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Original scientific article

FINGERPRINT AND EXPRESSION PROFILES OF OARE – 1 RETROTRANSPOSON IN MORPHOLOGICAL PARTS OF *Avena sativa* VARIETIES

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Avena sativa L. is the most typical cultivated oat species, highly valued as a good source of essential nutrients. Its genome stores a great mass of repetitive elements (86,95%) including *Ty1-copia* LTR-retrotransposons. Their activation in a stress environment was characterized in tobacco as a defence response. The genomic activity of OARE-1 was studied by PBS, IRAP DNA-based marker techniques and transcriptomic activity of OARE-1 was measured by qPCR in three varieties of oat including analysis of five morphological parts. Both DNA marker techniques were able to describe various profiles, PBS with 49 loci and PIC value of 0,303, IRAP with 218 loci and average PIC value of 0,291. A very variable fingerprint profiles were obtained, and no variety specificity was confirmed. The highest expression level of OARE-1 showed samples of root and the lowest levels of OARE-1 were expressed by chaff samples.

Keywords: common oat; DNA marker technique, PBS, IRAP, qPCR

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INTRODUCTION

Common oat (*Avena sativa* L.) is the most typical species among cultivated oats and belongs to the *Poaceae* family. Both grain and chaff are highly valued in human and animal diet. It is a good source of β-glucans, B-complex vitamins (especially B1), unsaturated fatty acids, proteins, minerals (e.g. magnesium, iron, potassium) (BUTT *et al*., 2008). Oat flour is not typically used for baking, more often it is served as porridge, oat flakes, breakfast cereals and often referred as a healthy meal.

The genus *Avena* covers 30 species, diploid, tetraploid or hexaploid, differing in genome compositions (LI *et al*., 2000). The hexaploid genome is highly dynamic, with significant loss of some gene family members, due to polyploidization and domestication. Up to now, different DNA-based marker techniques were applied to determine the differences in polymorphism of oat genomes. The first generation of DNA markers – RAPD (Randomly Amplified Polymorphic DNA) were used to describe the genetic structure in a set of tetraploid oats (BENCHACHO *et al*., 2002) or the genetic diversity relevant to local gene-pool of the oat (Sood et al., 2016). SNP markers (single nucleotide polymorphism) were associated in oat genome with short straw (TANHUANPÄÄ *et al*., 2006). Microsatellite-based DNA markers were reported to be divergent and effective in the analysis of mutant populations of *A. sativa* (SHARMA *et al*., 2016) as well as in dataset creations for landrace genotypes of this species (DZIUBIŃSKA *et al*., 2022). Gene specific SCoT (start codon targeted) markers were used for the characterization of genetic polymorphism among European oat varieties (BALÁŽOVÁ *et al*., 2017).

The genome of *A. sativa* is hexaploid composed of AACCDD sub-genomes (LEGGETT and THOMAS, 1995), with size 10,5 Gb, of which 9,35 Gb (86,95%) are repetitive elements, which is higher than in wheat and barley (PENG *et al*., 2022).

Half of these repetitive sequences were identified as long terminal repeat (LTR) transposons (81%), transposons (16%) and non-LTR-transposons (3%), according to assembled contigs from genomic clones of chromosome specific library (LUO *et al*., 2012), and about 60% *Avena* genome reads are related to LTR-retrotransposons with *Ty3-gypsy* and *Ty1-copia* superfamilies (LIU *et al*., 2019). *Ty1-copia* are ubiquitous among the plant kingdom (HIROCHIKA and HIROCHIKA, 1993) and are activated under specific conditions such as stress, developmental stages, or defense responses. Their activation can disrupt genes, create pseudogenes, or influence gene expression by epigenetic mechanisms (LI *et al*., 2020). The widespread presence of retrotransposons in the genome of oat allows the application of retrotransposon-based DNA marker techniques to study the polymorphism among its species and varieties of ecotypes (TOMÁS *et al*., 2016; NEČAJEVA *et al*., 2021; ANDROSIUK *et al*., 2023).

Seven groups of retrotransposons (OARE-1 to 7) belonging to *Ty1-copia* superfamily were designed for oat. OARE-1 is 8,665 bp long, both LTRs are 99% identical and the study assumed that the hexaploid oat genome contains at least 10,000 copies of it. OARE-1 responses to salicylic acid, a signaling mediator of plant defense, with strong upregulation, what indicates close connection between OARE-1 expression and plant defense to biotic stress (KIMURA *et al*., 2001; KIMURA *et al*., 2002). The linking of transposon and retrotransposon elements to such a wide range of stress has involved them to very effective and universal plant DNA-based markers (NEGI *et al*., 2016; PAPOLU *et al*., 2022). Up to now, the genotypes and the genetic variability of *Avena* spp. were studied and described to some extent using repetitive sequences, however the retrotransposon marker-based genetic variability is poorly studied for the genus, even though previous study confirmed the effectivity of PCR-based amplification of retrotransposon sequences for the species (KALENDAR *et al.*, 1999).

This study provides unique information about OARE-1 genomic polymorphism profiles and expression activity in the individual morphological parts of three *A. sativa* varieties. *Avena* specific LTR-retrotransposon OARE-1 was used to examine the intraspecific polymorphisms by IRAP (Inter-retrotransposon amplified polymorphism) and iPBS (Inter-primer binding site) primers designed to its sequence as well as its expression activity (figure 1). IRAP and iPBS are widely used retrotransposon-based marker systems. IRAP is dominant multiplex technique that produces transposon insertion site variations. It is highly specific and usually has a medium polymorphism and reproducibility. iPBS markers detect variations in primer binding sites in LTRs of retrotransposons. PBSs are highly conserved regions, which have role in the reverse transcription of the LTR-retrotransposons (AMITEYE, 2021).

MATERIALS AND METHODS

Three varieties and their 5 morphological parts of the common oat (*A. sativa* L.) were analyzed (Table 1). Commercially planted varieties of oat were used – Black Beauty, Dagles and Flenzan. Plant material was grown in field conditions in Straubenhardt (Germany) during the season 2020/2021 where the main source of water are only atmospheric rainfalls. During the season, a total of 767.02 mm of rainfalls. Plants were collected in situ and in cold transported to laboratories of Slovak University of Agriculture in Nitra (Slovak Republic), processed into 5 types of morphological parts (grain, leaf, stem, root, chaff) and frozen at – 20°C until the nucleic acid extraction.

Table 1. List of the plant material and its labelling.

Tissue / *Variety*	Chaff	Grain	Leaf	Stem	Root
Black Beauty	BC	BG	BL	BS	BR
Dagles	DC	DG	DL	DS	DR
Flenzan	FC	FG	FL	FS	FR

gDNAs were extracted by GeneJET Genomic DNA Purification Kit (Thermo Scientific™). The quality and quantity of DNAs were checked twice: spectrophotometrically by NanoPhotometer® P-360 (Implen) and by PCR amplification of ITS region by commercially available ITS1 and ITS4 primers (GRANDBASTIEN, 1998). Four primers were used to observe a retrotransposon's insertion, three (IRAP1, IRAP2 and IRAP3) were designed to LTR-retrotransposon ends of OARE-1 gag-pol pseudogene for polyprotein (GenBank: AB061327) (KIMURA *et al.*, 2002). The last one was designed to anneal the PBS region (GenBank: AB061327) (KIMURA *et al.*, 2002). Each primer required different cycling conditions, therefore the protocols are noticed in Table 2. PCRs were carried out with mastermix KAPA Taq HotStart (Roche), 1,200 nM primer, template DNAs (diluted 1:29) and nuclease-free water. Amplicons of each method were separated on 3% AGE, visualized by GelRed™ under UV light. PCR profiles were processed into binary matrices using Jaccard index with UPGMA method (URBANOVÁ *et* *al*., 2024) to create dendrograms of Jaccard genetic distances. PIC values were calculated by iMEC (GARCIA-VALLYE *et al*., 1999).

Table 2. Time-temperature protocols for genomic analyses.

Step	IRAP1	IRAP2	IRAP3	PBS	Number of cycles
P. denaturation	95°C (5 min)	95°C (5 min)	95°C (5 min)	95°C (5 min)	1
Denaturation	95°C (30 s)	95°C (30 s)	95°C (30 s)	95°C (30 s)	35
Annealing	56°C (40 s)	54°C (40 s)	62°C (40 s)	54°C (40 s)	
Elongation	72°C (2 min)	72°C (2 min)	72°C (2 min)	72°C (3 min)	
Final elongation	72°C (5 min)	72°C (5 min)	72°C (5 min)	72°C (5 min)	1

Total RNAs were extracted by Ribospin™ Seed/Fruit (GeneAll) kit following the manufacturer instructions. Immediately after the extraction, RNA were promoted by Maxima First Strand cDNA Synthesis Kit for RT-qPCR, using both oligodT and random hexamer primers, to prevent degradation. cDNAs were synthesized after standardization to 50 ng of RNA into a reaction. Activity levels of OARE-1 retrotransposon were observed by a qRT-PCR using OARE-M03 F/R primers (Table 3) designed for the same OARE-1 sequence as the genomic methods (GenBank: AB061327). Elizyme™ Green Mix Add Rox (Elisabeth Pharmacon®) was used in the qRT-PCR reaction together with 200 nM primers, ROX and 1 µl of template cDNA (diluted 1:99) together nuclease-free water. Thermal cycling protocol for OARE-1 was as follows: primary denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 10 s, annealing at 52°C for 10 s and elongation at 65°C for 10 s. Amplicon specificity was checked by product melt from 55 to 95°C. Elongation factor 1-α was used as a housekeeping gene that was confirmed as stable in expression during stress conditions. Thermal cycling protocol for housekeeping gene was as follows: primary denaturation at 95°C for 2 min; 40 cycles of denaturation at 95°C for 5 s, annealing at 59°C for 5 s and elongation at 65°C for 10 s. Amplicon specificity was checked by melt from 70 to 95°C. Samples of both qPCRs were analyzed in technical triplicates and standard curves were created by 4 dilutions: 1x, 10x, 100x, 1000x. Calculations were provided in Excel prepared program for qPCR data by delta delta Ct method (PFAFFL, 2001).

Table 3. List of used primers.

Name	Sequence (5‘-3‘)	T_m	T_a	outcome
OARE-M03F	ATC GGA TTA GGT GTG GTG AA	52,7°C	53°C	99 bp
OARE-M03R	GTT CAT CTA GTA ATT TGA TT	40,2°C		
EF1-F	AAGGAGGCAGCCAACTTCA	56,6°C	59°C	122 bp
EF1-R	AGCTCAGAAACTTGACAGC	55,6°C		
OARE-IRAP1	GGA ATT ATG CCC TAG AGG AA	50,5°C	55,5°C	polymorphism
OARE-IRAP2	ATC TGT TGG GTT GTA TTG TA	47,2°C	54°C	polymorphism
OARE-IRAP3	AGA TCG GAT CGG ATC GCG AG	59,3°C	61,5°C	polymorphism
PBS-OARE	GGT ATC AGA GCT AGA TCT AT	46,2°C	54°C	polymorphism

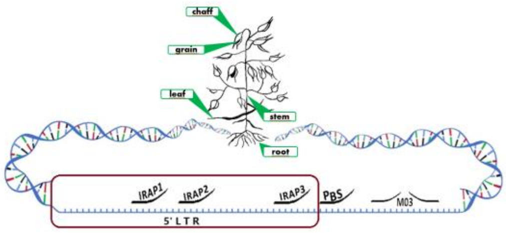


Figure 1. Positions of primers used in the study and parts/tissues of oat plant used as samples.

RESULTS

PBS marker technique

The PBS marker technique successfully generated polymorphic profiles for analyzed samples, except for the leaf sample from 'Dagles'. A total of 49 loci were detected in 14 lengths ranging from 340 bp to 1450 bp, longer amplicons may not have been captured due to limitations of the polymerase. The PIC value for the PBS marker was 0.303, the most common length was 410 bp, which occurred in 7 samples. Specifically, it was found in the chaff and root of 'Flenzan', as well as in all morphological parts of 'Black Beauty' except for the leaf sample. Additionally, it was present in the grain sample of 'Dagles'. It can be assumed that this specific length of 410 bp could potentially be considered as a control amplicon of PCR specificity due to its widespread occurrence. There were 5 unique lengths: 340 bp, 380 bp, 480 bp (‘Flenzan’ leaf), 765 bp (‘Black Beauty’ chaff) and 850 bp (‘Black Beauty’ root), none of the tissues from 'Dagles' exhibited any unique amplicons.

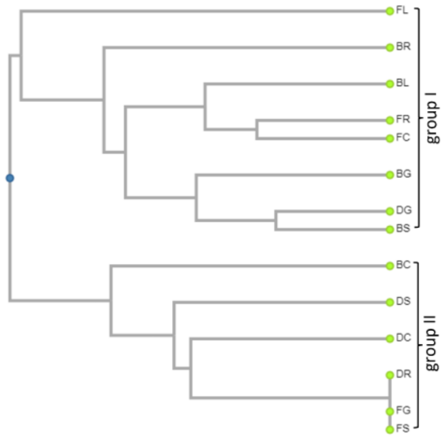


Figure 2. UPGMA dendrogram of Jaccard genetic distance values among 5 tissues of 3 oat varieties for PBS technique.

Figure 2 showcases the variation in PBS profiles, with the most distinct profile belonging to the leaf sample of 'Flenzan' that shares only one amplicon length of 500 bp with the other 4 samples. This linking ‘Flenzan’ leaf profile, as length of 410 bp, with the stem and chaff profiles of ‘Black Beauty’ and the grain profile of ‘Dagles’.

The samples were divided into 2 main clusters in the UPGMA dendrogram based on Jaccard genetic distance values. Group I is defined by the presence of amplicon length of 605 bp and a low number of loci. Half of the members in the group exclusively amplified this particular length. Each of the other members in group I showed an additional amplicon length: ‘Dagles’ chaff 400 bp, ‘Dagles’ stem 565 bp and ‘Black Beauty’ chaff a unique 765 bp (Figure 2).

Group II exhibited a higher diversity of the generated amplicons and interestingly included only the grain sample of ‘Dagles’. This suggests that the genome of the grain might be more active in OARE-1 retrotransposition events compared to other parts of the plant. The most similar profiles were created by grain profile of ‘Dagles’ and stem profile of ‘Black Beauty’ with a similarity of 71,4%. The leaf profile of ‘Flenzan’ was the most dissimilar varying from 83,3% with ‘Black Beauty’ grain to 100% with 9 other profiles.

The most of amplified fragments were generated in the profiles of the stem of ‘Black Beauty’ (amplified 7 bands) and the root of ‘Flenzan’ (amplified 6 bands). Both shared amplicons of 410 bp, 460 bp, 1,200 bp, 1,330 bp and 1,450 bp, and differed by lengths of 440 bp and 500 bp for ‘Black Beauty’ stem and 565 bp for the ‘Flenzan’ root profile.

IRAP marker technique

The three primers of IRAP were able to amplify 218 bands, one of which was monomorphic and 14 were unique. Dendrogram of genetic distances were created using Jaccard coefficient but calculated with both Jaccard and Dice. Jaccard index showed higher cophenetic correlation coefficients (CCC), that ranged from 0.86 to 0.94 as indicated in Table 4. CCC value shows how veracious the pairwise distances are presented in the dendrogram, as for the IRAP primers, IRAP1 had the highest CCC.

Table 4. List of statistical data for all three primers of IRAP.

				Primer	Polymorphism	PIC	CCC
IRAP1	100%	0.2282	0.9414	49	21	8	0.0-1.0
IRAP2	100%	0.2774	0.9286	59	19	5	0.0-1.0
IRAP3	94,74%	0.3687	0.8599	110	19	1	0.2-0.833*8

Comparing individual morphological parts alone, the most divergent fingerprint profiles of OARE-1 retrotransposon were observed in root tissues. The most contrasting profiles were the stem and the root of ‘Dagles’ which were 100% polymorphic by IRAP1 but identical by IRAP2. On the other hand, many profile pairs were 100% polymorphic by IRAP1 and IRAP2 and highly polymorphic (>0,5) by IRAP3 primer. Such pair profiles were created as follows:

- Root profile of ‘Flenzan’ with leaf of ‘Flenzan’ (0.643), ‘Black Beauty’ root (0.733), leaf (0.6), stem (0.733) profiles and ‘Dagles’ grain (0.643), leaf (0.538) and chaff (0.733)

- Grain profile of ‘Flenzan’ with chaff profile of ‘Dagles’ (0.7) and ‘Black Beauty’ grain (0.778), root (0.818), leaf (0.636) and stem (0.7) profiles.
- Chaff profile of ‘Dagles’ with leaf profile of ‘Dagles’ (0.75), ‘Flenzan’ leaf (0.727) and stem (0.615) profiles, and ‘Black Beauty’ leaf (0.667), grain (0.667) and chaff (0.667) profiles.

IRAP1

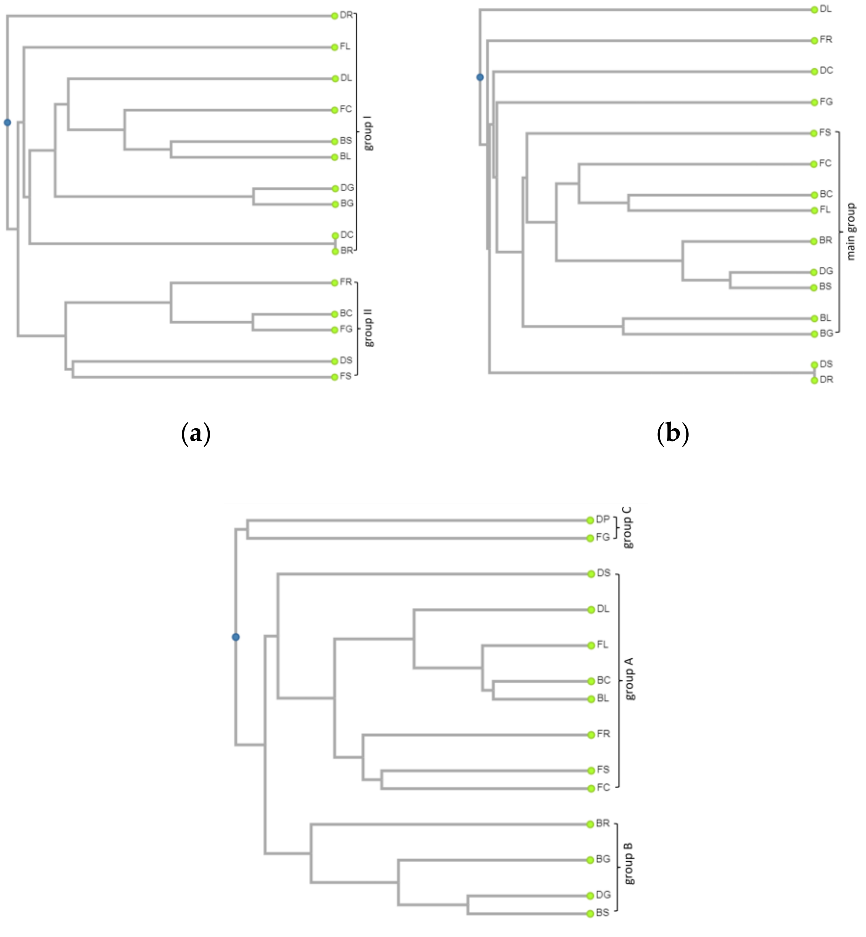
UPGMA created 2 groups and separated a unique root profile of ‘Dagles’ (Figure 3a). The bigger group I contained 9 samples including all leaf profiles, smaller group II involved five sample profiles. 59,04% of IRAP1 profile combinations were 100% polymorphic and only the chaff profile of ‘Dagles’ and the root profile of ‘Black Beauty’ were monomorphic. Primer IRAP1 created the most polymorphic data, although the total number of loci was the lowest. The comparison of the same morphological parts between the three varieties led to the observation that all the roots were 100% polymorphic, and on the contrary the grain of ‘Black Beauty’ and ‘Dagles’ were highly similar (75%). 8 amplicons were unique: 535 bp, 600 bp, 820 bp, 905 bp, 1,180 bp, 1,270 bp, 1,390 bp and 1,455 bp, the most frequent amplicon was 650 bp in size and was present in 6 profiles. The highest number of amplicons within one profile was five (‘Flenzan’ leaf and chaff, stem of ‘Black Beauty’).

IRAP2

The main cluster created by IRAP2 primers contained nine morphological part sample profiles, the others were joined one by one to the main cluster, and this grouping was caused by single unique bands in the sample profiles (Figure 3b). There was one monomorphic profile pair – stem and root of ‘Dagles’ where only a 500 bp long amplicon was amplified. The total number of loci was 59, 19 amplicon lengths varied between 155 bp to 1440 bp. Five amplicons were unique: 330 bp, 365 bp, 385 bp, 570 bp and 600 bp; the most frequently re-peated lengths were 300 bp (7x) and 1245 bp (6x). The main group clustered all morphological parts of ‘Black Beauty’, none of them were identical and no amplicon length was present in all morphological part profiles. The most similar profiles (75%), representing the main group, were the grain of ‘Dagles’ and the stem of ‘Black Beauty’. The most active profile was chaff of ‘Flenzan’ with 9 amplicons. Variety comparison of each morphological part showed that all three root accessions are 100% polymorphic by IRAP2 as same as by IRAP1 primer.

IRAP3

Primer IRAP3 was able to amplify the highest number of loci. Samples were clustered into 3 groups (Figure 3c). Group A, the biggest one, contains almost all ‘Flenzan’ morphological parts except the grain’s, group B contains root, grain, and stem profile of ‘Black Beauty’ together with grain profile of ‘Dagles’. The amplicon of 355 bp in size was present in every sample, and the amplicon of 800 bp was only absent in ‘Black Beauty’ root sample. Primer IRAP3 amplified 19 lengths from 135 bp to 1,100 bp. No profile was 100% polymorphic but also none of them were 100% similar. The highest (83,3%) dissimilarity was between the chaff profile of ‘Dagles’ and root profile of ‘Black Beauty’ variety. There was one unique amplicon of 620 bp that occurred in root profile of ‘Flenzan’, this accession was also the most active amplifying 12 amplicons.



(**a**) (**b**)

(**c**)

Figure 3. UPGMA dendrograms of Jaccard genetic distances among 5 tissues of 3 oat varieties by IRAP. (a) Dendrogram for primer IRAP1; (b) Dendrogram for primer IRAP2; (c) Dendrogram for primer IRAP3.

Expression level of OARE-1 by qPCR

Expression activity of OARE-1 was analysed by qPCR using specific primer pair (OARE-M03) bound behind 5’LTR of OARE-1 retrotransposon (Figure 4). The amplicon specificity was checked by the presence of a single peak in the dissociation curve with product melting temperature of 70,7°C, and by agarose electrophoresis. A qPCR effectivity was checked by standard curve with an outcome of 109.4%, which is acceptable for plant analyses. Elongation factor 1-α was used as a housekeeping gene, its effectivity was 99.7% and the melting temperature of the qPCR product was 85 °C, specificity was secondary confirmed on agarose gel. As a calibrator one of the samples was picked, specifically grain sample of ‘Flenzan’, calculated as the median sample value.

The highest (2- to 6-fold to calibrator) expression level of OARE-1 was recorded in root, on the contrary to chaff expressed the lowest levels. ‘Black Beauty’ leaf and stem and ‘Dagles’ showed no detected OARE-1 expression (Figure 4), it was detected by Ct values, but it was tens of thousands of times lower than the other samples.

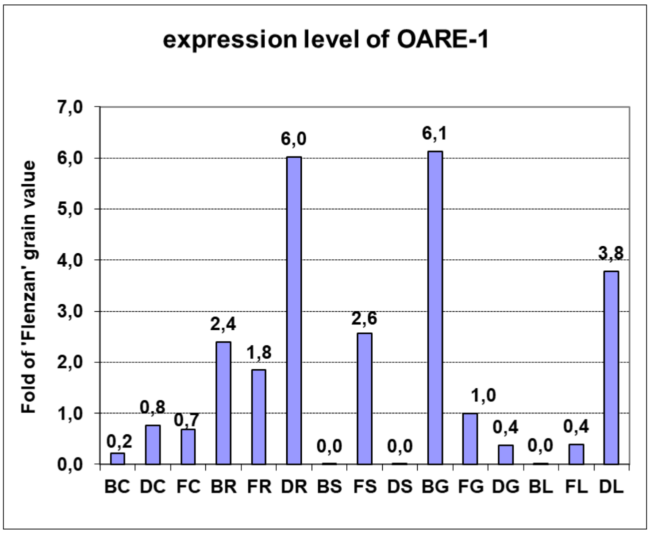


Figure 4. Graph shows OARE-1 retrotransposon expression levels comparing three varieties of *A. sativa* and their five tissues.

DISCUSSION

Retrotransposons are mobile genetic elements that code genes ensuring their intracellular retrotransposition modifying a plant genome. In recent years, they are used as molecular markers to improve agronomic traits, new variety breeding or to better understanding of a plant biology or their evolution (HASSAN *et al.*, 2023). Transcription and movement of retrotransposons can be stimulated by polyploidization, biotic or abiotic elicitors that influence genes or their epigenetic controlling process (GALINO-GONZÁLEZ *et al*., 2017). Stress-induced changes in retrotransposon activity may result in genome fluidity and its adaptation to new conditions (PAPOLU *et al*., 2022).

Comparative analysis of a retrotransposon-based technique REMAP (retrotransposon-microsatellite amplified polymorphism) and a repetitive sequences-based technique ISSR (inter simple sequence repeat polymorphism) were performed (PACZOS-GRZEDA and BEDNAREK, 2014) and REMAP was applied here to analyse the origin of different *Avena* hexaploids what confirmed retrotransposon-based markers as very effective. Interspecific variability of *Avena* species were analysed using REMAP and IRAP (inter-retrotransposon amplified polymorphism) and both were proved to be prospective molecular markers with sufficient generated amplicons for oat genetic studies (TOMÁS *et al*., 2016). The applicability of REMAP markers for studying of genomic changes under the abiotic stress resulted in the identification of the most tolerant varieties of *A. sativa* (DA SILVA OLIVEIRA *et al*., 2020). Recently, iPBS marker technique was reported for *Avena* spp. (ANDROSIUK *et al*., 2023) where monocots non-specific iPBS markers were applied to compare thirteen *Avena* species and a low genetic diversity was summarized. This study builds on the previous findings and enriches the knowledge about the movement of OARE-1 retrotransposons in oats grown under field conditions. This research confirms IRAP technique as a useful tool to study OARE-1 genetic variability among *A. sativa* varieties and their morphological parts due to 98.25% polymorphism detected with all three primers. Primer IRAP3 had the highest PIC value (0.37), the average of PIC values for all three primers was 0.29, similar to results of IRAP analyse in Brassica sp. (MAHJOOB *et al*., 2016) and slightly lower than in two *Triticum* spp., which compared interspecies genetic variations (TAHERI *et al*., 2018). The 355 bp length amplicon amplified by IRAP3 was very stable in all samples and we suppose, this amplicon is in oat specific.

The natural activity of plant retrotransposons (RAMALLO *et al*., 2008; HE *et al*., 2010; TAPIA *et al*., 2005) was studied previously by many different techniques (KIMURA *et al*., 2001; METCALFE *et al*., 2015; VORONOVA, 2019; ALZOHAIRY *et al*., 2012) including real-time PCR. A marker system with a potential to estimate the dosage of a particular retrotransposon was developed as a combination of TaqMan quantitative PCR (qPCR) combined with retrotransposon-based insertion polymorphism (RBIP) for sugarcane (METCALFE *et al*., 2015) but, to our knowledge, no real-time PCR-based analysis of OARE-1 retrotransposon expression was reported for oats grown in field conditions, up to now. Another situation is regarding of *A. sativa* housekeeping genes characteristics, where different types of genes were analysed and reported as suitable to be used as reference genes for gene expression studies of oat (JAROŠOVÁ and KUNDU, 2010; YANG *et al*., 2020; TAJTI *et al*., 2021). Elongation factor 1-α was proved to be a useful housekeeping gene for the oat because of its stable expression even under stress conditions (TAJTI *et al*., 2021). Stress such as wounding, fungal inoculation or UV light was related with up-expression of the OARE-1 retrotransposon (KIMURA *et al*., 2001). Retrotransposons could be the main means of adaptation to stress or new conditions during plant evolution because of their ability to reconstruct genome and transmit horizontally (GRANDBASTIEN, 1998). These abilities are used as a response to various stress signals and commented that expression of *Ty1-copia* retrotransposons is inducted ubiquitously among plants by stimuli associated with plant defence responses (KIMURA *et al*., 2002).

CONCLUSIONS

Analysing different morphological parts of three *Avena sativa* L. varieties, stability of fingerprints was obtained for OARE-1-based IRAP and iPBS markers. iPBS was able to distinguish all the analysed accessions and in the case of IRAP technique, two from three markers used in the study were able to distinguish them. Expression analysis of OARE-1 transcripts by qPCR technique described high differences in the expression levels of OARE-1 in individual morphological parts of analysed oat varieties, especially in roots. This corresponds to variability and specifity of retrotransposons as active part of plant genomes and pointed OARE-1 as abundant in the genome of oat.

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REFERENCES

ALZOHAIRY, A. M., M. A YOUSEF, S. EDRIS, B. KERTI, G. GYULAI, A. BAHIELDIN (2012): Detection of LTR Retrotransposons Reactivation induced by in vitro Environmental Stresses in Barley (*Hordeum vulgare*) via RT-qPCR. Life Sciences Journal, *9*(4): 5019-5026.

AMITEYE, S. (2021): Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon: *7*(10).

ANDROSIUK, P., S. E. MILARSKA, J. DULSKA, W. KELLMANN-SOPYŁA, J. SZABLIŃSKA-PIERNIK, L. B. LAHUTA (2023): The comparison of polymorphism among Avena species revealed by retrotransposon-based DNA markers and soluble carbohydrates in seeds. Journal of Applied Genetics, *64*(2): 247-264.

BALÁŽOVÁ, Ž., Z. GÁLOVÁ, M. VIVODÍK, L. PETROVIČOVÁ, R.H. GREGÁŇOVÁ (2017): Molecular variability of oat based on gene specific markers. Potravinarstvo Slovak Journal of Food Sciences, *11*(1): 332–337.

BENCHACHO, M., R. GUMA, M.P. DE LA VEGA, P. GARCÍA (2002): The genetic structure of tetraploid Avena: a comparison of isozyme and RAPD markers. Cellular and Molecular Biology Letters, *7*(2A): 465-470.

BUTT, M. S., M. TAHIR-NADEEM, M.K.I. KHAN, R. SHABIR, M.S. BUTT (2008): Oat: unique among the cereals. European journal of nutrition, *47*: 68-79.

DA SILVA OLIVEIRA, D. D. C., S.F. DA SILVEIRA SILVEIRA, R.V. FAGUNDES, D.D. WOLTER, F. MADABULA, V.F. DE OLIVEIRA, A. T. DE ARAUJO JR., C.F. BRUM PIANA, L. C. MAIA, A.C. DE OLIVEIRA (2020): Response of white oat to water stress. Communications in Plant Sciences, *10*: 18-26.

DZIUBIŃSKA, D., P. BOLC, G. KLOC, W. PODYMA, M. BOCZKOWSKA (2022): A comprehensive dataset of *Avena sativa* L. landraces phenotypes and genotype. Data in Brief, *41*: 107962.

GALINDO-GONZÁLEZ, L., C. MHIRI, M.K. DEYHOLOS, M.A. GRANDBASTIEN (2017): LTR-retrotransposons in plants: Engines of evolution. Gene, *626*: 14-25.

GARCIA-VALLVÉ, S., J. PALAU, A. ROMEU (1999): Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Molecular biology and evolution, *16*(9): 1125-1134.

GRANDBASTIEN, M.A. (1998): Activation of plant retrotransposons under stress conditions. *Trends in plant science*, *3*(5): 181-187.

GRZEDA, E. P. and P.T. BEDNAREK (2014): Comparative analysis of hexaploid Avena species using REMAP and ISSR methods. *Turkish Journal of Botany*, *38*(6): 1103-1111.

HASSAN, A. H., M.M. MOKHTAR, A. EL ALLALI (2023): TEMM: A Curated Data Resource for Transposon Element-Based Molecular Markers in Plants. In: *Plant Genomic and Cytogenetic Databases* (pp. 45-57). New York, NY: Springer US.

HE, P., Y. MA, G. ZHAO, H. DAI, H. LI, L. CHANG, Z. ZHANG (2010): FaRE 1: a transcriptionally active Ty1-copia retrotransposon in strawberry. Journal of plant research, *123*: 707-714.

HIROCHIKA, H. and R., HIROCHIKA (1993): Ty1-copia group retrotransposons as ubiquitous components of plant genomes. The Japanese Journal of Genetics, *68*(1): 35-46.

JAROŠOVÁ, J. and J. K., KUNDU (2010): Validation of reference genes as internal control for studying viral infections in cereals by quantitative real-time RT-PCR. BMC plant biology, *10*: 1-9.

KALENDAR, R., T., GROB, M., REGINA, A., SUONIEMI, A., SCHULMAN (1999): IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. TAG, *98*: 704-711.

KIMURA, Y., Y., TOSA, S., BETSUYAKU, Y., SASABE, R., TOMITA, J., MURAKAMI, H., NAKAYASHIKI, S., MAYAMA (2002): Oat retrotransposon OARE-1 is activated in both compatible and incompatible interactions with pathogenic fungi. Journal of general plant pathology, *68*: 8-14.

KIMURA, Y., Y., TOSA, S., SHIMADA, R., SOGO, M., KUSABA, T., SUNAGA,... AND S., MAYAMA (2001): OARE-1, a Ty1-copia retrotransposon in oat activated by abiotic and biotic stresses. Plant and cell Physiology, *42*(12): 1345-1354.

LEGGETT, J. M. and H., THOMAS (1995): Oat evolution and cytogenetics. In: *The oat crop: production and utilization* (pp. 120-149). Dordrecht: Springer Netherlands.

LI, C. D., B. G. ROSSNAGEL and G. J., SCOLES (2000): Tracing the phylogeny of the hexaploid oat Avena sativa with satellite DNAs. Crop Science, *40*(6): 1755-1763.

LI, J., J. YANG, Y. LI, and L., MA (2020): Current strategies and advances in wheat biology. The Crop Journal, *8*(6): 879-891.

LIU, Q., X., LI, X., ZHOU, M., LI, F., ZHANG, T., SCHWARZACHER and J. S., HESLOP-HARRISON (2019): The repetitive DNA landscape in Avena (Poaceae): chromosome and genome evolution defined by major repeat classes in whole-genome sequence reads. BMC Plant Biology, *19*: 1-17.

LUO, X., C. P., WIGHT, Y., ZHOU, N. A., TINKER (2012): Characterization of chromosome-specific genomic DNA from hexaploid oat. Genome, *55*(4): 265-268.

MAHJOOB, B., H. N., ZARINI, S. H., HASHEMI, F. V., SHAMASBI (2016): Comparison of ISSR, IRAP and REMAP markers for assessing genetic diversity in different species of Brassica sp. Russian journal of genetics, *52*: 1272-1281.

METCALFE, C. J., S. G., OLIVEIRA, J. W., GAIARSA, K. S., AITKEN, M. S., CARNEIRO, F., ZATTI, M. A., VAN SLUYS (2015): Using quantitative PCR with retrotransposon-based insertion polymorphisms as markers in sugarcane. Journal of experimental botany, *66*(14): 4239-4250.

ŅEČAJEVA, J., M., BLEIDERE, Z., JANSONE, A., GAILĪTE, D., RUŅĢIS (2021): Variability of seed germination and dormancy characteristics and genetic analysis of Latvian *Avena fatua* populations. Plants, *10*(2): 235.

NEGI, P.; A.N. RAI, P. SUPRASANNA (2016): *Moving through the Stressed Genome*: Emerging Regulatory Roles for Transposons in Plant Stress Response. Frontiers of Plant Science, *7*: 1448.

PAPOLU, P. K., M., RAMAKRISHNAN, S., MULLASSERI, R., KALENDAR, Q., WEI, L., ZOU, Z., AHMAD, K.K., VINOD, P., YANG, M., ZHOU (2022): Retrotransposons: How the continuous evolutionary front shapes plant genomes for response to heat stress. Frontiers in plant science, *13*, 1064847.

PENG, Y., H. YAN, L. GUO, C. DENG, C. WANG, Y. WANG, L. KANG, P. ZHOU, K. YU, X. DONG, X. LIU, Z. SUN, Y. PENG, J. ZHAO, D. DENG, Y. XU, Y. LI, Q. JIANG, Y. LI, L. WEI, J. WANG, J. MA, M. HAO, W. LI, H. KANG, Z. PENG, D. LIU, J. JIA, Y. ZHENG, T. MA, Y. WEI, F. LU, C. REN (2022): Reference genome assemblies reveal the origin and evolution of allohexaploid oat. Nature Genetics, *54*(8): 1248-1258.

PFAFFL, M. W. (2001): A new mathematical model for relative quantification in real-time RT–PCR. Nucleic acids research, *29*(9): e45-e45.

RAMALLO, E., R. KALENDAR, A. H. SCHULMAN, J. A. MARTÍNEZ-IZQUIERDO (2008): Reme1, a Copia retrotransposon in melon, is transcriptionally induced by UV light. Plant Molecular Biology, *66*: 137-150.

SHARMA, P., S., TIWARI, N., TRIPATHI, A. K., MEHTA (2016): Polymorphism analysis in advanced mutant population of oat (*Avena sativa* L.) using ISSR markers. Physiology and molecular biology of plants, *22*: 115-120.

SOOD, V. K., I. RANA, W. HUSSAIN, H. K. CHAUDHARY (2016): Genetic diversity of genus Avena from North western-Himalayas using molecular markers. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, *86*(1): 151-158.

TAHERI, M. T., S. S., ALAVI-KIA, S. A., MOHAMMADI, M. M., VAHED (2018): Assessment of genetic diversity and relationships among *Triticum urartu* and *Triticum boeoticum* populations from Iran using IRAP and REMAP markers. Genetic resources and crop evolution, *65*: 1867-1878.

TAJTI, J., M., PÁL, T., JANDA (2021): Validation of reference genes for studying different abiotic stresses in oat (*Avena sativa* L.) by RT-qPCR. Plants, *10*(7): 1272.

TANHUANPÄÄ, P., R., KALENDAR, J., LAURILA, A. H., SCHULMAN, O., MANNINEN, E., KIVIHARJU (2006): Generation of SNP markers for short straw in oat *(Avena sativa* L.). Genome, *49*(3): 282-287.

TAPIA, G., I., VERDUGO, M., YANEZ, I., AHUMADA, C., THEODULOZ, C., CORDERO, F., POBLETE, E., GONZÁLEZ, S., RUIZ-LARA (2005): Involvement of ethylene in stress-induced expression of the TLC1. 1 retrotransposon from *Lycopersicon chilense* Dun. Plant Physiology, *138*(4): 2075-2086.

TOMÁS, D., J., RODRIGUES, A., VARELA, M. M., VELOSO, W., VIEGAS, M., SILVA (2016): Use of repetitive sequences for molecular and cytogenetic characterization of Avena species from Portugal. International Journal of Molecular Sciences, *17*(2): 203.

URBANOVÁ, L. S., FARKASOVÁ, I., SPEVÁKOVÁ, M., KYSEĽ, V., ŠIMORA, M., KAČÁNIOVÁ, J., ŽIAROVSKÁ (2024): DNA-Based Variability of Length Polymorphism of Plant Allergens Coding Genes Homologs in Selected Lamiaceae Herbs. OBM Genetics, *8*.

VORONOVA, A. (2019): Retrotransposon expression in response to in vitro inoculation with two fungal pathogens of Scots pine (*Pinus sylvestris* L.). BMC Research Notes, *12*, 1-7.

YANG, Z., K., WANG, U., AZIZ, C., ZHAO, M., ZHANG (2020): Evaluation of duplicated reference genes for quantitative real-time PCR analysis in genome unknown hexaploid oat (*Avena sativa* L.). Plant Methods, *16*: 1-14.

PROFILI OTISKA PRSTIJA I EKSPRESIJE OARE-1 RETROTRANSPOZONA U MORFOLOŠKIM DELOVIMA SORTI *Avena sativa*

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Izvod

Avena sativa L. je najtipičnija gajena vrsta ovsa, visoko cenjena kao dobar izvor esencijalnih hranljivih materija. Njen genom sadrži veliku masu ponavljajućih elemenata (86,95%), uključujući Ty1-copia LTR-retrotranspozone. Njihova aktivacija u stresnom okruženju kod duvana okarakterisana je kao odbrambeni odgovor. Genomska aktivnost OARE-1 je proučavana PBS i IRAP tehnikama DNK markera, a transkriptomska aktivnost OARE-1 je merena qPCR metodom kod tri sorte ovsa, uključujući analizu pet morfoloških delova. Obe tehnike DNK markera su bile u stanju da opišu različite profile, PBS sa 49 lokusa i PIC vrednošću od 0,303, IRAP sa 218 lokusa i prosečnom PIC vrednošću od 0,291. Dobijeni su veoma varijabilni profili otisaka prstiju i nije potvrđena specifičnost sorte. Najviši nivo ekspresije OARE-1 pokazali su uzorci korena, a najniži nivoi OARE-1 ekspresovani su uzorcima pleve.

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