

MicroRNA-Based and Proteomics Fingerprinting of *Avena sativa* L. Genotypes [†]

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Abstract: Regulatory potential of microRNAs in growth, metabolism and stress adaptation is well known. 15 common oat genotypes was performed by miRNA-based markers. Markers involved in MiRNAs sequences provide an effective type of putative functional markers. Markers involved to photosynthesis regulation, growth, and biomass production (miR408, miR156) generated 40% of all amplified loci. Those associated to nutrition accumulation and homeostasis (miR827 and miR399) generated 29% and stress-responsive markers (miR398 and miR858) 31%. By proteomics approaches (SDS and A-PAGE electrophoresis) was detected sufficient diversity between the analyzed samples, and genetic related dendrograms were constructed based on the electrophoretic profiles.

Keywords: miRNAs; SDS; A-PAGE; UPGMA; common oat

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1. Introduction

Due to the significantly changing climatic conditions, it is necessary to apply approaches to identify the genomic potential of plant genetic resources for adaptation to abiotic stress and thus provide a platform for successful genotype selection. The plant organism must cope with environmental stresses in natural and agricultural conditions. A genetic background allows plants to adapt to the environment by various mechanisms at the molecular level. From this point of view, genome screening by functional markers may provide knowledge for identifying added value of plant genetic resources.

MicroRNAs (miRNAs) have been identified to be involved in regulation of plant stress responses [1–3]. The high conservation of miRNA sequences allowed to develop an effective type of putative functional markers [4–7]. The fundamental potential of miRNA-based markers relies on the primer design based on the consensus sequences of mature miRNAs which are part of the stem-loop structures. The advantages of this marker system include high polymorphism, reproducibility, and inter-species transferability [4,5]. The abundance of mature miRNAs, which is linked to the expression of MIRNA genes, varies greatly among miRNAs, tissue types or developmental stages, indicating the spatially and temporally regulated expression patterns of plant miRNAs [8,9]. Polymorphism amplified by the application of miRNAs markers indicates changes in miRNA loci sequences, which may result in changes in target gene regulation [4,10].

Plant seed storage proteins are characterized by a polymorphism due to the existence of several discrete forms because of heterozygosity. The polymorphism results from

the in vivo hybridization of protein subunits, controlled by independent genes of different chromosome loci that are expressed at different times. Polymorphism can also be induced by post-translational changes in proteins, such as acylation, glycosylation, phosphorylation, deamination, decarboxylation, methylation, etc. [11].

The aim of our study was to detect the polymorphism of miRNA-based markers to characterize the oats genotypes. By proteomics approaches was detected diversity between the analyzed samples.

2. Materials and Methods

2.1. Biological Material

Grains of oat varieties (*Avena sativa* L.) Vaclav, Valentin, Vendelin, Viliam, Vit, Flamingsgarand, Flamingsregent, Fuchs, Arne, Magne, Calibre, Cascade, Lord, Senator and Marloo were analyzed. Samples were obtained from the Gene Bank of Seed Species of the Slovak Republic NPPC VÚRV in Piešťany.

2.2 Selection of Markers and Primers Design

The suitable microRNA markers were selected based on their functional involvement in *Poaceae* family (wheat, barley, maize, rice) [12–15]. Database miRBase (<https://www.mirbase.org>) (release 22.1) [16] was used for search and browse of microRNA sequences. Sequences-based homology search was done by BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast>) and CLUSTAL OMEGA (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) algorithms. The primers were designed according to the methodologies [4,5].

2.3 MicroRNA-Based Marker Assay

Plants were grown in pot experiments under controlled growth chamber conditions (23 °C, 16/8h photoperiod, 50% humidity). After 15 days of growth, bulk leaf samples (10 plants) were taken for DNA extraction. Total genomic DNA was extracted using the NucleoSpin® Plant II isolation kit (Macherey-Nagel) and quantified by the Implen NanoPhotometer®. The miRNA-based markers assays were performed based on protocols [4,5], modified by [17]. MiRNA-based PCR amplicons were separated on 15% TBE-UREA PAGE gels and scored for their length and gel migration characteristics by GeneTool™ gel analysis software (Syngene).

2.4 SDS-PAGE

Electrophoretic analysis of oat seed stock protein polymorphism was performed on SDS-PAGE according to [18]. Electrophoretic profiles of individual varieties were scanned with a GS-800 Calibrated Densitometer (BioRad) and evaluated using Doc-It LS Image analysis and GelAnalyzer. A dendrogram using the Jaccard similarity coefficient and the UPGMA algorithm for statistical interpretation of electrophoreograms was constructed in the DendroUPGMA program, which is available online (<http://genomes.urv.cat/UPGMA/>).

3. Results and Discussion

3.1. MiRNA-Based Assay

For genomic screening of 15 oat genotypes originated from six countries (Austria-AUS-1, Australia-AUT-2, Canada-CAN-2, Deutschland DEU-3, Slovakia SVK-5 and Sweden SWE-2) were used six different miRNA-based markers (miR156, miR398, miR399, miR408, miR827 and miR858). In total 849 miRNA-based loci were amplified, which represents an average of 57 fragments per genotype. Balanced amplification was also observed depending on the origin of the genotype. The range of amplified fragments

varied from 46 (Flamingsregent, DEU) to 67 (Senator, AUT). The representation of individual types of miRNA loci was marker specific.

A predominance of miR398 (22% of the total number of amplified fragments) marker was observed in the genome of the analyzed oat samples. MiRNA398 (miR398) is considered as stress-responsive miRNA involved in plant stress regulation mechanism [1,12]. MiR398 has been reported to be associated with various stress conditions as oxidative stress [19], water deficit [20], salt stress and abscisic acid stress [21]. Due to optimal and controlled growth conditions, it is not possible to predict the influence of any of the above stress factors, considering the short time interval (15 days) of growth. The predominant presence of miR398 loci may indicate the genomic potential of the analyzed genotypes in terms of their tolerance to abiotic stress, whereas miR398 represents a highly conserved miRNA in diverse monocots and dicots, and it can be assumed that it is important for plants stress tolerance [1,12]. The highest number of loci (16) was recorded in genotype Vaclav and the lowest in genotype Valentine (9) both originated from Slovakia.

As the second most represented type of miRNA-based marker was miR408 (21% of the total number of amplified fragments) (Figure 1). The highest number of loci (14) was recorded in genotypes Cascade (CAN) and Magne (SWE) and the lowest in genotypes Vendelin and Vit (9) both from Slovakia. Studies have shown that miR408 is involved in the development, light signaling pathway and biotic stress reactions as well as biomass production [2,3]. Constitutive expression of miR408 affects various stages of development and promotes intense plant growth and seed yield by increasing the efficiency of photosynthesis. Therefore miR408 is likely to have a pleiotropic effect on plant growth and development [22].

Similar to a marker miR408, the marker miR156 having 19% representation within amplified loci is also considered as the most conserved miRNAs indicating that its role is essential for the development and existence of plants [23,24]. The highest number of loci (4) was recorded in genotype Calibre (CAN) and the lowest (9) in genotypes Lord and Senator (AUT), Marloo (AUS) and Vendelin (SVK).

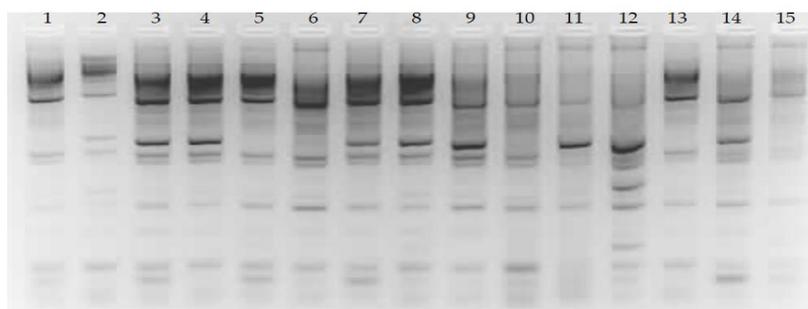


Figure 1. A representative genome fingerprinting of oat samples based on electrophoretic separation of “touch-down” PCR products amplified by miR408-based primers. Genotypes: 1–Arne, 2–Calibre, 3–Cascade, 4–Flamingsgarand, 5–Flamingsregent, 6–Fuchs, 7–Lord, 8–Magne, 9–Marlov, 10–Senator, 11–Vaclav, 12–Valentin, 13–Vendelin, 14–Viliam, 15–Vit.

The representation of the marker miR827 was recorded at the level of 16% of the total number of amplified fragments. MiR827 plays an important role in regulatory mechanisms related to nutrient homeostasis, especially phosphorus. It is characterized by increased activity in conditions of phosphorus deficiency [25,26]. The highest number of amplified miR827 loci (15) was recorded in genotype Senator (AUT) and the lowest (4) in Vendelin (SVK) and Flamingsregent (DEU).

Several studies confirmed the roles of miR399 in regulation of phosphate accumulation and homeostasis [27]. However, its responsiveness may be species- and tissues/organs specific [28]. In genotype Vit (SVK) was recorded the highest number (15) of

miR399 loci and the lowest (3) in Calibre from Canada. Total representation of the marker miR399 was 13% of the total number of amplified fragments.

And finally, the least represented type of markers was miR858 (9%), with the highest number of loci (9) in genotype Magne (SWE). For multiple genotypes (Calibre, Flamingsgarand, Flamingsregent and Vaclav) was not recorded the amplification. The role of miR858 was described in regulation of flavonoid biosynthesis. Flavonoids are known for their antifungal activity and miR858 functions as negative regulator of plant immunity by controlling biosynthesis of flavonoids [29]. We can therefore assume that the activity of this type of marker was below the level of detection.

3.2. SDS-PAGE Analyses

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is considered to be one of the most common methods suitable for the identification and differentiation of plant genotypes based on their electrophoretic profiles. SDS-PAGE has also been shown to be suitable for the detection of oat genetic diversity. By the action of a constant electric current, the grain storage proteins of individual genotypes were divided into three groups based on different molecular weights, namely HMW-GS (high molecular weight glutelin subunits), LMW-GS (low molecular weight glutelin subunits) and prolamins, as well as residual albumins and globulins, which have the lowest weight [11,30].

Electrophoreograms show that the molecular weight of HMW-GS oats was 85 kDa to 80 kDa, LMW-GS ranged from 65 kDa to 30 kDa, and residual albumins and globulins ranged from 28 kDa to 4 kDa. The results further show that on average in the samples there were 3.77% HMW-GS (range 2.71–5.58%), 42.55% LMW-GS and prolamins (range 40.21–44.64%) and 53.69% of residual albumins and globulins (49.89–55.47%) (Figure 2). These results correspond to the findings of other authors [11,30–33].

The dendrogram (SDS-PAGE) divided the analyzed varieties into 2 main clusters, while the FUCHS variety was separately separated from the other varieties, which means that it is genetically the most distant from the other varieties. In the second cluster, the remaining 14 varieties were separated into two further sub-clusters.

There were 4 varieties in the first sub-cluster and the remaining 10 were in the second sub-cluster. The genetically most similar in terms of glutelin polymorphism were Senator and Marloo, then Ame and Cascade. Similar dendrograms because of research were achieved in their work [30–33].

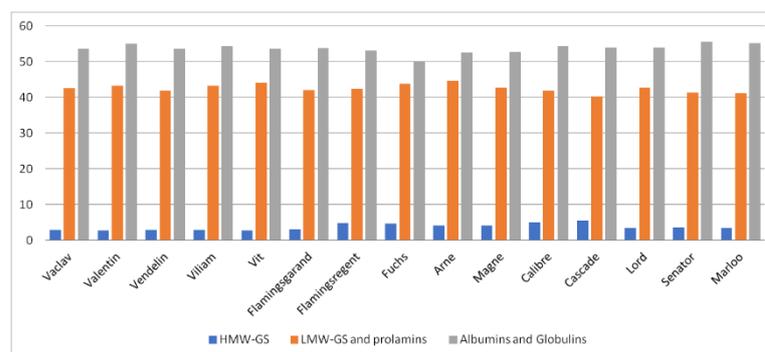


Figure 2. Percentage of protein fraction in genotypes of oat analyzed by SDS-PAGE.

4. Conclusions

Because of the functionality of miRNA-based markers, their application is appropriate in terms of characterizing the added value of plant genetic resources. The genome screening points out that all tested genotypes should have sufficient genetic background in order to provide biomass while having the potential for adaptability to environmental conditions. From this point of view, we can assume that genotypes Magne (SWE) and

Vaclav (SVK) have available higher stress adaptation potential as the number of amplified loci of the stress-sensitive markers miR398, miR858 and miR408 was the highest. On the other side, in genotype Vendelin (SVK) was observed the lowest amplification of miR156-, miR408- and miR827-based markers associated to plant growth and nutrition homeostasis. SDS -PAGE analysis allows to detect the genetic diversity of tested oat genotypes based on glutelin polymorphism.

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

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