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MOLECULAR VARIABILITY OF OAT BASED ON GENE SPECIFIC MARKERS

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ABSTRACT

Oat *(Avena sativa* L.*)* is a grass planted as a cereal crop. Cultivation of oat is increasing in the recent years because of its good nutrition value. The aim of our study was to analyze genetic variability of oat accessions based on SCoT markers. Eighteen primers were used to study polymorfism of 8 oat genotypes. All 18 primers produced polymorphic and reproducible data. Altogether 153 different fragments were amplified of which 67 were polymorphic with an average number of 3.72 polymorphic fragments per genotype. The number of polymorphic fragments ranged from one (SCoT9, SCoT62) to nine (SCoT40). The percentage of polymorphic bands ranged from 14.29% (SCoT9) to 60% (SCoT59) with an average of 41.62%. Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). The diversity index of the tested SCoT markers ranged from 0 (SCoT9, SCoT62) to 0.878 (SCoT40) with an average of 0.574. The polymorphic information content ranged from 0 (SCoT9, SCoT62) to 0.876 (SCoT40) with an average of 0.524. Dendrogram based on hierarchical cluster analysis using UPGMA algorithm grouped genotypes into two main clusters. Two genotypes, Taiko and Vok were genetically the closest. Results showed the utility of SCoT markers for estimation of genetic diversity of oat genotypes leading to genotype identification.

Keywords: *Avena sativa* L.; SCoT technique; genetic diversity; polymorphism; dendrogram

INTRODUCTION

Cereals belong to a group of key foods of plant production. Oat together with corn and barley is the most used for feed but for human nutrition is used only a little. Cultivated oats are hexaploid cereals belonging to the genus *Avena* L., which is found worldwide in almost all agricultural environments. Recently, oats have been receiving increasing interest as human food, mainly because the cereal could be suitable for consumptions by celiac patients **(Gálová et. al., 2012)**. In the Nordic countries and Northern Europe it became a well-established crop both for food and feed. Oat belongs to alternative cereals which are used mainly as a supplement to traditional species of cereals **(Daou and Zhang, 2012)**.

Recently, the studies of genetic diversity based mainly on the molecular analysis. Worldwide collections of oats were described by several types of dominant molecular markers, for example AFLP **(Fu et al., 2003)**, RAPD **(Baohong et al., 2003)** and ISSR **(Boczkowska and Tarczyk, 2013)**. With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called SCOT **(Collard and Mackill, 2009)** was developed based on the short conserved region flanking the ATG start codon in plant genes. SCOT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis **(Collard and Mackill, 2009)**. In principle, SCOT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer **(Collard and Mackill, 2009; Gupta et al. 1994)**. SCoT marker system has gained popularity for its superiority over other dominant DNA marker systems like RAPD and ISSR for higher polymorphism and better marker resolvability **(Gorji et al., 2011; Que et al. 2014; Satya et al., 2015; Zhang et al., 2015)**. Suitability of SCoT markers system has been successfully employed in genetic diversity analysis and fingerprinting of a number of agricultural and horticultural crop species, such as peanut **(Xiong at al., 2011)**, tomato **(Shahlaei et al., 2014)**, citrus **(Mahjbi et al., 2015)**, date palm **(Al-Qurainy et al., 2015)**, ramie **(Satya et al., 2015)**, castor **(Kallamadi et al., 2015)**, maize **(Vivodík et al., 2016)** and mango **(Gajera et al., 2014)**.

The aim of our study was to detect genetic variability among the set of 8 oat genotypes using 18 SCoT markers and to testify the usefulness of a used set of SCoT primers for the identification and differentiation of oat genotypes.

MATERIAL AND METHODOLOGY

Eight oat (*Avena sativa* L.) genotypes were used in the present study. Seeds of oat were obtained from the Gene Bank of the Slovak Republic of the Plant Production Research Center in Piešťany. Genomic DNA of rye cultivars was isolated from 100 mg freshly-collected leaf tissue according to GeneJETTM protocol (Fermentas, USA). The concentration and quality of DNA was checked up on 1.0% agarose gel coloured by ethidium bromide and detecting by comparing to λ-DNA with known concentration.

SCoT analysis: For analysis 18 SCoT primers were chosen (Table 2) according to the literature **(Collard a Mackill, 2009)**. Amplification of SCoT fragments was performed according to **(Collard a Mackill, 2009)** (Table 2.). Polymerase chain reaction (PCR) was performed in 15 μL mixture in a programmed thermocycler (Biometra, Germany). Amplified products were separated in 1% 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 100 bp standard lenght marker (Promega).

Data analysis: For the assessment of the polymorphism between castor genotypes and usability of SSR markers in their differentiation diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)** were used. The SCoT bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands and to prepare a dendrogram. 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.

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. For detecting polymorphisms new molecular marker system called SCoT **(Collard a Mackill, 2009)** was developed which tag coding sequences of the genome. SCoT marker system had initially been validated in the model species rice (*Oryza sativa*) **(Collard and Mackill 2009)**.

For the molecular analysis of 8 oat genotypes 18 SCoT primers were used. PCR amplifications using 18 SCoT primers produced total 153 DNA fragments that could be scored in all genotypes. The selected primers amplified DNA fragments across the 8 genotypes studied with the number of amplified fragments varying from 4 (SCoT62)

Table 1 List of analyzed genotypes of oat.

No.	Genotype of oat (*Avena* spp. L.)	Country of origin	Breeding year
1.	Azur	Czech Republic	2004
2.	Dalimil	Czech Republic	2004
3.	Vok	Czech Republic	2004
4.	Revisor	Netherland	1996
5.	Taiko	Netherland	2004
6.	Argentina	Italy	2004
7.	Euro	Austria	1995
8.	Vilma	Sweden	2004

Table 2 List of used SCoT markers.

SCoT primer	Sequence of primers (5´-3´)	Anealing temperature [°C]
SCoT 6	CAACAATGGCTACCACGC	50
SCoT 8	CAACAATGGCTACCACGT	50
SCoT 9	CAACAATGGCTACCAGCA	50
SCoT 12	ACGACATGGCGACCAACG	50
SCoT 23	CACCATGGCTACCACCAG	50
SCoT 26	ACCATGGCTACCACCGTC	50
SCoT 28	CCATGGCTACCACCGCCA	50
SCoT 29	CCATGGCTACCACCGGCC	50
SCoT 30	CCATGGCTACCACCGGCG	50
SCoT 36	GCAACAATGGCTACCACC	50
SCoT 40	CAATGGCTACCACTACAG	50
SCoT 44	CAATGGCTACCATTAGCC	50
SCoT 45	ACAATGGCTACCACTGAC	50
SCoT 54	ACAATGGCTACCACCAGC	50
SCoT 59	ACAATGGCTACCACCATC	50
SCoT 60	ACAATGGCTACCACCACA	50
SCoT 61	CAACAATGGCTACCACCG	50
SCoT 62	ACCATGGCTACCACGGAG	50
SCoT 63	ACCATGGCTACCACGGGC	50
SCoT 65	ACCATGGCTACCACGGCA	50

to 17 (SCoT40) and the amplicon size varied from 200 to 3000 bp. Of the 153 amplified bands, 67 were polymorphic with an average of 3.72 fragments per primer (Table 3). The percentage of polymorphic bands ranged from 14.29% (SCoT9) to 60% (SCoT59) with an average of 41.62%. The polymorphic information content (PIC) values varied from 0 (SCoT9, SCoT62) to 0.876 (SCoT40) with an average of 0.524 and index diversity (DI) value ranged from 0 (SCoT9, SCoT62) to 0.878 (SCoT40) with an average of 0.574 (Tab.3). The most polymorphic SCoT40 marker is showed on Figure 2.

A dendrogram was constructed from a genetic distance matrix based on profiles of the 18 SCoT primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 8 diverse accessions of oat was clustered into two main clusters (Figure 1). The first cluster contained unique genotype Azur coming from the Czech Republik. Second cluster contained 7 genotypes of oat which were further subdivided into two subclusters (2a, 2b). Subcluster 2a contained unique Austrian genotype Euro and rest of genotypes (6) were included in the subcluster 2b. Genetically the closest were two genotypes, Vok (coming from the Czech Republik) and Taiko (coming from Netherland).

Lower average polymorphism (21%) obtained by SCoT technique was detected by **Kallamadi et al. (2015)** who analysed molecular diversity of castor (*Ricinus communis* L.). Out of 36 SCoT primers tested, all primers produced amplification products but only 10 primers resulted in polymorphic fingerprint patterns. Out of a total of 108 bands, 23 (21%) were polymorphic with an average of 2.1 polymorphic bands per primer. The total number of bands per primer varied from 5 and 20 in the molecular size range of 100 – 3000 bp. The PIC/DI varied from 0.06 for SCoT28 to 0.45 for SCoT12 with an average of 0.24.

On the other side, higher polymorphism with SCoT primers has been reported in crops like peanut **(Xiong et**

Table 3 Statistical characteristics of the SCoT markers used in oat.

SCoT marker	Number of all fragments	Number of polymorphic fragments	Percentage of polymorphic bands (%)	DI	PIC	PI
SCoT6	8	4	50.00	0.715	0.697	0.026
SCoT8	8	2	25.00	0.486	0.368	0.160
SCoT9	7	1	14.29	0.000	0.000	1.000
SCoT12	5	2	40.00	0.500	0.375	0.375
SCoT23	6	2	33.33	0.497	0.374	0.376
SCoT26	8	3	37.50	0.561	0.467	0.285
SCoT28	10	5	50.00	0.719	0.688	0.087
SCoT29	10	4	40.00	0.722	0.690	0.091
SCoT30	12	7	58.33	0.778	0.769	0.034
SCoT36	7	4	57.14	0.648	0.589	0.176
SCoT40	17	9	52.94	0.878	0.876	0.002
SCoT54	9	3	33.33	0.625	0.586	0.148
SCoT59	10	6	60.00	0.741	0.735	0.040
SCoT60	6	2	33.33	0.500	0.375	0.375
SCoT61	8	4	50.00	0.613	0.542	0.078
SCoT62	4	1	25.00	0.000	0.000	1.000
SCoT63	9	3	33.33	0.667	0.617	0.038
SCoT65	9	5	55.56	0.688	0.681	0.046
Average	8.50	3.72	41.62	0.574	0.524	0.241
Total	153	67				

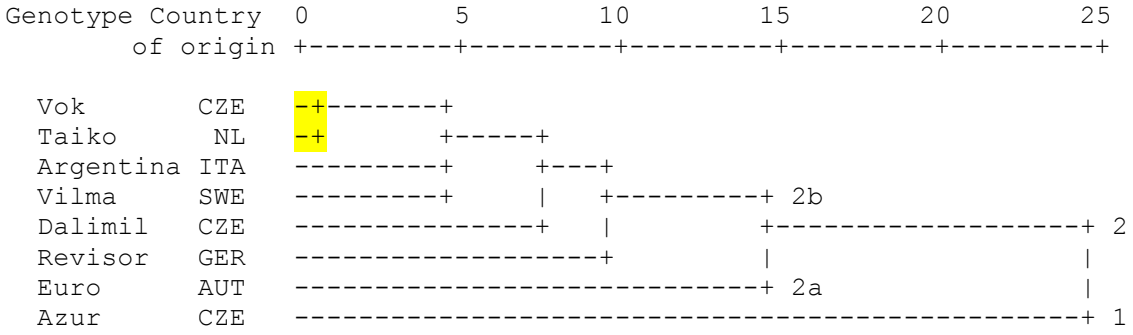

Figure 1 Dendrogram of 8 oat genotypes prepared based on 18 SCoT markers.

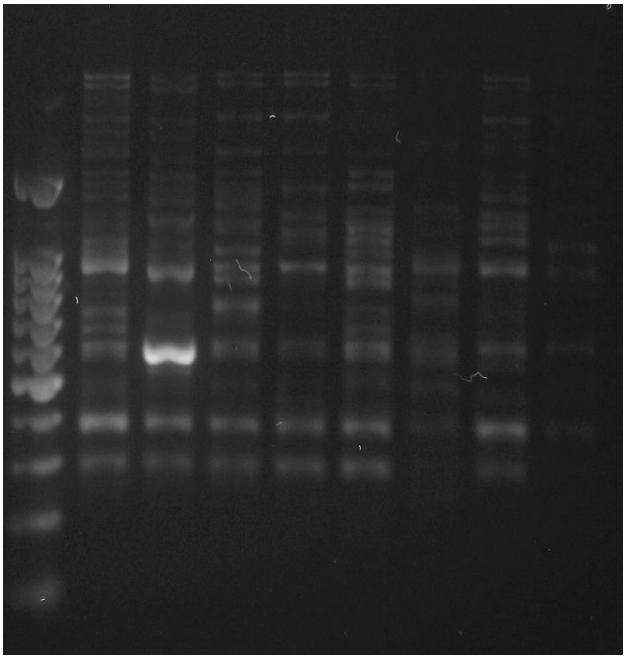

Figure 2 Electrophoreogram of SCoT40 marker.

al., 2011), cicer **(Amirmoradi et al., 2012)**, mango **(Luo et al., 2010)**, ramie **(Satya et al., 2015)**, sugarcane **(Que et al., 2014)**, Chinese bayberry **(Fang-Yong and Ji-Hong, 2014)**, pepper **(Tsaballa et al., 2015)**, castor **(Kallamadi et al., 2015)**, maize **(Vivodík et al., 2016)**.

Satya et al. (2015) used 20 SCoT markers to assess genetic diversity and population structure of indigenous, introduced and domesticated ramie (*Boehmeria nivea* L. Gaudich.). A total of 155 genotypes from five populations were investigated for SCoT polymorphism, which produced 136 amplicons with a range of 4 to 10 bands per primer, of which 119 (87.5%) were polymorphic. Percent polymorphism varied from 20% to 100%, with 3 – 10 polymorphic bands per primer. Polymorphism information content ranged from 0.25 to 0.93 with an average of 0.69. **Gajera et al. (2014)** used 19 SCoT primers for amplification among 20 cultivars which yielded a total of 117 clear and bright loci. Number of loci varied from 4 to 10 with an average of 6.16 loci per primer. Of 117 loci, 96 loci (79.57%) were found to be polymorphic, the number of polymorphic loci varied from 2 to 10 with an average of 5.05 loci per primer. The detected polymorphism per primer among the tested cultivars ranged from 50% (SCoT26) to 100 % (SCoT-33, SCoT-40, and SCoT-51). In our study we detected by SCoT26 primer of percentage of polymorphic bands 37.5%. Also **Luo et al. (2010)** found high percentage of polymorphism (76.2 %) using SCoT markers in analysis of diversity and relationships among mango cultivars. **Que et al. (2014)** used 20 SCoT primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. Tventy SCoT primers amplified 176 DNA fragments, of which 163 were polymorphic (92.85%). **Fang-Yong and Ji-Hong (2014)** assessed the genetic diversity of 31 germplasm resources of Myrica rubra of China using 38 SCoT markers. Authors detected 298 reproducible bands of which 251 were polymorphic (84.23%).

Level of polymorphism in analysed oat genotypes was also determined by calculated polymorphic information content (PIC) (Table 3). Lower PIC values compare to our analysis (0.524) were detected by **Tsaballa et al. (2015)** and **Kallamadi et al. (2015)**. **Tsaballa et al. (2015)** analyzed genetic variability among the 30 landraces and one commercial Greek cultivar of pepper (*Capsicum annuum* L.) using 6 SCoT primers. They detected PIC values ranged from 0.123 (SCoT33) to 0.258 (SCoT15), with an average value of 0.232 per primer. **Kallamadi et al. (2015)** detected average PIC/DI vales from 0.06 (SCoT28) to 0.45 (SCoT12) with an average of 0.24 in analysis of genetic diversity in 31 accessions of castor representing seven geo-graphic areas by 36 SCoT markers.

Similar values of PIC were detected by other authors **(Luo et al. 2010; Gajera et al. 2014; Que et al. 2014; Gao et al. 2014; Fang-Yong et al. 2014; Jiang et al. 2014; Huang et al. 2014; Satya et al. 2015, Hajibarat et al., 2015)** and these values presented a high level of polymorphism of genotypes detected by SCoT markers. **Huang et al. (2014)** assessed the genetic diversity of six Hemarthria cultivars using seven SCoT primers. They calculated PIC values ranged from 0.471 to 0.758 with an average of 0.612. **Hajibarat et al. (2015)** used a set of 9 SCoT primers to fingerprint 48 chickpea genotypes. PIC values ranged from 0.43 to 0.47 with an average value of 0.45 per primer. Higher PIC values were detecte by **Que et al. (2014)** who used assessed the genetic diversity among 107 sugarcane accessions using 20 SCoT markers and calculated PIC values from 0.783 to 0.907 with a mean of 0.861.

For the revealing of the genetic relationships among the cultivars it is necessary to construct a dendrogram.In the study **Que et al. (2014)**, used 20 SCoT primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. Unweighted pair group method of arithmetic averages (UPGMA) cluster analysis of the SCoT marker data divided 107 sugarcane accessions into six clusters. **Jiang et al. (2014)** used start codon-targeted (SCoT) markers to analyze the diversity and genetic relationships among 95

orchardgrass accessions. In total, 273 polymorphic bands were detected with an average of 11.4 bands per primer. The UPGMA dendrogram separated 95 accessions into 7 main clusters according to the geographical origin. **Kallamadi et al. (2015)** by analysis of genetic diversity of 31 accessions of castor using 36 SCoT markers constructed the UPGMA dendrogram based in which the accessions of castor separated into two major clusters (11 and 17 accessions). Three accessions failed to cluster with others accessions. **Rajesh et al. (2015)** constructed dendrogram using corresponding genetic similarity coefficients obtained from UPGMA analysis and determined the clustering pattern among the coconut accessions. Coconut accessions grouped into two main clusters. Cluster analysis supported population genetic analysis and suggested close association between introduced and domesticated genotypes. **Gajera et al. (2014)** constructed dendrogram of the 20 mango cultivars using 19 SCoT primers which clustered into two major groups based on the SCoT data analysis with UPGMA.

Recent advances in genomic research has resulted in a change of preference from the use of random DNA markers to gene-targeted, functional markers and the development of novel DNA-based marker systems **(Poczai et al., 2013)**. Functional markers developed from the transcribed region of the genome have the ability to reveal polymorphism, which might be directly related to gene function **(Poczai et al., 2013)**. Start codon targeted polymorphism (SCoT) is a simple and novel marker system first described by **Collard and Mackill (2009)**, which is based on the short conserved region flanking the ATG translation start codon in plant genes. The technique is similar to RAPD or ISSR in that a single primer acts as the forward and the reverse primer, amplicons can be visualized by standard agarose gel electrophoresis, without the need for costly automated electrophoresis systems **(Collard and Mackill, 2009)**. The higher primer lengths and subsequently higher annealing temperatures ensure higher reproducibility of SCoT markers, compared to RAPD markers **(Rajesh et al., 2015)**. **Gorji et al. (2011)** presented that SCoTs markers were more informative and effective, followed by ISSRs and AFLP marker system in in fingerprinting of potato varieties.

CONCLUSION

The present work reported utilization of SCoT markers for the detection of genetic variability of oat genotypes. In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus of oat accessions originated from various regions. The hierarchical cluster analysis divided oat genotypes into 2 main clusters. SCoT markers are generated from the functional region of the genome; the genetic analyses using these markers would be more useful for crop improvement programs. Polymorphism revealed by SCoT technique was abundant and could be used for molecular genetics study of the oat accessions, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, construction of linkage maps, conservation of the genetic resources of oat species and QTL mapping.

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