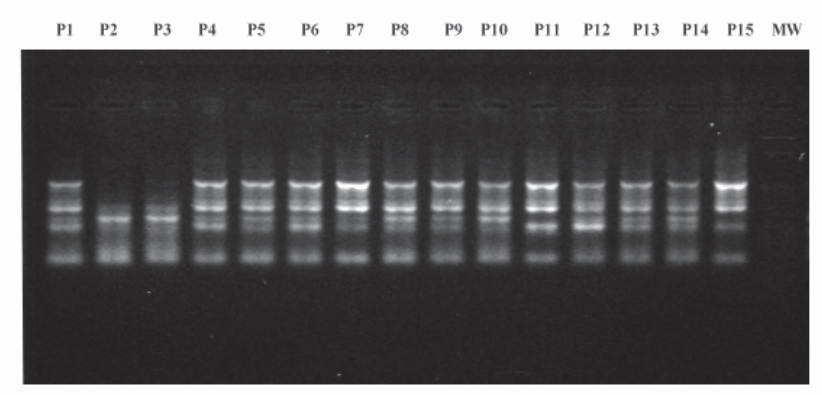
Table 3. The length of the petiole and stipule of 15 apple rootstock clones
Tabela 3. Długość ogonków liściowych i przylistków 15 klonów podkładek jabłoni

Number of clone Numer klonu	Average length of – Średnia długość	
	petiole ogonka liściowego (mm)	stipules przylistków (mm)
1	18.0 a*	3.1 a
2	16.4 a	5.6 a
3	15.1 a	5.9 a
4	19.6 a	6.7 a
5	13.9 a	2.6 a
6	14.8 a	3.9 a
7	15.4 a	8.6 a
8	12.7 a	9.7 a
9	16.2 a	4.6 a
10	14.4 a	4.3 a
11	10.9 a	4.2 a
12	12.2 a	3.7 a
13	11.4 a	3.5 a
14	13.1 a	4.7 a
15	12.8 a	4.6 a

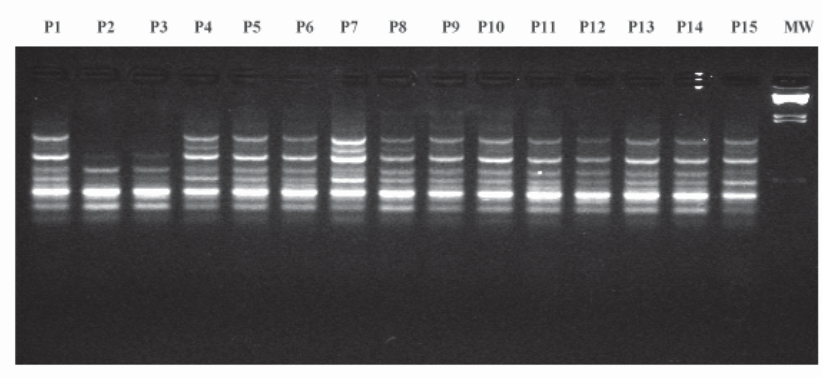
*For explanation, see table 1
*Wyjaśnienie, patrz tabela 1



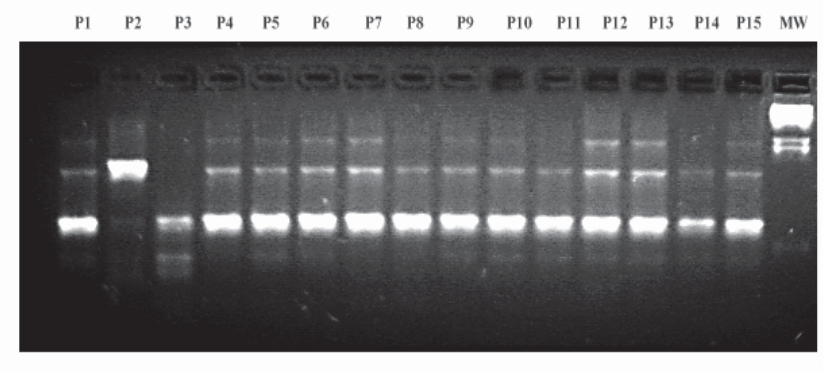
Phot. 1. The model of stripes of apple rootstock clones obtained using primer OPL-1
Fot. 1. Wzory prążkowe klonów podkładek jabłoni uzyskane przy użyciu startera OPL-1



Phot. 2. The model of stripes of apple rootstock clones obtained using primer OPL-8
Fot. 2. Wzory prążkowe klonów jabłoni uzyskane przy użyciu startera OPL-8



Phot. 3. The model of stripes of apple rootstock clones obtained using primer OPL-17
Fot. 3. Wzory prążkowe klonów jabłoni uzyskane przy użyciu startera OPL-17



Phot. 4. The model of stripes of apple rootstock clones obtained using primer UBC-52
Fot. 4. Wzory prążkowe klonów jabłoni uzyskane przy użyciu startera UBC 52

The area of a leaf plate was the smallest for clones number 11 and 13. The biggest area of a leaf plates was observed for clones number 1, 3 and 7 (tab. 2). The area of a leaf for clone no 11 was consistent with the smallest parameters of length and width mentioned earlier.

The smallest mean value of the length of petiole was found for clone number 11, and the biggest for clones number 4 (tab. 3). However, the results obtained for individual clones did not differ considerably.

On average the smallest length of stipule was observed for clone number 5, and the biggest for clone number 8 (tab. 3). Numerical values of this feature did not differ much, as well.

The examined clones did not differ significantly in colour of leaves and shoots, which was inherited from B9 rootstock. It was marked as Greyed – Purple (187 B). With the development of plants, leaves changed their colour for green, which could be recognized as Green Group (141 B). The colour of shoots was marked as Greyed – Purple (187 A).

Authors [Engel 1986, Masseron and Roche 1993, Kolasiński 1996, Wertheim 1997, Baab 1998] conducting studies on rootstocks often obtained big morphological diversity, even within the range of sub-clones of one rootstock, as e.g., M.9. These sub-clones differed also in vigour of growth, however, not more than 10–15% [Hrotko 2007]. In the literature it is very rare to find information on the subject of determination of individual inconstancy of rootstocks. Similar studies were conducted by Maćkowiak [1995]. Sub-clones of M.9 rootstock collected from different regions of Poland constituted research material. The author noticed differences in the morphology of shoots, leaves and buds of various sub-clones, which could have resulted from different ontogenetic stage of plants development. In the studies one could observe differences giving witness to variability of many features of the obtained hybrids.

Genetic studies. On the basis of the conducted genetic studies with the use of PCR--RAPD method, it was found that clones number 2 and 3 differed from others in number and intensity of stripe patterns. Starters OPL-1, OPL-8 and OPL-17 as well as UBC 52 were especially useful in the process of recognition of genetic differences of these clones (phot. 1, 2, 3 and 4).

CONCLUSIONS

1. There was a different term of the beginning of vegetation period for the studied clones.

2. Morphological measurements of individual clones showed significant differences in lengths of internodes, length and area of leaf plate and in number of thorns.

3. Clones number 5, 11, 12 and 13, characterized with shorter internodes and smaller area of a leaf plate, were rootstocks with a smaller vigour of growth.

4. Genetic variability, especially for clones number 2 and 3, was observed, as well as a big usefulness of starters OPL-1, OPL-8 and OPL-17 and also UBC 52 for genetic studies of these clones was found

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ZMIENNOŚĆ FENOLOGICZNA, MORFOLOGICZNA I GENETYCZNA 15 KLONÓW PODKŁADEK DLA JABŁONI

Streszczenie. W wielu krajach, poza hodowlą twórczą, prowadzona jest także selekcja klonalna w celu uzyskania najbardziej wartościowych podkładek dla jabłoni. W Katedrze Dendrologii i Szkółkarstwa Uniwersytetu Przyrodniczego w Poznaniu w latach 2008––2010 prowadzono doświadczenie, którego celem było określenie stopnia zmienności morfologicznej, fenologicznej i genetycznej 15 klonów, powstałych z krzyżowania podkładek A2×B9. Prowadzone obserwacje potwierdziły zróżnicowanie cech omawianych klonów. Stwierdzono różnice w morfologii pędów, liści, pąków i terminie rozpoczęcia wegetacji. Analiza wyników pozwoliła wyodrębnić cztery klony charakteryzujących się pożądaną redukcją siły wzrostu. Były to klony oznaczone jako nr 5, 11, 12 i 13, które mogą okazać się wartościowymi podkładkami dla jabłoni. Wykazano także za pomocą techniki PCR-RAPD zróżnicowanie genetyczne klonów nr 2 i 3.

Słowa kluczowe: nowe klony, podkładki dla jabłoni, zmienność

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