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Proceeding Paper

Genetic Diversity of Oat Genotypes Using SCoT Markers †

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Abstract: The aim of this study was to analyze the genetic variability of 22 oat genotypes, of which 20 genotypes were common oats and 2 were naked oats, using 7 SCoT primers. Out of 40 fragments that were totally amplified, 26 were polymorphic, with an average number of 3.71 polymorphic fragments per genotype. The average percentage of polymorphism was 65.67%. The value of the polymorphic information content (PIC) ranged from 0.305 (SCoT12) to 0.674 (SCoT8), with an average value of 0.506. Using hierarchical cluster analysis, a dendrogram was constructed. The genotypes of oats were divided into two main groups. Two naked oats (*Avena nuda* L.), Czech genotype Izak and Slovak genotype Hronec, grouped side by side in subcluster Ia. The used SCoT markers showed the ability to identify and differentiate genotypes of the common and naked oats.

Keywords: *Avena* L.; gene-specific markers; genetic variability; dendrogram; polymorphism

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1. Introduction

Cereals belong to the group of key foods of plant production. Thanks to their nutritional value and high usability, they are grown all over the world. Oat together with maize and barley is the most important cereal used for livestock feed, but it is still underappreciated in human nutrition. Oat belongs to the alternative cereals that are most often used as a supplement to traditional types of cereals [1]. Due to its resistance to cold weather and its ability to grow on poor soil, oat has earned a place among traditional cereals [2]. The soluble component of oat fiber helps to lower cholesterol and glucose in the blood, helps the proper functioning of the gastrointestinal tract, reduces the risk of colon cancer and supports the body's immunity [3]. Along with wheat, oat is the most clinically studied cereal associated with celiac disease. Although it belongs to the same group of cereals as wheat, barley and rye, its protein fractions differ, and it is similar to pseudocereals, which are non-toxic for celiac people [3,4].

Recently, modern breeding methods have come to the fore, in which it is possible to monitor hereditary changes at the DNA level. There is currently a large number of techniques for detecting DNA polymorphisms, each of which has its advantages and disadvantages [5]. The advantages of these tools are knowledge of gene functions and the ability to map the entire genome. Amplification techniques based on polymerase chain reaction are the most often used techniques to identify and differentiate individual varieties [6]. These techniques include AFLP, RAPD, ISSR and STMS [5–9], and in recent years, also the SCoT technique [10]. The ideal technique should provide accurate and reproducible results in a short time and, at the same time, should not be costly. The SCoT

technique has the potential among various DNA marker systems to gain popularity and dominance over techniques such as RAPD and ISSR due to its higher polymorphism and ability to map the coding region of the genome [11,12]. It is already considered a useful DNA marker system that is highly conserved in all plant species. The suitability of the SCoT marker system has been successfully employed in genetic diversity analysis and fingerprinting of the number of agricultural and horticultural crop species, such as pepper [13], ramie [11] and castor [12,14], but also many cereals, such as wheat [15], maize [16], rye [17] and oat [18,19].

The aim of this work was to identify and characterize 22 genotypes of oat (*Avena* L.) using 7 SCoT markers, determine the genetic relationships of the analyzed oat samples and verify the effectiveness and usability of the SCoT method for the identification and differentiation of the analyzed genotypes of oat.

2. Materials and Methods

2.1. Biological Material

Twenty common oat (*Avena sativa* L.) and two naked oat (*Avena nuda* L.) genotypes were used in this study originating from ten different countries in the world (Russia—1 genotype; Austria—1 genotype; Poland—3 genotypes; Czech Republic—2 genotypes; Czechoslovakia—1 genotype; Canada—4 genotypes; Germany—5 genotypes; Slovakia—2 genotypes; Sweden—2 genotypes; France—1 genotype). Seeds of oat were obtained from the Gene Bank of the Slovak Republic of the Plant Production Research Center in Piešťany. Genomic DNA of oat cultivars was isolated from 100 mg freshly collected seedlings according to the GeneJET^{TM} protocol (ThermoScientific, Waltham, MA, USA). The concentration and quality of DNA were checked on a Biodrop (Biochrom, Ltd., Cambridge, UK).

2.2. PCR Conditions

For analysis, 7 SCoT primers were chosen (Table 1) according to the literature [10]. Amplification of SCoT fragments was performed according to [10]. Polymerase chain reactions (PCRs) were performed in a 15 µL mixture in a programmed thermocycler (Biometra, Germany).

Table 1. List of used SCoT markers.

SCoT Primer	Sequence of Primers (5′-3′)	Anealing Temperature [°C]
SCoT 8	CAACAATGGCTACCACGT	50 °C
SCoT 9	CAACAATGGCTACCAGCA	50 °C
SCoT 12	ACGACATGGCGACCAACG	50 °C
SCoT 23	CACCATGGCTACCACCAG	50 °C
SCoT 26	ACCATGGCTACCACCGTC	50 °C
SCoT 28	CCATGGCTACCACCGCCA	50 °C
SCoT 29	CCATGGCTACCACCGGCC	50 °C

2.3. Electrophoresis of DNA

Amplified fragments were separated on 1.5% agarose gels in 1× TBE buffer. The gels were stained with ethidium bromide and documented using a gel documentation system, UVP PhotoDoc-t®. The size of amplified fragments was determined by comparing with the standard length marker Quick-Load® Purple 2-Log DNA ladder (New England Biolabs, Inc., Ipswich, MA, USA).

2.4. Data Analysis

The data from electrophoregrams were converted to binary matrices on the basis of the presence (1) or absence (0) of each fragment.

Based on the number of alleles per locus and their frequency, values of the polymorphism information content (PIC) for each marker [20] were calculated.

A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS professional statistics version 17 software package was constructed.

3. Results and Discussion

For the detection of the genetic diversity of 22 oat genotypes, 7 SCoT markers were used. In total, 40 DNA fragments were detected, of which 26 were polymorphic (Table 2), with an average number of polymorphic fragments of 3.71. Using the marker SCoT8, the highest number of polymorphic fragments (5) was detected. The highest percentage of polymorphic bands (80%) was detected by SCoT23, and the lowest (33.33%) was detected by the SCoT12 marker. The average percentage of polymorphic bands was 65.67%. The size of the DNA fragments ranged from 250 to 3000 bp and was estimated using a 2-Log DNA length marker.

Table 2. Statistical characteristics of the SCoT markers used in oat.

SCoT Marker	Number of All Fragments	Number of Polymorphic Fragments	Percentage of Polymorphic Bands (%)	PIC
SCoT8	7	5	71.42	0.674
SCoT9	6	4	66.66	0.537
SCoT12	6	2	33.33	0.305
SCoT23	5	4	80.00	0.390
SCoT26	6	4	66.66	0.547
SCoT28	4	3	75.00	0.519
SCoT29	6	4	66.66	0.567
Average	5.71	3.71	65.67	0.506
Total	40	26		

A higher percentage of polymorphism was detected in the work by [11], who determined the genetic diversity in 155 varieties of ramie *(Boehmeria nivea* L. Gaudich.) using 24 SCoT markers. SCoT markers produced 136 amplicons with 87.5% polymorphism. An example of a high percentage of polymorphism was also offered by the work of [13], in which the authors analyzed annual pepper (*Capsicum annum* L.) using SCoT and ISSR markers. Using SCoT markers, 48 of the 53 amplified fragments in the pepper genotypes were determined to be polymorphic. The average percentage of polymorphism reported was up to 88.99%. Lower polymorphism compared to our results was reported by [12], who analyzed the genetic diversity of common ricin using SCoT markers. They detected 108 fragments in total, of which 23 (21%) were polymorphic. The authors of [19] detected lower polymorphism (46.55%) in an analysis of 36 oat genotypes using 5 SCoT primers.

The polymorphic information content (PIC) is the basic indicator that characterizes molecular markers by the frequency and diversity of fragments in individual genotypes. The PIC values ranged from 0.305 (SCoT12) to 0.674 (SCoT8), with an average value of 0.506. The most suitable marker was the SCoT8 marker, where the PIC values were higher than 0.6, and it thus showed the highest polymorphism of all the SCoT markers tested.

Higher PIC values were reported in studies of crops such as pepper [13], castor [14], durum wheat [15], maize [16] and rye [17].

In [17], genetic variability among a set of 45 rye genotypes was detected using 8 SCoT markers. The average PIC value of the SCoT primers used was estimated at 0.835, which indicates the high resolving power of the used molecular markers. The authors proved the SCoT technique to be a rapid, reliable and practicable method for revealing polymorphism in rye cultivars. The authors of [16] evaluated the genetic variability of 40 maize genotypes originating from different European countries using 20 SCoT markers. The average PIC value obtained from the SCoT analysis was 0.739. In that study, the authors proved the usefulness of the used SCoT technique as a successful method for estimating the genetic diversity of old maize genotypes and recommended it for the conservation of genetic

resources. Higher PIC values were also reported by [11], with PIC values ranging from 0.25 to 0.93, with an average value of 0.69, who detected the genetic diversity of ramie genotypes (*Boehmeria nivea* L. Gaudich.) using 24 SCoT markers. In contrast, lower PIC values were obtained in the study by [13], who analyzed the genetic variability of thirty varieties of annual pepper (*Capsicum annum*) using SCoT and ISSR markers. Six SCoT markers used showed an average PIC value of 0.212, which is a lower value compared to our analysis. A lower average PIC value (0.24) compared to our results was reported by [12], who analyzed the genetic diversity of common ricin using SCoT markers. A lower average PIC value (0.154) was also obtained by [19] in the analysis of 36 oat genotypes using 5 SCoT primers. They explained the low values of the PIC and low percentage of polymorphism as the result of a relatively narrow gene pool, with genotypes originating from three different countries sharing borders.

Using data obtained from DNA analysis of oat genotypes, a dendrogram was prepared. The dendrogram was constructed based on the principle of hierarchical cluster analysis using the UPGMA algorithm. Genotypes were divided in two main clusters (I, II), with cluster I composed of 13 genotypes and cluster II composed of 9 genotypes, which were further subdivided into subclusters (Ia, Ib, IIa, IIb). The genetic relationships between individual genotypes were revealed in the dendrogram. In subgroup Ia, genotypes Amur originating from Germany and Amursky utes originating from Russia were genetically the closest based on analyses (Figure 1). Two genotypes of naked oat (Hronec, Izak) grouped closely in subgroup Ia.

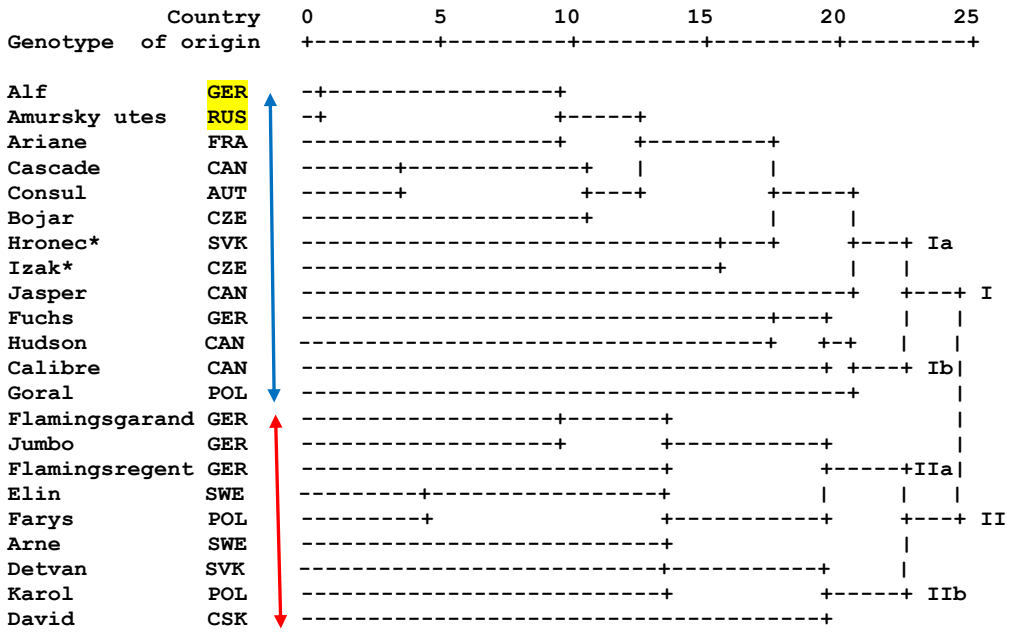

Figure 1. Dendrogram of 22 oat genotypes based on 7 SCoT markers. Note: RUS—Russia; AUT—Austria; POL—Poland; CZE—Czech Republic; CSK—Czechoslovakia; CAN—Canada; GER—Germany; SVK—Slovakia; SWE—Sweden; FRA—France. Genotypes marked * are species of *Avena nuda* L. (Hronec, Izak).

The authors of [17] constructed a dendrogram of a set of 45 rye genotypes using hierarchical cluster analysis, where rye genotypes were divided into two main clusters. They concluded that the used SCoT markers could distinguish between the various *Secale* species. The authors of [16] analyzed 40 old genotypes of maize from different European countries using 20 SCoT markers and constructed a dendrogram based on hierarchical cluster analysis using the UPGMA algorithm. In the dendrogram, the maize genotypes divided into two main clusters. They concluded that the polymorphism revealed by the SCoT technique was so abundant and could be used for molecular genetic study of the

maize accessions, the improvement in the current breeding strategies and conservation of old maize genotypes. In order to determine the genetic diversity, a dendrogram of thirty annual pepper genotypes (*Capsinul annum* L.) was constructed in the study of [13]. Genetic variability between 30 genotypes and 1 commercial Greek cultivar for industrial use was evaluated using SCoT markers. All genotypes were clearly distinguished in the dendrogram. The authors suggested that genotyping of Greek peppers using molecular markers will help farmers to select higher-quality and productivity cultivars. In the analysis of 36 oat genotypes, ref. [19] used only 5 SCoT primers to construct a UPGMA dendrogram. The authors were able to differentiate oat genotypes and thus confirm the applicability of SCoT markers in the analysis of oat genotypes.

4. Conclusions

The average value of the PIC for the used SCoT markers was higher than 0.5, which means that sufficient polymorphism was detected in the chosen oat genotypes. In the UPGMA dendrogram, 22 oat genotypes were divided into two main clusters (I, II). It was possible to distinguish all analyzed genotypes of oat in the constructed dendrogram based on seven SCoT markers. The two genetically closest varieties, Alf originating from Germany and Amursky utes originating from Russia, grouped in subcluster Ia. Two genotypes of naked oat (Hronec, Izak) grouped closely in subgroup Ia. SCoT markers were revealed as a powerful tool for the assessment of genetic diversity in oat cultivars.

Based on the results obtained, SCoT markers showed sufficient polymorphism between the observed genotypes of common and naked oats, so the technique is suitable for the identification and differentiation of genotypes of common and naked oats. SCoT markers were revealed to be suitable for application in the process of breeding and detecting new genotypes containing important genes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECPS2021-11926/s1.

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