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**MOLECULAR RESEARCH ON THE GENETIC DIVERSITY OF  
POLISH VARIETIES AND LANDRACES OF *PHASEOLUS COCCINEUS*  
L. AND *PHASEOLUS VULGARIS* L. USING THE RAPD AND AFLP  
METHODS**

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**Abstract:** The aim of our research was to evaluate the genetic diversity among 25 commercial varieties registered in Poland and 14 landraces of *Phaseolus vulgaris* var. *nanus* Asch. (the dwarf common bean) and *Phaseolus coccineus* L. (the runner bean) maintained in the National Centre of Plant Genetic Resources in Radzików. An additional goal of this study was to compare the precision and efficiency of two techniques of PCR (RAPD and AFLP), used to estimation the genetic diversity of bean. The breeding varieties of bean were registered in the period between 1950 and 2000. The landraces, collected during expeditions conducted from 1985 to 1988, mainly originated from the eastern and southern part of Poland. In the plant genetic diversity research of RAPD and AFLP markers are commonly used. Complex electrophoresis pictures of DNA fragments were taken, and revealed a considerable polymorphism. The polymorphic fragments were obtained on the basis of 6 differentiating primers using the RAPD method and 15 differentiating primers using the AFLP method. *P. vulgaris* and *P. coccineus* accessions formed distinct groups. Each of the RAPD and AFLP analyses allowed for the unique distinguishing of all accessions.

**Key Words:** RAPD, AFLP, *Phaseolus vulgaris*, *Phaseolus coccineus*

## INTRODUCTION

The genus *Phaseolus* includes several wild and cultivated species, originated in the New World, such as *P. vulgaris* (the common bean) and *P. coccineus* (the runner bean). In post-Columbian times, these two most important species spread widely. They are currently amongst the most popular grain legumes in Poland. Although new cultivars are displacing landraces in Poland, it is still possible to

find farmers who grow landraces of beans for self-consumption and for sale at farm markets. It is still most common for collecting missions in Poland to turn up local populations of beans [11].

The evaluation of morphological differences is a traditional method of evolutionary and pedigree relationship determination. It was particularly useful in bean, in which numerous phenotypic differences occur (e.g. colour, size, pattern and shape of seeds). However, only molecular markers provide information which is independent of environmental influences or the plant's development phase. Therefore, techniques of DNA analysis have become more and more important. Methods based on polymerase chain reaction – PCR – are widely used in research. One of the most popular methods is the RAPD (Randomly Amplified Polymorphic DNA) technique [3, 8, 18, 19, 20]. It makes it possible to quickly examine genetic material in a large number of samples at a relatively low cost. In earlier research conducted in the PBAI-Radzików, the modified RAPD technique with a system of primers containing additional DNA sequences partly complementary to the semi-conservative sequences of intron-exon junctions proved to be very useful in a variety of plant species [12]. These primers, also known as semi-random primers, were successfully used by Weining and Langridge [18] to target diverse regions of the genome in cereals.

Another commonly used method in DNA research is Amplified Fragment Length Polymorphism (AFLP). AFLP marker technology allows efficient DNA fingerprinting and an analysis of large numbers of polymorphic fragments on polyacrylamide gels. The AFLP technique is based on the detection of DNA restriction fragments amplified by PCR, and can be used for DNA of any origin or complexity [17].

The AFLP technique has major advantages over other PCR-based fingerprinting techniques. It is a fast method, requires no prior sequence knowledge and gives access to a very large range of polymorphisms, because of access to the complete genome (the non-expressed DNA is also subject to analysis).

AFLP analysis has emerged as a popular method for genetic mapping, species identification and phylogenetic analysis [5, 14, 15, 16].

The objective of this research was to determine the genetic variability existing among landraces of *P. vulgaris* and *P. coccineus*, and among commercial varieties cultivated in Poland, and to adapt a method for their distinction. We hoped to obtain information on the level of intervarietal divergence, which is essential for plant breeders. The additional practical goal was the elimination of possible duplicates. They resulted from difficulties in distinguishing collecting landraces from commercial varieties grown in home gardens.

## MATERIALS AND METHODS

### Plant material

Twenty five commercial varieties registered in Poland and 14 landraces of *Phaseolus vulgaris* and *Phaseolus coccineus* (Tab. 1) taken for the preliminary

analysis are maintained in the National Centre for Plant Genetic Resources at Radzików. The commercial varieties of bean were registered in the period from 1950 to 2000. The landraces were collected during expeditions conducted from 1985 to 1988, and they mainly originated from the eastern and southern part of Poland.

Tab. 1. Polish varieties and landraces of *Phaseolus vulgaris* and *Phaseolus coccineus* used for the RAPD and AFLP analyses.

Accession number	Name	Type	Origin	Seed size
<i>Phaseolus vulgaris</i>				
PL 180137	Atut	CV	Selected from materials of English origin	
PL 181407	Augustynka	CV	Bomba x mutant 63B Kaiser Wilhelm	medium
PL 181415	Aura	CV	Bor x Wiejska	large
PL 180014	Biała Wyborowa	CV	Selected from the Niewyczerpana variety	medium
PL 180039	Bomba	CV		large
PL 180015	Bor	CV	Złota Saxa x Perlówka	small
PL 181657	E 0952	L	Landrace from Różanka near Włodawa	
PL 181520	E 0956	L	Landrace from Różanka near Włodawa	
PL 181519	E 1004	L	Landrace from Kolonia Orzechów	
PL 181660	E 1172	L	Landrace from Wirkowice, Lublin Upland	
PL 181513	E 1204	L	Landrace from Góra Grabowiec	
PL 181509	E 1452	L	Landrace from Jeziorna near Tomaszów Lubelski	
PL 181510	E 1478	L	Landrace from Bircza	
PL 181671	E 1683	L	Landrace from the Lublin region	
PL 180018	Florentynka	CV	Line 11 Bomba x mutant Kaiser Wilhelm	medium
PL 180017	Igołomska	CV	Landrace Siarkowa from Zakliczyn x mutant Kaiser Wilhelm	large
PL 180010	Jubilatka	CV	Bomba x local landrace Malinowa 2x Cocco Blanc	large
PL 181408	Justynka	CV	Bomba x mutant Kaiser Wilhelm	Medium
	Katarzynka	CV	Szubińska x local landrace Złoty Deszcz Kujawski	large
PL 181991	Krakowska	CV	Sans Rival x Cud Francji	medium
	Longina	CV	Wiejska x Asta	large
	Małopolanka	CV		large
	Mela	CV	Allerfrueste Weisse x Perlówka	small
PL 180024	Michigan	CV		small
	Nida	CV		medium
PL 181992	Perlówka	CV	Mutant Zucker Perle Perfection	small
PL 181411	Polanka	CV	Cannellini 2x Cannellini x SIG	large

Accession number	Name	Type	Origin	Seed size
PL 182003	Prosna	CV		large
PL 180080	PV 196	L	Landrace from Kajanka, Białystok	
PL 180067	PV 93	L	Landrace from Włosienica, Bielsko-Biała	
PL 180069	PV 95	L	Landrace from Włosienica, Bielsko-Biała	
PL 180007	Słowianka	CV	Line 11 Bomba x mutant 63B Kaiser Wilhelm	medium
PL 181406	Wenta	CV	Allerfrueste Weisse x Perlówka	small
PL 180011	Wiejska	CV	Selected from landrace from the Lublin region	large
<i>Phaseolus coccineus</i>				
PL 181990	Felicja	CV	Cross landraces from region Wrocław, Kraków, Kielce	
PL 181989	Piękny Jaś	CV	Selected from local landrace	
PL 181986	Landrace Kasiłan	L	Landrace from Kasiłan	
PL 181988	Landrace Kraśnik	L	Landrace from Kraśnik	
PL 181987	Landrace Tyszowce	L	Landrace from Tyszowce	

CV - commercial variety ; L- landrace

### DNA isolation

DNA isolation was performed according to the CTAB procedure [4, 10, 13]. Genomic DNA was isolated from approximately 1 g of fresh leaves of 15 plants of each variety or population taken for the study.

### RAPD and primers

RAPD reactions were carried out according to method described by Rafalski *et al.* (1998). In the experiments, primers were used with sequences partly complementary to the semi-conservative sequences of the intron-exon junctions (Tab. 2). This type of primer provides a considerably higher polymorphism than

Tab. 2. Primers used in the PCR reactions generating reproducible polymorphisms.

Name	Sequence	
	5`	3`
ET 1/18	<b>ACTTACCTGAGGCGCGAC</b>	
ET 2/18	<b>ACTTACCTGCTGGCCGGA</b>	
ET 4/18	<b>ACTTACCTGCCTGCCGAG</b>	
ET 6/18	<b>ACTTACCTGCCTACGCGG</b>	
IT 1/18	<b>CCGGCAGGTCAGGTAAGT</b>	
IT 2/18	<b>GCAGAGGGCCAGGTAAGT</b>	

RAPD primers; besides, this system is universal for plants (it is based on sequences commonly flanking introns in plants). Using them, fragments located in the transcriptional part of the genome were subjected to amplification.

The fragment of each primer sequence written in bold is complementary to the semi-conservative sequences of the intron-exon junctions. The remaining part is random. The ET (exon targeting) primers duplicate DNA fragments of the exons, and the IT (intron targeting) primers duplicate DNA fragments of introns (Tab. 2).

Six primers, selected from a set of 50, were used in this study. The primers were selected on the number of DNA fragments generated, and polymorphisms were detected. DNA fragments were analysed using Fragment NT analysis software.

### AFLP and primers

For the AFLP reactions, sets of chemicals provided by Applied Biosystems were used. The analyses were conducted with the application of the AFLP method with fluorescent primers, version o AFLP, using ABI-PRISM 377. On the set of a 64 primer combination, bought from Applied Biosystems, the optimal primer combination was established [1]. The eight most polymorphic primers were chosen for the investigation proper (Tab. 3). The minimum height of the peak taken for the analysis was 100 points. The range of the analysis was from 35 to 500 bp. Electrophoresis was conducted on a 36 cm long 4.5% polyacrylamide denaturing gel, for 4 h at 2400 V. Pictures of bands were taken using Genescan software. The zero-one template was generated using Genotyper software.

Tab. 3. Selection of AFLP primer combinations for the *Phaseolus vulgaris* and *Phaseolus coccineus* investigation.

	MseI- CAA	MseI- CAC	MseI- CAG	MseI- CAT	MseI- CTA	MseI- CTC	MseI- CTG	MseI- CTT
EcoR I- ACT FAM	X						X	X
EcoR I- ACA FAM			X			X		
EcoR I- AAC NED								
EcoR I- ACC NED								
EcoR I- AGC NED								
EcoR I- AAG JOE	X							X
EcoR I- AGG JOE								
EcoR I- ACG JOE				X				

## RESULTS AND DISCUSSION

### RAPD

A total of 397 polymorphic bands were produced, of which only five were detected in all the *P. coccineus* accessions, and only four in the *P. vulgaris* accessions. Based on combined banding patterns, all 39 accessions were identified.



cluster (G3) is formed by ecotypes collected in south-eastern Poland and all the varieties originated from the Kaiser Wilhelm mutant. The last group (G4) is formed by the registered varieties and three landraces: PV 196 (the Białystok region) and PV 95, PV93 (the Bielsko-Biała region). The applied primers made it possible to distinguish the two species of *Phaseolus*, the landraces and the registered varieties of *Phaseolus vulgaris*. *Phaseolus coccineus* accessions form a closely related group, unlike *Phaseolus vulgaris*, which displays broad genetic diversity. These results confirm the efficiency of the applied RAPD markers for the identification of genotypes of *Phaseolus*.

### AFLP

A total of 381 polymorphic bands were produced, of which 34 were exclusively detected in all the *P. coccineus* accessions and 10 in the *P. vulgaris* accessions. The number of AFLP fragments generated allowed us to distinguish all the accessions used. The cluster analysis was based on the Jaccard distance and the UPGMA (Unweighted Pair Group Method Average) method. The similarity level ranged from 0.24 to 0.80.

The evaluated genotypes formed four clusters (Fig. 2). *Phaseolus coccineus* comprised one isolated cluster (G1). The other three clusters are created by *Phaseolus vulgaris* but the composition of the groups is different than that obtained by the RAPD method. The last group, G4, is formed only by a single variety "Biała wyborowa", and the outstanding position of this variety cannot be explained by morphology or origin. The *Phaseolus coccineus* accession forms a closely related group, unlike *Phaseolus vulgaris*, which displays broad genetic diversity. The results clearly discriminate the two species. The results confirm the efficiency of AFLP markers for the identification of genotypes of *Phaseolus*. However, genetic similarity among accessions estimated by the AFLP method, as with RAPD, were not related with the seed morphological characteristics which define each variety or landrace.

The common bean, *Phaseolus vulgaris*, comprises two major domesticated groups, namely the Mesoamerican and Andean gene pools [7]. The Andean beans have larger seeds than the Mesoamerican ones [6]. The groups formed in our studies by RAPD and AFLP analysis are not correlated with seed weight (Tab. 1). There is also no relation between the formed clusters and their belonging to particular gene pools. As indicators of gene pools, three landraces were used with previously determined genetic background. Wołoszczyńska *et al.* [21] stated that the PV 196 and PV 95 accessions belong to the Middle American gene pool and the PV 93 accession belongs to the Andean gene pool. The obtained results do not allow for conclusions on the genetic background of groups formed with the presence of these accessions. According to the investigation conducted by Zimniak-Przybylska and Przybylska [22], a majority of the cultivated forms of *Phaseolus* from Europe are of Andean origin. Maciel *et al.* [9] studied genetic variability among the cultivars and landraces of the common bean of south-Brasil, using the RAPD method. They found a high

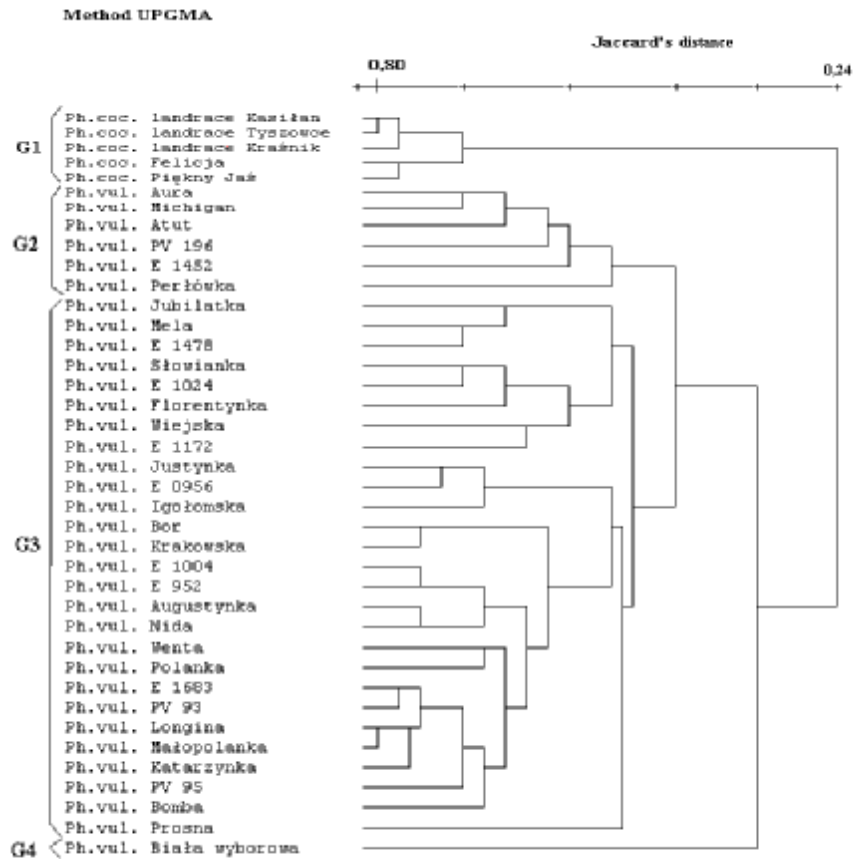


Fig.2. Relationships between the investigated bean genotypes using AFLP data.

correlation among the formed clusters and seed weight and that accessions belonged to specific gene pools. Similar results were obtained by Galvan *et al.* [6], in studies on Northwestern Argentinian varieties of the common bean. However such a relationship was not found by Alvarez *et al.* [2] in their analysis of the Spanish populations of the common and runner bean. The analyses performed in this study indicate that investigated accessions Polish traditional varieties of the common and runner bean are genetically distinct and can be clearly distinguished between using the phenograms from commercial obtained by varieties using the RAPD and AFLP methods.

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