biology and life sciences forum

MDPI

Proceeding Paper

Genetic Diversity of Oat Genotypes Using SCoT Markers †

Milan Chnapek [1], Lucia Mikolasova [1], Martin Vivodik [1], Zdenka Galova [1], Zuzana Hromadova [1], Katarina Razna [2] and Želmíra Balážová [1,*]

[1] Department of Biochemistry and Biotechnology, Institute of Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; milan.chnapek@uniag.sk (M.C.); xmikolasova@uniag.sk (L.M.); martin.vivodik@uniag.sk (M.V.); zdenka.galova@uniag.sk (Z.G.); xhromadovaz@uniag.sk (Z.H.)

[2] Department of Genetics and Plant Breeding, Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia; Katarina.Razna@uniag.sk

* Correspondence: Zelmira.Balazova@uniag.sk

† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: The aim of the study was to analyze the genetic variability of 22 oat genotypes, of which 20 genotypes were common and two naked oats, using 7 SCoT primers. Out of 40 fragments 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 polymorphic information content (PIC) ranged from 0.305 (SCoT12) to 0.674 (SCoT8) with an average value of 0.506. Using hierarchical cluster analysis the 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. Used SCoT markers showed the ability to identify and differentiate genotypes of the common and naked oat.

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

Citation: Chnapek, M.; Mikolasova, L.; Vivodik, M.; Galova, Z.; Hromadova, Z.; Razna, K.; Balážová, Ž. Genetic Diversity of Oat Genotypes Using SCoT Markers. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Fernando Lidon

Published: 29 November 2021

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, are our 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, oats have earned a place among traditional cereals [2]. The soluble component of oat fibre 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, oats are the most clinically studied cereal associated with celiac disease. Although it belongs to the cereals as wheat, barley and rye, its protein fractions differ and 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 large number of techniques for detecting DNA polymorphisms, each of which have 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 the polymerase chain reaction are the most often used to identify and differentiate individual varieties [6]. There are included techniques like AFLP, RAPD, ISSR, 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 for 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. Suitability of SCoT markers system has been successfully employed in genetic diversity analysis and fingerprinting of number of agricultural and horticultural crop species, such as pepper [13], ramie [11], castor [12,14], but also many cereals, such as wheat [15], maize [16], rye [17], but also oat [18,19] and many others.

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 analysed oat samples and verify the effectiveness and usability of the SCoT method for identification and differentiation of the analysed 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 the 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 GeneJET™ protocol (ThermoScientific, USA). The concentration and quality of DNA was checked up on Biodrop (Biochrom, Ltd.**)**.

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 (PCR) were performed in 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 in 1,5% agarose gels in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system UVP PhotoDoc-t®. Size of amplified fragments was determined by comparing with standard lenght marker Quick-Load® Purple 2-Log DNA ladder (New England Biolabs, Inc.).

2.4. Data Analysis

The data from electrophoreograms were converted to binary matice on the base of presence (1) or absence (0) of each fragment.

Based on the number of alleles per locus and their frequency, values of 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 genetic diversity of 22 oat genotypes 7 SCoT markers were used. Totally 40 DNA fragments were detected of which 26 were polymorphic (Table 2) with an average number of polymorphic fragments 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 in the SCoT12 marker. 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 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 is also offered by the work of [13], in which they analysed 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, respectively. The average percentage of polymorphism studies reported was up to 88.99%. Lower polymorphism compared to our results was reported by [12], who analysed the genetic diversity of common ricin using SCoT markers. They detected totally 108 fragments of which 23 (21%) were polymorphic. [19] detected lower polymorphism (46.55%) in the analysis of 36 oat genotypes using 5 SCoT primers.

The polymorphic information content (PIC) is the basic indicator that characterize 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 thus showed the highest polymorphism of all the SCoT markers tested.

Higher PIC values were reported in a study of crops like pepper [13], castor [14], durum wheat [15], maize [16], rye [17] and others.

In the study [17] detected genetic variability among the set of 45 rye genotypes using 8 SCoT markers. The average PIC value of the SCoT primers used was estimated at 0.835 that indicates high resolving power of used molecular markers. They have proved the SCoT technics to be a rapid, reliable and practicable method for revealing of polymorphism in the rye cultivars. [16] evaluated the genetic variability of 40 maize genotypes originating from different European countries using 20 SCoT markers. An average PIC value obtained from the SCoT analysis was 0.739. In the study they have proven the

usefulness of used SCoT technique as a successful method for estimating the genetic diversity of old maize genotypes and recommended it for the conservation of the genetic resources. Higher PIC values were reported also by [11] with PIC values ranged 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 analysed 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. Lower average PIC value (0.24) compared to our results was reported by [12], who analysed the genetic diversity of common ricin using SCoT markers. Lower average PIC value (0.154) obtained also [19] in the analysis of 36 oat genotypes using 5 SCoT primers. They explained low values of PIC and percentage of polymorphism by a relatively narrow gene pool, 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), cluster I with 13 genotypes and cluster II with 9 genotypes which were further subdivided to the subclusters (Ia, Ib, IIa, IIb). The genetic relationships between individual genotypes were revealed in the dendrogram. In subgroup Ia genotypes Amur originated from Germany and Amursky utes originated from Russia were genetically the closest based on analyses (Figure 1). Two genotypes of naked oats (Hronec, Izak) grouped closely in the subgroup Ia.

[17] constructed dendrogram of the set of 45 rye genotypes using hierarchical cluster analysis where rye genotypes divided into two main clusters. They conclude that used SCoT markers could distinguish between the various *Secale* species. [16] analysed 40 old genotypes of maize from different European countries using 20 SCoT markers and constructed the dendrogram based on hierarchical cluster analysis using UPGMA algorithm. In the dendrogram the maize genotypes divided into two main clusters. They concluded that the polymorphism revealed by SCoT technique was so abundant and could be used for molecular genetic study of the maize accessions, and for the improvement of 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 one commercial Greek cultivar for industrial use was evaluated using SCoT markers. All genotypes were clearly distinguished in the dendrogram. The authors suggest that genotyping of Greek peppers using molecular markers will help farmers to select higher quality and productivity cultivars. [19] in the analysis of 36 oat genotypes used only 5 SCoT primers to construct UPGMA dendrogram. They were able to differentiate oat genotypes and thus confirm the applicability of SCoT markers in analysis of oat genotypes.

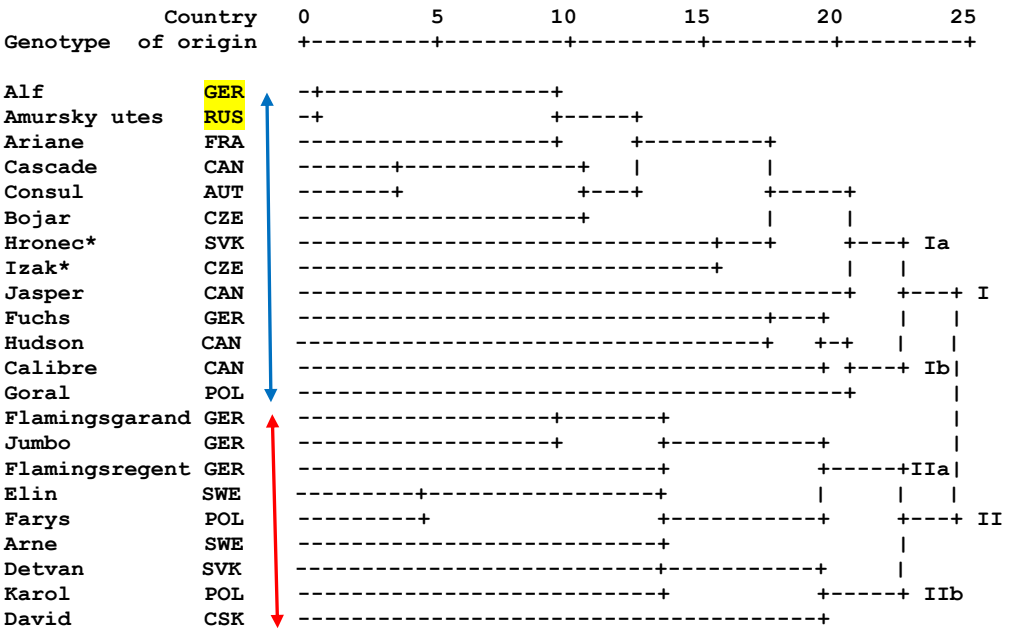

Figure 1. Dendrogram of 22 oat genotypes based on 7 SCoT markers. Note: RUS—Russia, AUT—Austria, POL—Poland, CZE—Czech Republik, CSK—Csechoslovakia, CAN—Canada, GER—Germany, SVK—Slovakia, SWE—Sweden, FRA—France. Genotypes marked * are species of *Avena nuda* L. (Hronec, Izak).

4. Conclusions

Average value of PIC for used SCoT markers was higher than 0.5, that means sufficient polymorphism 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 analysed genotypes of oat in the constructed dendrogram based on 7 SCoT markers. Genetically the closest were two varieties, Alf originating from Germany and Amursky utes originating from Russia, grouped in the subcluster Ia. Two genotypes of naked oats (Hronec, Izak) grouped closely in the subgroup Ia. SCoT markers revealed as a powerful tool for 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 identification and differentiation of genotypes of common and naked oats. SCoT markers reveal to be suitable for application in the process of breeding and detecting new genotypes containing important genes.

Author Contributions: L.M., M.V., K.R., Z.H., Z.G. methodology and investigation; Ž.B., M.C. writing and editing; M.C., software. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author (Želmíra Balážová) upon reasonable request.

Acknowledgments: This publication was supported by the project VEGA No. 1/0291/21, KEGA No. 026SPU-4/2021, KEGA No. 027SPU-4/2021 and by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and inovative food, Drive4SIFood 313011V336, cofinanced by the Euruopean Regional Development Fund.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Daou, C.; Zhang, H. Oat Beta-Glucan: Its Role in Health Promotion and Prevention of Diseases. *Compr. Rev. Food Sci. Food Safety* **2012**, *11*, 355–365.
2. Boczkowska, M.;, Tarczyk, E. Genetic diversity among Polish landrances of common oat (*Avena sativa* L.). *Gen.Res.Crop Rev.* **2013**, *60*, 2157–2169.
3. Gálová, Z.; Palenčáková, E.; Chňapek, M.; Balážová, Ž. *Use of Cereals, Pseudo-Cereals and Legumes in a Gluten-Free Diet*, 1st ed; Slovak University of Agriculture: Nitra, Slovakia, 2011; pp. 38–39.
4. Gaborská, D. Can celiacs consume oats—A still solved and unresolved issue. *Nutr. Food.* **2007**, *62*, 162–163.
5. Gálová, Z.; Balážová, Ž.; Chňapek, M.; Vivodík, M.; Oslovičová, V. *Protein and DNA Markers of Wheat*, 1st ed; Slovak University of Agriculture: Nitra, Slovakia, 2011; pp. 86–96.
6. Gregáňová, Ž. *Molecular Markers in the Identification and Characterization of Summer Wheat*; Dissertation work; University of Agriculture: Nitra, Slovak, 2005.
7. Vivodík, M.; Balážová, Ž.; Gálová, Z.; Kuťka Hlozáková, T. Differentiation of ricin using RAPD markers. *Pak. J. Bot.* **2015**, *47*, 1341–1345.
8. Vyhnánek,T.; Trojan, V.; Štiasna, K.; Presinszká, M.; Hřivna, L.; Mrkvicová, E.; Havel, L. Testing of DNA isolation for the identification of Hemp. *Potravin. Slovak J. Food Sci.* **2015**, *9*, 393–397.
9. Žiarovská, J.; Senková, S.; Beţo, M.; Ražná, K.; Masnica, M.; Labajová, M. ISSR markers as a tool to distinguish Idt and SSS populations of Zea mays L. *J. Cent. Eur. Agric.* **2013**, *14*, 489–499.
10. Collard, B.Y.;, Mackill, D. Start codon targeted (SCoT) polymorphism: Asimple, novel DNA marker technique for generating gene-targeted markers inplants. *Plant Mol. Biol. Rep.* **2009**, *27*, 86–93.
11. Satya, P.; Karan, M.; Jana, S.; Mitra, S.; Sharma, A.; Karmakar, P.G.; Rayb, D.P. Start codon targeted (SCoT) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (*Boehmeria nivea* L. Gaudich.), a premium textile fiberproducing species. *Meta Gene* **2015**, *3*, 62–70.
12. Kallamadi, P.R.; Nadigatlab, V.P.R.; Mulpuriba, S. Molecular diversity in castor (*Ricinus communis*). *Ind. Crop. Prod.* **2015**, *66*, 271–281.
13. Tsaballa, A., Ganopoulos, I., Timplalexi, A., Aliki, X., Bosmali, I., Irini, N.O., Athanasios, T., Madesis, P. Molecular characterization of Greek pepper (Capsicumannuum L) landraces with neutral (ISSR) and gene-based (SCoT and EST-SSR) molecular markers. *Biochem. Syst. Ecol.* **2015**, *59*, 257–259.
14. Vivodík, M.; Saadaoui, E.; Balážová, Ž.; Gálová, Z.; Petrovičová, L. Genetic diversity and population structure in Tunisian castor genotypes (*Ricinus communis* L.) detected using SCoT markers. *Potravin. Slovak J. Food Sci.* **2018**, *12*, 143–149.
15. Etminan, A.; Pour-Aboughadareh, A.; Mohammadi, R.; Ahmadi-Rad, A.; Noori, A.; Mahdavian, Z.; Moradi, Z. Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnol. Biotechnol. Equip.* **2016**, *30*, 1075–1081.
16. Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L. Start codon targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) genotypes. *Potravin. Slovak J. Food Sci.* **2016**, *10*, 563–569.
17. Petrovičová, L.; Balážová, Ž.; Vivodík, M.; Gálová, Z. Detection genetic variability of *Secale cereale* L. by scot markers. *Potravin. Slovak J. Food Sci.* **2017**, *11*, 197–202.
18. Balážová, Ž.; Gálová, Z.; Vivodík, M.; Petrovičová, L.; Hornyák Gregáňová, R. Molecular variability of oat based on gene specific markers. *Potravin. Slovak J. Food Sci.* **2017**, *11*, 332–337.
19. Cieplak, M.; Okoń, S.; Werwińska, K. Genetic Similarity of *Avena sativa* L. Varieties as an Example of a Narrow Genetic Pool of Contemporary Cereal Species. *Plants* **2021**, *10*, 1424.
20. Weber, J.L. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics* **1990**, *7*, 524–530.