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In silico Analyzes of miRNAs Associated with Root and Tuber in *S. commersonii*

Aysel Ozgul KORAL[1], Mine TURKTAS[2*]

[1]*Cankiri Karatekin University, Faculty of Science, Biology Department, Cankiri, Turkey*
[2]*Gazi University, Faculty of Science, Biology Department, Ankara, Turkey*

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Abstract

Potato is an industrial plant that is produced and consumed globally due to its cheapness, high yield in the unit area, high nutritional values. It is used in many different fields. It has been stated that wild species with various characteristics can be used in studies to increase productivity because they have greater rate of genetic variation than their domesticated relatives. One of the wild species of potato found in nature is *S. commersonii* Dunal. It is more resistant to many stresses than cultivated potato *S. tuberosum* L. Also, its tuber has better quality due to the fact that it contains a higher proportion of dry matter. With the aim of determining the effects of miRNAs in tuber production and root characteristics relation we aimed to detect miRNAs in two transcriptome libraries of *S. commersonii*. In this study miRNAs were evaluated for the first time in the wild potato transcriptome data using *in silico* analysis. A number of miRNAs were identified, and their potential roles in tuber were discussed.

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Author ID (ORCID Number)

A. O. Koral, 0000-0002-1206-5130
M. Turktas, 0000-0001-8089-3774

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1. INTRODUCTION

Potato is a plant belonging to the Solanaceae family that is consumed heavily as a vegetable in the fourth place in the world. It is an industrial plant that is produced and consumed globally due to its cheapness, high yield in the unit area, high nutritional values and being used in many different fields. Although there are 2000 genera of potato plant in nature, about 180 of these can produce tubers. Yet, only eight of these tuber-producing genera have been cultured for use in the food field (Dilsiz & Yorgancılar, 2018).

It has been stated that wild species with various characteristics can be used in studies to increase productivity, because they have greater rate of genetic variation than their genetically bred relatives (Hajjar & Hodgkin, 2007). Therefore it has been proposed that wild potato species contain important characters such as disease resistance and therefore the wild species are used in breeding studies.

S.commersonii is a tuber-bearing wild potato. It has a higher quality tuber structure and stach content, yet it is more resistant to many stresses when compared to the commonly grown cultivated species (*S. tuberosum* L.) (Hanneman & Bamberg, 1986). Due to these important features, its genome was recently sequenced (Aversano et al., 2015). The genome size is about 80 Mb, and the study revealed that there are several genes effective in stress response in the genome of *S. commersonii* which are not present in *S. tuberosum* L. Moreover, duplications have been found in some gene regions in the wild potato genome suggesting that they provide advantages in adaptation to stress.

During the root formation system of the potato plant in the soil, white extensions occur between the roots which are called stolons. With the swelling of the ends of the stolons, tubers are formed. The tubers of the

*Corresponding Author, e-mail mturktas@gazi.edu.tr

plants are rich in starch. Consequently, tubers of the potato plant are the main carbohydrate source in many diets. Formation of stolons, the underground stems, in potato is known as tuberization. Tuberization is a complex process and affected by several factors. Various mechanisms are involved in tuberization in potato plant. In tuber formation, signals are received and processed in the leaves of the plant and these mobile signal molecules are transported towards the stolon tip (Chapman, 1958). As the signal molecules reach to stolon end, the cell growth at the stolon end begins to longitudinal growth. This causes bulging of the stolon. Following that, the starch synthesis in the leaves and the transport of these starches to the stolon form tuber development signals with the accumulation of starches at the tip of the stolon.

The tuberization in potato is a dynamic process and occurs with the interaction of many environmental and internal factors. Although, the physiology of tuberization is well-known, the molecular mechanisms of tuber formation in potatoes has not been completely elucidated. Thus, understanding of the tuberization process is vital for improvement of tuber quality and yield.

One of the molecules in the formation of tuber in the potato plant involved in this process is microRNAs (miRNAs) (Natarajan et al., 2017). They are non-coding small RNA molecules which repressing their target genes in many biological processes. Due to these important roles, studies have been conducted on the detection of miRNAs in many organisms, such as computer-based *in silico* methods in which the motif content and secondary structures of miRNAs are analyzed.

Until now, there are studies performed on miRNA analysis in potato. It has been reported that potato-specific miR193, miR152 and conserved miR172-1, and miR172-5 were significantly expressed in the tuberization development process in potato (Lakhotia et al., 2014). Besides, it has been shown that miR164-1, miR399-1, miR157-1, miR171-1 have key roles in tuberization (Kondare et al., 2018). Besides, potato-specific miR53, miR172, and miR399, which are miRNAs transportable in vascular tissues, are found to be effective in tuberization (Marín-González & Suárez-López, 2012). Some of those miRNAs target genes involved in signaling pathways, giberellic acid, auxin, jasmonic acid, signaling, while the targets of many miRNAs are still unknown.

It is also known that production of tuber is associated with root characteristics such as mass, lenght (Ahmadi et al., 2017). Therefore we aimed to evaluate this relation in term of miRNAs in root and tuber. We performed *in silico* analysis of miRNAs in transcriptome libraries of *S. commersanii* root and tuber. This is the first study on detailed miRNA analysis in underground organs of the wild potato using *in silico* analysis. Comparative analysis of wild potato will increase our knowledge of the usage of wild species in crop breeding and improvement.

2. MATERIAL AND METHOD

In this study transcriptome libraries of *S. commersonii* root and tuber with the accession numbers of SRR1687231 and SRR1687232, respectively, were analysed. The raw data was obtained from NCBI databank using SRA-Toolkit (v. 2.11.1). The miRNA sequences were obtained from miRBase (22.1). Since there is any *S. commersonii* miRNA is available in the databank, miRNA sequences of *S.tuberosum* (SolTub3.0) which is the most closely related species was used.

Blast+ (v. 2.12) was used for homology analysis between the *S. tuberosum* miRNAs and *S. commersonii* transcriptome sequences. E-value was set as 0.01. The resulting sequences were analysed, and the read counts of the identified miRNAs were extracted by in home perl script. The blast analysis was also performed on the cDNA data of *S.tuberosum* in order to exclude false positive miRNAs from the results by analyzing the mRNA target regions of the matching reads. The protein coded sequences were extracted from the data. The miRNAs showing 2-fold difference between the libraries were assigned as differentially expressed miRNAs.

3. RESULTS AND DISCUSSION

RESULTS As a result of the bioinformatics analysis, total of 47 miRNAs were idendified. Among them, 32 miRNAs showed different expression levels between the transcriptome libraries. The miRNAs of Stu-miR477b-3p, Stu-miR482a/b-3p, Stu-miR5303e/f/g/h/i/j, Stu-miR6023, Stu-miR6026-3p, Stu-miR6027, Stu-

miR7122-5p, Stu-miR7982a/b, Stu-miR7983-3p, Stu-miR7997c, Stu-miR8011b-3p, Stu-miR8011b-5p, Stu-miR8026, Stu-miR8030-3p were up-regulated in root transcriptome library. While Stu-miR7987 was found to be expressed more in the tuber than the root. Although some miRNAs with very low read counts showed difference in expression between the libraries, they were not classified as differential miRNAs due to their low read count. The 15 miRNAs showed similar expression levels between the libraries. The read counts are given in Table 1.

Table 1. *Read counts of miRNAs in S. commersonii root and tuber transcriptome libraries*

miRNA name	**SRR1687231_1 (Root)**	**SRR1687232_1 (Tuber)**
stu-miR156d-3p	1	0
stu-miR156f-3p	2	0
stu-miR477a-3p	2	0
stu-miR477b-3p	5	0
stu-miR482a-3p	12	1
stu-miR482b-3p	5	0
st-miR482d-3p	2	0
stu-miR482e-3p	2	0
stu-miR5303a	25	20
stu-miR5303b	25	20
stu-miR5303c	25	20
stu-miR5303d	25	20
stu-miR5303e	26	3
stu-miR5303f	38	9
stu-miR5303g	154	40
stu-miR5303h	93	36
stu-miR5303i	154	40
stu-miR5303j	90	16
stu-miR6023	43	14
stu-miR6026-3p	6	0
stu-miR6027	10	0
stu-miR7122-5p	16	1

Table 1. *(continued)*

miRNA name	SRR1687231_1 (Root)	SRR1687232_1 (Tuber)
stu-miR7981-3p	1	3
stu-miR7982a	9	0
stu-miR7982b	9	0
stu-miR7983-3p	7	1
stu-miR7987	0	5
stu-miR7996a	1	0
stu-miR7996b	1	0
stu-miR7996c	1	0
stu-miR7997c	9	0
stu-miR8001a	2	0
stu-miR8001b-5p	1	0
stu-miR8006-5p	1	3
stu-miR8007a-3p	3	0
stu-miR8007a-5p	0	1
stu-miR8007b-3p	1	0
stu-miR8011b-3p	4	1
stu-miR8011b-5p	4	1
stu-miR8014-5p	1	0
stu-miR8020	4	4
stu-miR8025-3p	2	0
stu-miR8026	5	0
stu-miR8029	1	0
stu-miR8030-3p	2	0
stu-miR8031	1	0
stu-miR8032b-3p	36	18

4. DISCUSSION

Tuberization is an important process in potato and several mechanisms play parts in the formation of tuber in the potato plant. Among these mechanisms, miRNAs play vital roles, but there is still insufficient information about the effects of miRNA on tuberization in potato. Moreover, in wild potato which has high quality tuber structure there is any information. For that reason, we aimed to evaluate the miRNA influences in root and tuber of wild potato.

The relation between tuber production and root characteristics is known (Ahmadi et al., 2017). The comparative analyses indicated that many miRNAs are differentially expressed in tuber and root which shows organ specific regulation of the miRNAs in wild potato. Besides, the differentially expressed miRNAs are mainly related with tuberization. The results proved that the presence of miRNAs in tuberization process in wild potato.

Kondhare et al. (2018) found that Stu-miR8006-5p expression was increased in stolon tissues. The miRNA affects on phosphatese 2c which plays role in abscisic acid (ABA) signaling (Fujii et al., 2009). Moreoever, it was proposed that ABA is vital in potato tuberization (Marschner et al., 1984). Since the expression pattern of miR8006-5p in our analysis was found to be similar to the previous study, the results showed that miR8006 act on tuberization via ABA signaling.

The other differentially expressed miRNAs detected in *S. commersonii* were miR482a and miR482b. It has been reported that the expression of Stu-miR482a-3p in stolon tissue is increased in *S. tuberosum* and it takes part in the initial phase of tuberization (Chi et al., 2015). In another study, it was reported that this miRNA targets both StARF8 (auxin response factors 8), which is involved in the auxin synthesis pathway and plays an activator role in tuberization, and StSUT1 (sucrose transpoter 1) gene, which is involved in sucrose transport and plays an activator role in tuberization (Kondhare et al., 2020). Thus, it appears that auxin signaling is essential in the initial tuberization step. A situation consistent with this finding was also found in our results. Observing that Stu-miR482a-3p is significantly suppressed in tuberous tissue, it can be understood that it is required only in the early stage of tuberization. On the other hand, although the effect of Stu-miR482b-3p has not been not known exactly, its relationship with tuberization was detected for the first time in this study.

Kondare et al. (2018) indicated that Stu-miR5303f and Stu-miR5303g were involved in stolon-tuber transition in *S. tuberosum*. Moreover Stu-miR5303g regulates Ca2+ release and expression of inositol monophosphatase 3 gene. It is also involved in ABA signaling (Jia et al., 2019). Thus, it appears that Ca2+ release and ABA signaling is essential in the initial tuberization step. It has been reported that Stu-miR5303f/g stimulates TAS-like loci of unknown function in tuberization (Kondhare et al., 2018). Confirming the fact that Stu-miR5303 is needed in early tuberization stage, in our study Stu-miR5303 e, f, g, h, i, j forms were found to be suppressed in tuber. Stu-miR5303e, h, j, i have not been not known exactly, their relationship with tuberization was detected for the first time in this study.

There is another tuberization related miRNA known as miR477. It has been stated that miR477a/ b-5p is involved in the expression regulation of DELLA which is a GRAS transcription factor (Kim et al., 2005) and required for radial formation and development in the root and early stolon in tuberization in *S. tuberosum* (Kondhare et al., 2018). In our study, the expression of miR477a/ b was increased in root. However, it should be noted that the miRNA counts are very low in the libraries. Therefore, the read count are insufficient to make a firm decision.

In our study it was found that Stu-miR6023 was suppressed in tuber. Stu-miR6023 controls StuPME21575 which is a pectinesterase (Yan et al., 2020). It is known that tuber pectin methyl esterase activity (PME) is a potential factor influencing the textural properties (Ross et al., 2011). It is concluded that down regulation of miR6023 in tuber stimuleted the expression of pectinesterase in starch and changed the pectin content in wild potato tuber. It is speculated that the differences in pectin structure between wild and domesticated potato might be related with miRNAs.

miR7983 is a *Solanaceae* species specific miRNA and cleaves hydroxyproline-rich glycoprotein (HRGP) which is a major group of wall glycoproteins (Kondhare et al., 2018). HRGPs are involved in numerous

processes such as cell wall integrity pathway (Johnson et al., 2017). Our analysis indicated that differential expression of miR7983 might be realeted with cell wall architecture heterogeneity between root and tuber in wild potato.

It was found that Stu-miR6026-3p targets GA 2-oxidase gene (StGA2ox1) which is a key giberellic acid metabolic gene and involved in stolon-to-tuber transition potato (Kondhare et al., 2018). Confirming the fact that suppression of miR6026 results in tuber development, Stu-miR6026-3p was down-regulated in tuber in our study.

One of the major categories of small RNAs is phased secondary small interfering RNAs (phasiRNAs) (Liu et al., 2020). It was shown that miR7122 triggers phasiRNA production (Xia et al., 2013). In our study expression of miR7122 was repressed in tuber. Although functions of the phasiRNA are still poorly defined, this is the first study showing their contribution in root and tuber interaction.

In silico analysis revealed that miR6027 was up-regulated in root transcriptome library. In literature it was shown that miR6027 represses Sw-5b gene which is resistance gene (de Oliveira et al., 2018). The miRNA also plays a role in phasiRNA biogenesis (Seo et al., 2018). Therefore this is the first study indicating the role of miR6027 in tuberization. On the other hand since both miR7122 and miR6027 which are invovled in phasiRNA biogenesis were up-regulated in root, it can be inferred that phasiRNAs have important functions in potato root.

miR7982 is another stress related miRNA which was identified in *Cajanus cajan* (Shanmugavadivel et al., 2016). In our study, it was observed that Stu-miR7982a and b were suppressed in tuber and were detected for the first time in relation to tuberization.

miR7997c targets bZIP transcription factors targeting several genes involved in defense responses, growth and development (Cheng et al., 2016). Our study revealed its role in root and tuber involvement for the first time.

Until now, any information has been found in the literature on which pathways stu-miR8011b-3p, 5p, stu-miR8032b-3p, stu-miR8026, stu-8011b-3p and stu-8011b-5p are involved. However, these miRNAs were significantly suppressed in tuber tissue in our study, and their relationship with tuber and root relevance was detected for the first time. Therefore, it is necessary to investigate the genes affected by these miRNAs with further studies.

5. CONCLUSION

In this study, significant differences were determined in terms of miRNA expression level of tuber and root. As a result of *in silico* analysis of tuber and root transcriptome libraries of *S. commersonii* that the most miRNAs were found to be effective in cell wall architecture, textural properties, tuberization. Moreover, involvement of some miRNAs in root and tuber relation was detected for the first time, and this study serves important data in understanding the roles of miRNAs in wild potato. Howoever, with the purpose of understanding the efficiency of these miRNAs furher functional analyzes should be performed. The miRNAs should be clarified in more detail, so that the obtained data could be transferred to *S. tuberosum* for breeding studies in terms of increasing tuber quality.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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