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The potential of specific profilin based fingerprinting differs in legume species

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Abstract

One requirement for using germplasm in agricultural development initiatives is its characterization, which necessitates knowledge of the genetic polymorphism and relationships among the individual varieties. Up to now, different DNA markers were utilized for this purposed, one of the newest are those for coding regions. Here, we aimed to investigate polymorphism and genetic relationships among 24 varieties of *Cicer arietinum* L. and 23 varieties of *Pisum sativum* L. using profilin based fingerprinting. PCR approach was used to generate homologue amplicons of plant profilins and UPGMA grouping for visualization of obtained fingerprint similarity. Amplification results showed different results for analysed legume species, where the higher polymorphism at the level of 96% was obtained within the accessions of pea varieties, as for chickpea fingerprints a very similar profiles were generated with only a limited amplicons of a total of three different length of 150 bp, 182 bp and 348 bp. This information could be useful in breeding strategies for the improvement of chickpea and pea accessions.

Keywords: *Cicer arietinum* L., Length polymorphism, *Pisum sativum* L., Profilin, DNA markers, Fingerprinting.

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Contents

Contribution of this paper to the literature

Allergen based DNA marker techniques provide a valuable insight to polymorphism of plant coding regions. Here, profilin based polymorphism was firstly applied to analyse the variability among pea and chickpea germplasm.

1. Introduction

Genetic diversity of plants and its description is still of great importance in the research. Plants are inevitable parts of our daily lives, but many of their characteristics are still unknown. The group of legumes provide a basic part of human nutrition in many areas worldwide [1] that is why the interest in their germplasm genetic variability is actual and different studies were published previously [2-5]. Especially molecular and DNA marker-based characterization of legume germplasm has the great potential in determining of genetic diversity and identification of unique variants that can be used in breeding programmes. In this study, selected varieties of *Cicer arietinum* L. and *Pisum sativum* L. were analysed. Both of this species are well characterized in their germplasm variability using different DNA based markers. Investigation of chickpea gerplasm by RAPD (Randomly Amplified Polymorphic DNA) provided a low degree of polymorphism with high coefficients of genetic similarity [6]. Inter-simple sequence repeat (ISSR) markers were found to be dependent on the characteristics of their 3′anchores sequences in the ability to generate polymorphism in chickpea accessions and a combination of the principal component anaysis (PCA) together with cluster analysis in data analysis is important for informativeness of generated data and extracting the valuable informations [7, 8]. In this technique, more primers need to be used to analyse germplasm thoroughly [9]. Retrotransposon based DNA marker techniques such as iPBS (inter Primer Binding Sites polymorphism) were reported to be able to generate up to the 100% polymorphism in chickpea variability analysis [10]. Start codon targeted (SCoT) polymorphism is efficient in genetic diversity analysis of chickpea depending on the primer combinations and the genetic similarity can range from 84 to 98%, but many of primer combination can be monomorphic in its results [11]. Investigation of pea germplasm by RAPD were performed previously with good results in obtained polymorphism where clear pattern of clustering according to the source of germplasm were showed in different studies [12-14]. Microsatellite based markers were reported to differentiate successfully among parent and hybrid genotypes of pea [15] and provided a high level of polymorphism among pea genotypes [16]. SCoT are more informative marker compared to ISSR and RAPD markers for discrimination and identification of *P. sativum* subspecies [17]. Retrotransposon based marker techniques were proved to assess distinctness of pea germplasm, especially in cases where the time frame plays an important role. RBIP technique (Retrotransposon based information polymorphism) was reported to be robust and easy to score method, while multilocus IRAP (Inter retrotransposon amplified polymorphism) produced informative fingerprint already in a single analysis [18].

DNA markers are actually developed based on *in silico* prediction and the of potential of these markers was reported to be efficient [19]. *In silico* approach in allergen coding-based DNA markers is based on the identification of conserved parts in the amino acid and genomic sequences of individual homologs of plant allergens. Finding the similarity in alignments, specific as well as degenerate primers are designed [20]. Plant allergens and their protein and nucleotide sequences share a high degree of homology [21, 22] what allow to predict DNA markers for them, too. Previously, Bet v 1 based amplified polymorphism was conformed to be informative in various of plant species, as the genomic sequences of this pollen allergen of birch has the high level of conservation in its epitops [23, 24]. Here, an abundant plant allergen profilin wase used based on the designation of primer pair to its specific conservative part. When using a nonspecific profilin based primer pairs, the profilin based amplified polymorphism method (PBAP) was effective generating of specific fingerprints in all of the analysed genotypes of soybean and groundnut [25, 26] here, specific one was used.

The aims of the present study were (1) analyzing of the potential of specific legume profilin based marker technique to reveal the polymorphism among legume species and genotypes, (2) characterizing and comparing the polymorphism based on the specific profilin based fingerprints generated for *Cicer arietinum* L. and *Pisum sativum*, L.

2. Materials and Methods

2.1. Biological Material

Seeds of legume species *Cicer arietinum* L. and *Pisum sativum* L. were obtained from GeneBank of Slovak Republic, Piešťany. Different numbers of individual varieties were randomly selected to be able analyse the natural intraspecie variability as old land, ancient as well as modern varieties were represent in a final number of 24 for chickpea and 23 for pea. Young plants were obtained *in vitro* from sterilized seeds in basal Murashige and Skoog medium [27] with day-length 15h and 20°C.

2.2. DNA Extraction

Total genomic DNA was extracted by GeneJET™Plant Genomic DNA Purification Mini Kit (Thermo Scientific, Waltham, MA, USA) following the manufacturer instruction. Quantity and quality of extracted DNA was analysed spectrophotometrically by NanoPhotometer™ (Implen) and functionality in PCR was checked by ITS primers [28].

2.3. Specific Profilin Based Polymorphism Analysis

Primers for specific profilin based polymorphism fingerprints were designed using the *in silico* analysis of their conserved sequences [29]. In PCRs, DreamTaq™ DNA polymerase together with 600 nmol of each primer was utilized. PCR conditions of time and thermal profile was: 95 °C for 5 minutes (95 °C for 45 seconds; 55 °C for 45 seconds; 72 °C for 35 seconds) 40x plus final 72 °C for 10 minutes. Obtained amplicons were separated in 2% agarose gel stained by GelRed®. Amplified fingerprints were transformed into binary matrices and based on this,

distance matrices and dendrograms of genetic dissimilarity were prepared using UPGMA analysed based on Jaccard index [30]. Dendrograms of profilins were created by DendroUPGMA software [31].

3. Results and Discussion

In the case of obtained fingerprints for *Cicer arietinum* L., a very similar profiles were generated with only a limited amplicons of a total of three different length of 150 bp, 182 bp and 348 bp. A total of six groups were obtained in the constructed dendrogram, that are part of three branches with the sparation of varieties Calia, Sefiros and Farihame with the same fingerprint profile (Figure 1). The first group of genotypes were characterized with only one amplified fargment with the length of 150 bp from which genotypes Yialousa and Kalika were separeted having one more amplicon with the length of 348 bp. Second branch of the dendrogram comprises from groups with amplicon of the 182 bp length and those that have this one plus amplicon with the lenght of 348 bp.

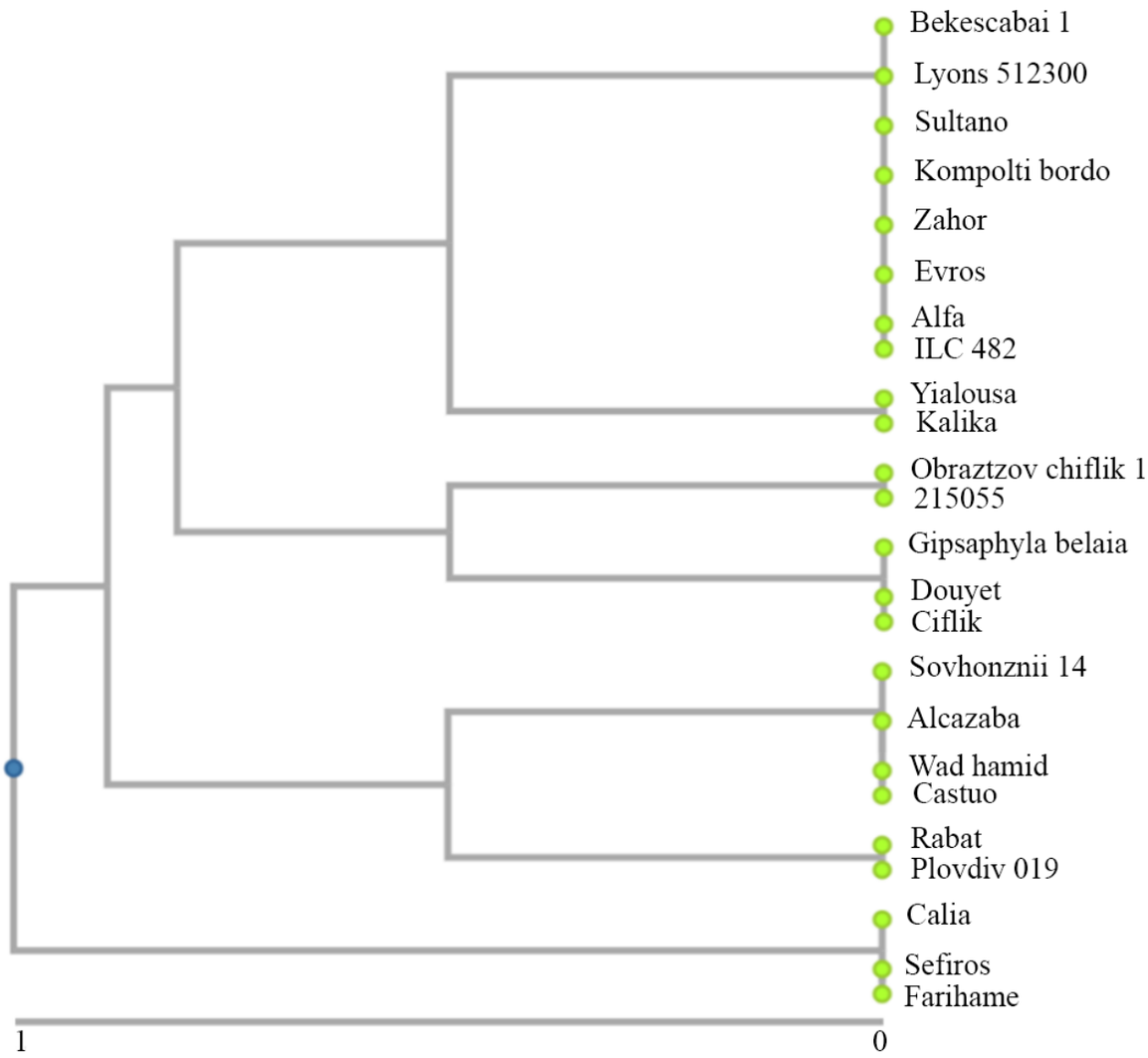

Figure 1. Dendrogram of chickpea accession using the profilin specific fingerprinting.

For *Pisum sativum* L., very different specific profilin fingerprints were obtained. For individual varieties, from 7 to 12 amplicons were generated with the length within the range from 95 bp up to the 1350 bp. The polymorphism in the analysed set of pea varieties was of 96%. Different groups were obtained in the constructed dendrogram, with a separation of varieties Lancet, Libochovicky urodny, Frostar and Parade (Figure 2). The most of analysed accessions were grouped with the average Jaccard coefficient of genetic similarity of 0.47 and two of analysed pea varieties (Cicero and Skagid) have the same profile of generated amplicons. An amplicon of the length 95 bp was amplified in all of the analysed pea varieties.

Profilins belong to actin binding molecules and are defined as plant panallergens, what provide them as markers universal in their use [32]. For legume species - soybean and groundnut genotypes, profilins were previously utilized by using degenerated primer pair in PBAP (profilin based amplified polymorphism) [25, 26]. For both of this species, polymorphic profiles were generated. In the set of groundnut accessions, all were distinguishable but profiling based marker technique, but in soybean, a couple of varieties (Sciaming and Krajina) produced the same fingerprint profiles. PBAP fingerprints of *Arachis hypogaea* L. were distributed in the length ranging from 78 bp up to the 1642 bp and for soybean from 118 bp up to 1000 bp. This correspond to the finding of this study, that profilin based fingerprints are specie specific for legumes with different levels of polymorphism generated. Such differences in the ability of generating polymorphism and distinguishing of analyzed genotypes are in concordance with natural variability of profiling homologs in plants. Multiple proteins belonging to profilins were found to have their sequences in higher plants. They are divided into two classes that differ in their distinct expression patterns, mainly in vegetative and reproductive plant tissues [33]. Actuallly, more than four hundred plant profilin proteins sequences are stored at NCBI (National Center for Biotechnology Information) gene database [34]. It was reported previously that even small changes in the sequence of amino acid can alter the

biochemical properties of profilin substantially and a results in sequence diversity among profiling genes with their evolutionary origin in the polyphyletic mode [35].

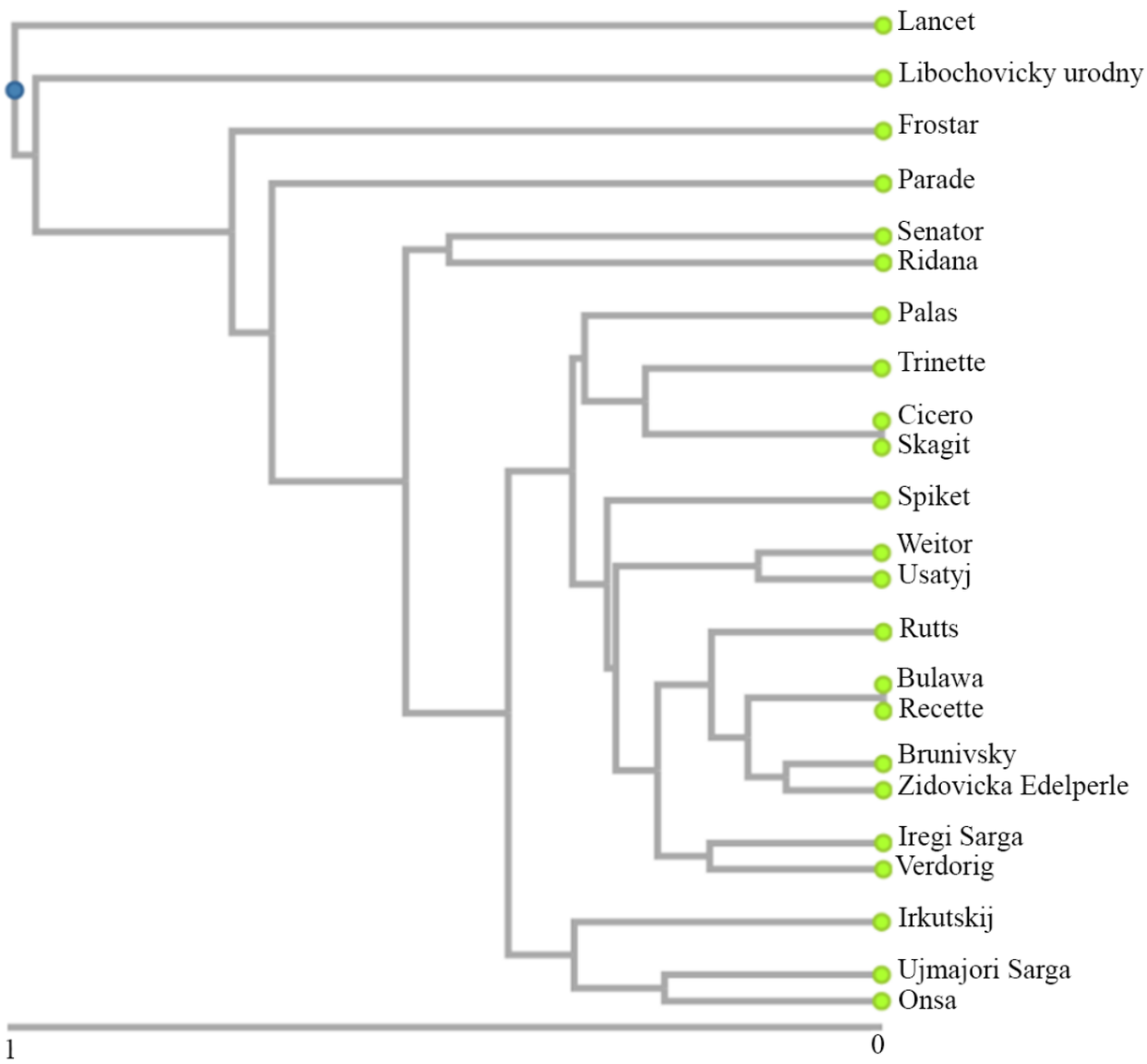

Figure 2. Dendrogram of pea accession using the profilin specific fingerprinting.

The results obtained in this study provide data that profilin based generated polymorphism characteristics provide a good source of germplasm variability in the analysis of legumes.

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