

MicroRNA-based markers as a tool to monitor the barley (*Hordeum vulgare* L.) response to soil compaction

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Article Details: Received: 2020-03-26 | Accepted: 2020-05-19 | Available online: 2020-09-30

<https://doi.org/10.15414/afz.2020.23.03.139-146>



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Plants are often exposed to adverse environmental conditions that can significantly interfere with their genomic response. Soil compaction induced by heavy field machinery represents a major problem for crop production mainly due to restricted root growth and penetration into soil and therefore reduced water and nutrient uptake by the plants. Tested hypotheses were to declare whether the plant's genome responds to soil compaction and whether the microRNA-based markers are suitable to determine this response. A long term field scale experiment was established in 2009 where different levels of soil compaction are researched from the soil and crop point of view. The analyzed barley (*Hordeum vulgare* L.) plants were collected during the growing season in 2019. The effect of soil compaction was analysed by four different DNA-based markers corresponding to miRNA sequences of dehydration stress-responsive barley miRNAs (hvu-miR156, and hvu-miR408) and nutrition-sensitive markers (hvu-miR399 and hvu-miR827), within the leaf, stem and root tissues of barley plants. Our preliminary data support hypotheses that plant genome response was tissue-specific due to significant induction of the biomarkers to dehydration and nutrition stress. The most affected part of the plant by dehydration, were roots and lack of nutrient supply was most pronounced on leaves.

Keywords: abiotic stress, crop yield, miRNAs, root growth

1 Introduction

Compaction is regarded as one of the main causes of soil degradation and it is addressed in the proposed European Soil Framework Directive (Brussel's European Commission, 2006). Traffic compaction has adverse effects on the physical, chemical and biological properties of soils; thus, affecting important soil processes and functions, and crop productivity (Antille et al., 2019).

Soil compaction results from vehicular traffic, which causes an increase in soil strength (Chancellor and Schmidt, 1962). This restricts root development and penetration into the soil, which therefore reduces water and nutrient uptake by the plant, and translates into reduced crop yield (Unger and Kaspar, 1994; Lipiec and Hatano, 2003). Several studies (Canarache et al., 1974; Schäfer-Landefeld et al. 2004; Vero et al., 2014) have shown that traffic-induced compaction can cause reductions in soil porosity of up to 70% or greater,

which occurs primarily in the larger drainage pores, with significant disruption in pores connectivity (Berisso et al., 2012). Annual application of axle load reduces seedling emergence, grain yield, soil water storage and crop water used efficiency (Radford et al., 2001).

In Europe, the extent of soil compaction is estimated to be approximately 33 million ha (Van den Akker and Canarache, 2001). Recent research has showed that compaction is the most widespread kind of soil physical soil degradation in central and eastern Europe. About 25 million ha were deemed to be lightly compacted while a further 36 million ha were more severely affected (ESDAC, 2020).

Estimates for Slovakia suggest that approximately 600,000 ha of arable land are affected by compaction, which results in yields of winter cereal crops being reduced by up to 10% to 20% on average compared with achievable yields in central Europe in most years (Fulajtar,

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2000). An assessment of the state of soil compaction in Slovakia (Houšková, 2002) indicated that the majority of medium- and medium to heavy-textured soils, which are primarily used for arable cropping, exhibited dry bulk densities that were at or above the threshold values suggested by authors (Lhotský et al., 1991) for those soils. Further studies (Bielek, 2003; Bielek et al., 2005) indicated that up to 25% of the arable land in Slovakia has a productivity index reduced by 10% or greater as a result of soil compaction, which is exacerbated by their natural susceptibility to such compaction (Houšková and Montanarella, 2008). Kobza (2018) reported that nowadays there is over 200 000 hectares of compacted soils and 500 000 hectares are under the risk of compaction.

Physiological processes of plants affected by soil compaction are discussed in many studies (Kobaissi et al., 2013; Shukor et al., 2015; Colombi and Keller, 2019; Singh et al., 2020). As a main problem is address the low root system expansion rates due to compacted soil. Consequently the low levels of oxygen in soil reduced supply of water and nutrients leads to significant decrease of leaf development and finally decrease of biomass yield (Colombi and Keller, 2019; Correa et al., 2019). Mechanical penetration resistance (impedance) is one of the major limitations to root growth in compacted soils even under moderately wet conditions (Bengough et al., 2011). The increased soil strength results in decreased plant vigor and root numbers. The conditions for root growth in such soils are limited and aggravated by drought stress (Rivera et al., 2019). The effect of soil compaction on phenotype variability of root system is influenced by crop species including major mono- and dicotyledonous crops, genotype and stage of growth (Batey, 2009; Colombi and Walter, 2017).

MicroRNAs are endogenous, single-stranded, non-coding molecules of about 21-25 nt in size, which have an important regulatory role in plant growth and development (Axtell et al., 2011; Kruszka et al., 2012; Barvkar et al., 2013), biological and metabolic processes (Bartel, 2004; Cuperus et al., 2011; Reis et al., 2015) and regulates various developmental and physiological processes (Jones-Rhoades et al., 2006; Bej and Basak, 2014). DNA-based markers corresponding to miRNA sequences have been developed as a new type of functional markers (Fu et al., 2013). They represent a highly efficient, stable, reproducible and protocol-portable method of genotyping in the area of marker techniques (Yadav et al., 2014). A system of genotyping based on miRNAs markers was applied to *Brassica* sp. (Fu et al., 2013), *Setaria* sp. (Yadav et al., 2014) as well as to rice (Mondal and Ganie 2014; Ganie and Mondal, 2015). MicroRNA markers combine the advantages of

relatively high polymorphism, reproducibility, inter-species transferability, and ease of use with predicted functionality. Polymorphism amplified by the application of miRNAs markers indicates changes in miRNA loci sequences, which may result in changes in target gene regulation.

The aim of research into the impact of soil compaction was to identify the barley (*Hordeum vulgare* L.) genome response by stress-responsive miRNA-based markers. A prerequisite for the research was that the plants are exposed to a lack of soil moisture and nutrients due to soil compaction.

2 Materials and methods

2.1 Experimental site

Plant material was collected at an experimental site, where a long-term field scale experiment on Controlled traffic farming was established in growing season 2009/2010. The 16 ha experimental field is located at University farm in Koliňany on silty loam Haplic Luvisol. The different intensity of soil compaction is introduced through the controlled traffic of machinery at the field. The layout of the experiment and all the details are described in works of (Galambošová et al., 2017; Macák et al., 2018). Data from soil proximal sensors were collected at two areas with different soil compaction conditions (Figure 1): CTF-004B – Crop bed – non compacted soil (no field traffic since 2009/2010) and CTF-004C – Traffic line – permanent traffic line of the CTF system, all field traffic at this line since 2009/2010. The differences in soil structure conditions have been regularly measured. To show the difference, values of penetrometric resistance of the two variants is provided in Figure 1.

2.2 Genomic analysis

For molecular analyzes, barley plant material from the following experimental variants was used: samples designated CTF-004B (control variant) and CTF-004C (stress variant). The total genomic DNA was extracted from roots, stems and leaves of the bulk sample of three plants and homogenized in liquid nitrogen in accordance with the protocol by (Khanuja et al., 1999) The extracted DNA was quantified by the Implen NanoPhotometer®, and diluted to 70 ng·µl⁻¹. In order to explore the pedocompaction effect on the barley genome, four types of microRNAs markers have been used. We tested the barley genome response through drought-sensitive (miR156 and miR408) and nutrition-deficiency-sensitive biomarkers (miR399 and miR827) of microRNA (Kantar et al., 2010; Kehr, 2013; Hajyzadeh et al., 2015;). Genomic analyses were performed based on following studies (Cuperus et al., 2011; Bej and Basak,

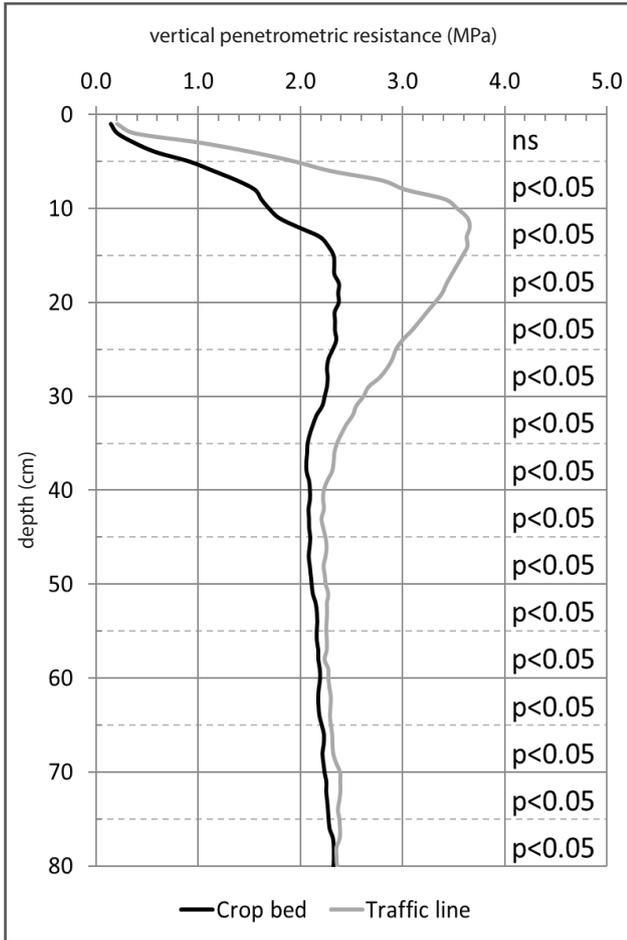


Figure 1 Intensity of soil compaction in experimental zones represented by vertical penetration resistance (crop bed and trafficked lane)
 Note: average of gravimetric soil moisture content in depth horizons 0–20 cm, 20–40 cm and 40–80 cm were 20.6%, 22.7%, 23.7%

2014), with modifications (Hlavačková and Ražná, 2015; Ražná and Hlavačková, 2017).

3 Results and discussion

Hvu-miR156 and hvu-miR408 are identified as dehydration stress-responsive miRNAs mediating barley genome response to drought stress factor (Kantar et al., 2010). Due to dehydration stress, these molecules are activated. Figure 2 illustrates tissue-specific genome reaction to dehydration stress detected by both markers hvu-miR156 and hvu-miR408. In the case of a hvu-miR156 marker was stress-induced activity detected mainly in leaf and stem tissues (Figure 2a). However in marker hvu-miR408 significant induction could be observed in stems and root tissues of the tested samples (Figure 2b). Induction of miR156 activity was detected in barley leaves and stems, whereas there were minimum changes for miR156 in roots. This data is consistent with the findings by (Kantar et al., 2010); however, in rice was found that miR156 were down regulated upon drought stress (Zhou et al., 2010). It seems that the drought biomarkers response is not only tissue-specific but also species-specific (Kruszka et al., 2014) moreover in the case of miR408 marker its response might be cultivar-specific (Melnikova et al., 2016).

Many plant miRNAs contribute to plant genome response to abiotic stress factors (Bej and Basak 2014; Zhang, 2015; Rajwanshi et al., 2014; Sun et al., 2019). MicroRNA-based markers are considered functional molecular markers because they are designed from sequences corresponding to pre-miRNA or mature miRNAs and therefore they are able to predict phenotypes controlled by miRNA. Observed polymorphism indicates sequence

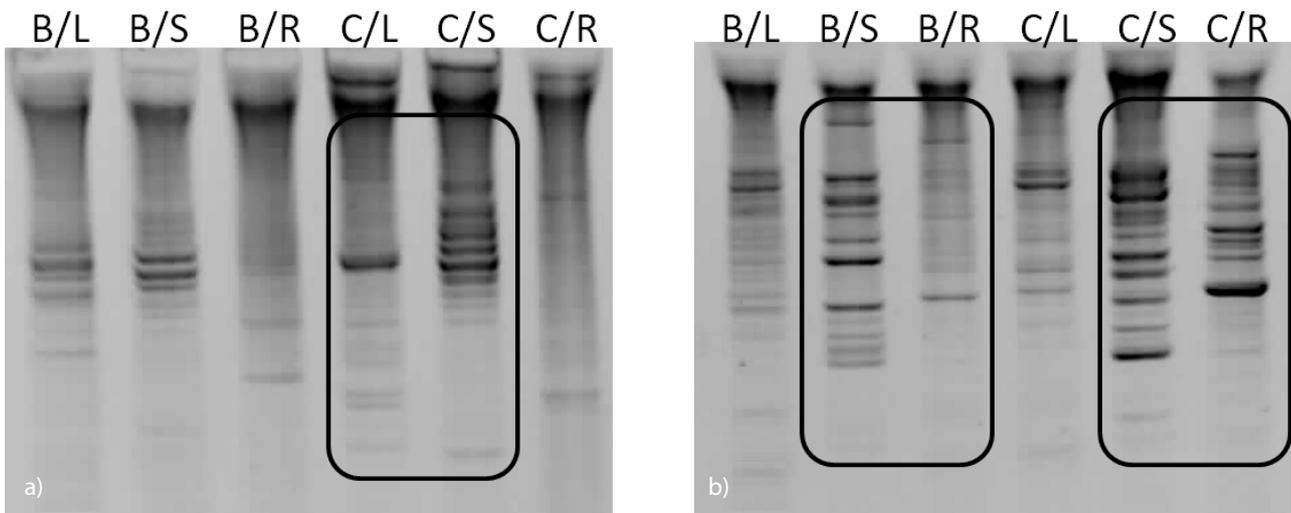


Figure 2 Profile of DNA fragments amplified by marker hvu-miR156 (a) and hvu-miR408 (b) in barley control (B) and stressed (C) samples
 L – leaves, S – stems, R – roots

variability or changes in miRNA loci as a consequence of modified regulation of targeted genes (Fu et al., 2013; Yadav et al., 2014).

Digital profiles of the electrophoreograms obtained by the marker hvu-miR408 showed, in comparison to control samples, significant induction of the biomarker to dehydration stress as a consequence of pedocompaction effect (Figure 3). Our findings are supported by the several studies where the activity of miR408 was induced as a result of water deficiency (Kantar et al., 2010; Zhou et al., 2010; Hajyzadeh et al., 2014). The role of miR408 in the regulatory mechanisms associated with plant genome response to dehydration stress was proved by Bej and Basak (2014) and Ma et al. (2015).

Stress-induced activity of hvu-miR408 marker was detected mainly in stem and root tissues, which corresponds to the results of Trindade et al. (2010), where strong up-regulation of miR408 in roots of *Medicago truncatula* and down-regulation of its target gene was observed. A minor decrease in activity of miR408 marker in leaves under drought stress was observed (Figure 1), which is in line with the results of Ma et al. (2015), where a minor decrease of miR408 level under drought stress was observed in *Arabidopsis leaves*. According Liu et al. (2008) the miR408 was included into category of miRNAs

whose target genes respond to several stresses. However, the research of Hajyzadeh et al. (2014) proved that miR408 is involved in drought stress regulation and increased level of miR408 is important to drought tolerance. There is evidence that many of the miRNAs (including miR156 and miR408) are involved in the stress tolerance and developmental processes which make it possible to assume that these miRNAs might be co-regulated by both environmental factors and developmental stimulus (Liu et al., 2008; Zhou et al., 2010; Ma et al., 2015; Zhang, 2015).

Another important factor to be considered in terms of miRNAs expression pattern is the specification of miRNAs molecules belonging to certain miRNA family. Observations showed that miRNAs encoded by different genes representing the same MIR gene family might differ in their expression regulation pattern (Kruszka et al., 2014). As it can be seen in the study of (Zhou et al., 2010) where significant down regulation of miR408e of sugar cane was observed in response to drought stress. The differential response of miR408 in reported studies could also be due to the different developmental stages of testes plant, stress intensity, growth conditions and methods employed to monitor miRNAs activity (Zhang, 2015).

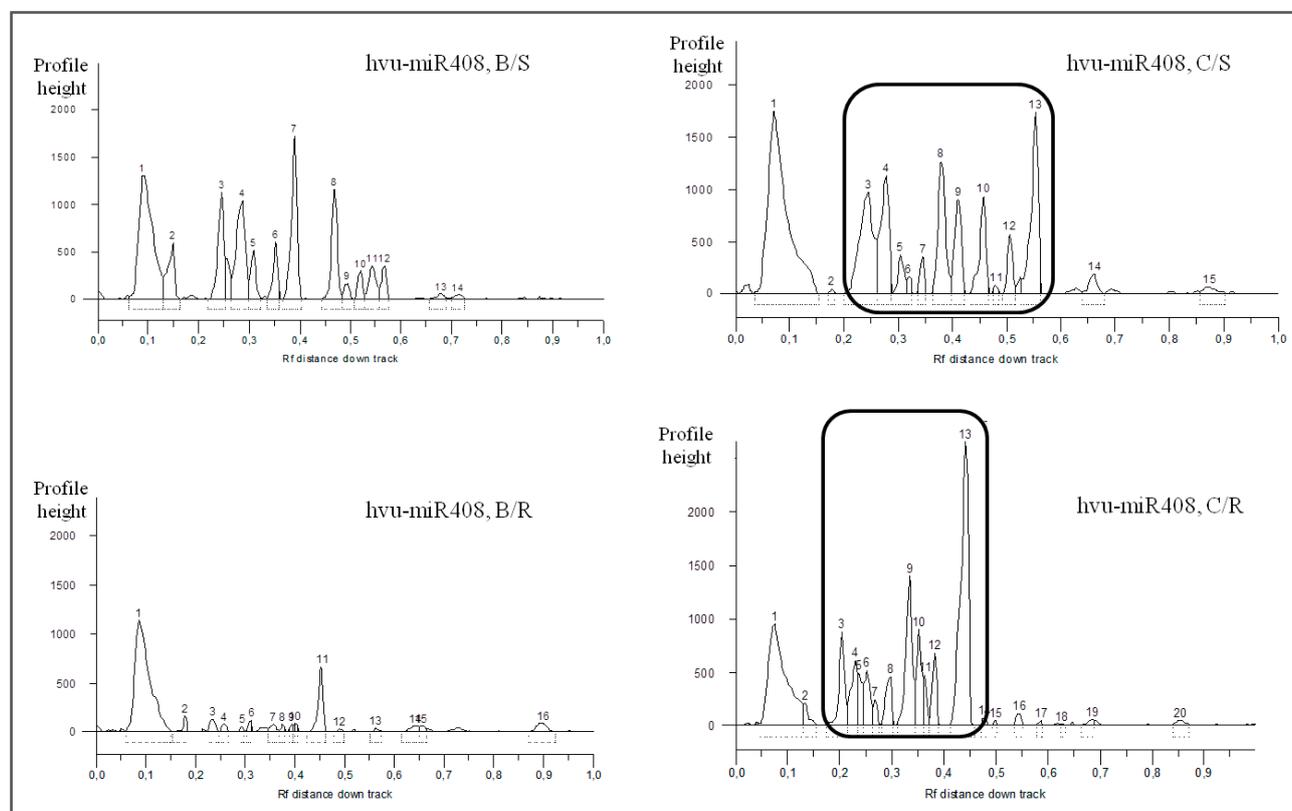


Figure 3 Digital profiles of the electrophoreograms obtained by the hvu-miR408 marker. Control (B) and stressed (C) samples L – leaves, S – stems, R – roots

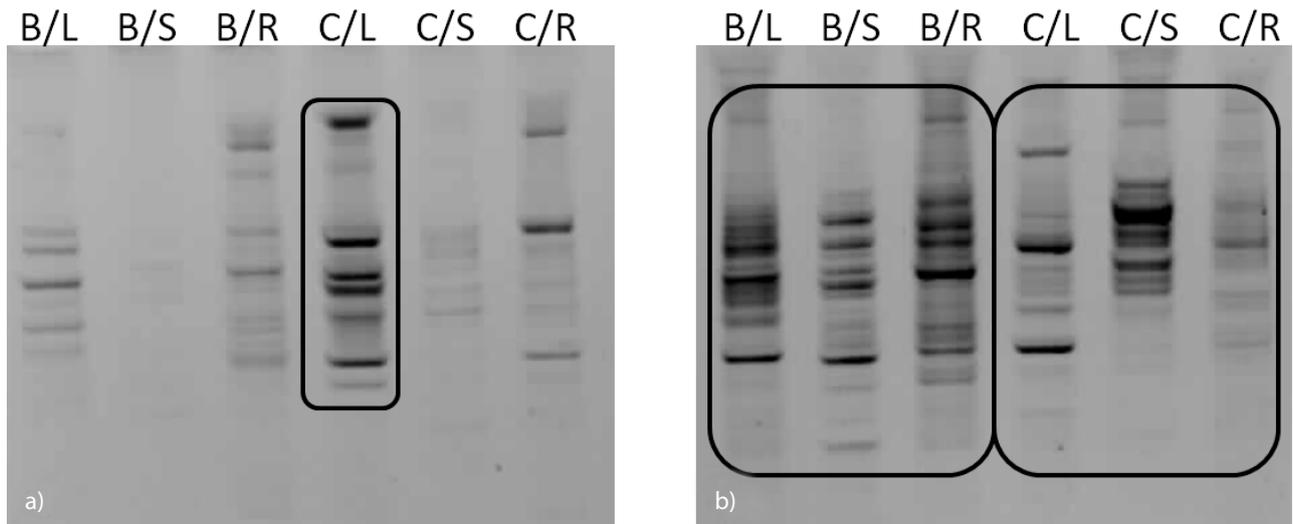


Figure 4 Profile of DNA fragments amplified by markers hvu-miR399 (a) and hvu-miR827 (b) in control (B) and stressed (C) samples
 L – leaves, S – stems, and R – roots

Two other types of nutrition-sensitive markers, hvu-miR399 and hvu-miR827 belong to the most conserved miRNA families in barley genome (Wu et al., 2014), which points to their importance in plants genome. They play an important role in regulatory mechanisms related to nutrient homeostasis (Hsieh et al., 2009; Schreiber et al., 2011; Wu et al., 2014; Melnikova et al., 2015; Melnikova et al., 2016). We assumed that as a consequence of water deprivation the plants will suffer of nutrition deficiency. Both types of markers are characterized by differentially expressed activity under different nutrient deprivation (Hsieh et al., 2009; Soumitra et al., 2015; Melnikova et al., 2016). Expressive activity of hvu-miR399 marker could be observed in leaves tissue of barley plants (Figure 4a). That

is in line to results of studies (Lin et al., 2008; Pant et al., 2008) which confirm the role of miR399 in shoot-to-root movement in the initial response to Pi deprivation.

On the other site the activity of the miR399 biomarker might be also connected to nitrogen deficiency (Soumitra et al., 2015), which indicates a more general role of this marker in mineral homeostasis. There is an evidence that NITROGEN LIMITATION ADAPTATION (NLA) gene, which is regulated by miR827, participates on plant adaptation under low- nitrogen conditions, which at the same time induce the activity of miR399 (Kant et al., 2011; Soumitra et al., 2015).

The activity of the hvu-miR827 marker was not only tissue specific (Figure 4b) but also unique with respect to

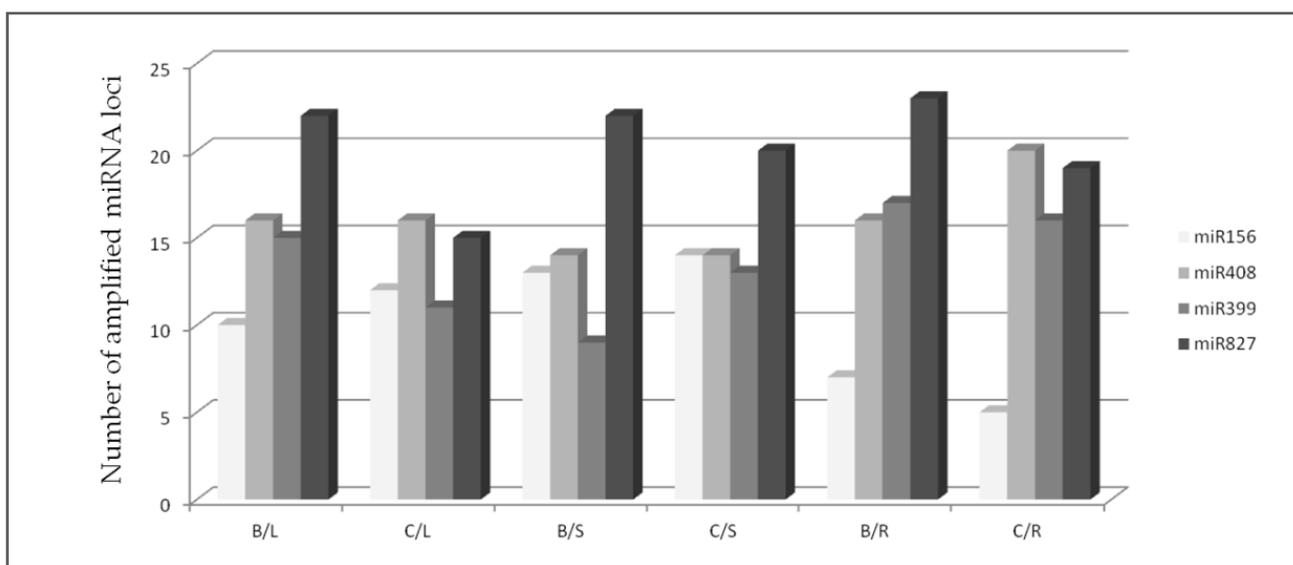


Figure 5 Amplified miRNA loci by individual markers in control (B) and stressed (C) samples
 L – leaves, S – stems and R – roots

control and test variants. Surprisingly, we did not observe the activity increase of this marker due to the phosphate deficiency as recorded in several studies (Hsieh et al., 2009; Schreiber et al., 2011; Soumitra et al., 2015;). On the contrary, the repression of miR827 in individual tissues was observed with the strongest reduction in leaves (Figure 5). Searching for the reasons of this pattern activity, we found out that expression of microRNA827 is due to nitrogen deprivation down-regulated (Liang et al., 2015; Soumitra et al., 2015). There is an antagonistic interaction between the accumulation of phosphate and decrease of nitrogen supply (Kant et al., 2011). In view of the above, we could state that observed up-regulation of hvu-miR399 and down-regulation of hvu-miR827 indicated insufficient supply of plant tissues with nitrogen under stress of pedocompaction.

Traffic induced compaction is a major problem of today's crop production as almost 85% of area is trafficked annually in a conventional cropping system (Kroulík et al., 2011). This is considered to be one of the factors of yield stagnation (Keller et al., 2019). Effect of soil compaction on crop and yield has been described in many studies. For examples the research of (Chamen et al., 1995; Arvidsson 1999; Radford et al., 2007) and others indicated reduced water and nutrient use efficiencies, and therefore yield of winter cereal crops grown in soils are affected by traffic compaction. Due to these, technologies to minimise the negative effect of machinery traffic on soil and crop has been developed and adopted across the world (Chamen 2015). The significant effect of traffic compaction on crop development and yield at the experimental is described in following studies (Rajwanshi et al., 2014; Galambošová et al., 2017; Antille et al., 2019). Difference in yield between the non-trafficked area and areas with different level of compaction ranged from 9 to 33% for spring barley (Galambošová et al., 2017). The contribution of this study is in the novel and interdisciplinary approach of plant genome response analysis to soil compaction by miRNA-based molecular markers.

4 Conclusions

We can conclude that the plant genome responds to the external abiotic stress factor. The molecular markers based on microRNAs sequences made it possible to detect changes in the level of genomic polymorphism. The number of amplified miRNAs loci was statistically significantly dependent on the stress-sensitive marker applied. As a result of the mechanical compression of the soil, the plants were exposed to a lack of moisture, which also limited the intake of nutrients. This consequently led to reduction of plant growth parameters and reduced yields. We consider such an interdisciplinary approach to a given issue to be innovative.

Acknowledgments

This study is funded by EU co-finance project ITMS 26220220180 Building "AgroBioTech" Research Centre.

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