Characterization of circular RNAs and their role in wilt stress tolerance in soybean

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Abstract

Soybean (Glycine max) is an important oil seed crop and widely grown legume in India as well as in the world. The yield of soybean is severely affected by Wilt disease caused by a fungus Fusarium oxysporum. Several coding and non-coding RNAs in plant cells may directly or indirectly play a role in exhibiting stress tolerance to wilt. Here, an attempt has been made to identify the role of circular RNAs (circRNAs), a type of non-coding RNAs, in wilt stress tolerance mechanism. The study also aims at identification of circRNAs that act as endogenous target mimics (eTMs) or as sponges to miRNAs that may regulate the genes involved in wilt stress tolerance. The study reveals the presence of 24 differentially expressed circRNAs (DEcircRNAs) between the transcriptomes of wilt affected and control samples out of which 18 were acted as eTMs for 10 microRNAs (miRNAs). These miRNAs in turn found to regulate 275 genes that fall under 14 Gene Ontology (GO) based functional categories. The GO analysis further shown the annotation term ‘stimulus response’ for most of the DEcircRNAs, which were found in various vital activities like polygalacturonase activity, carbohydrate metabolic process. Hence, it is predicted that the identified miRNAs got absorbed by the circRNAs that acted as sponges or eTMs and the miRNA regulated genes are likely to be freed up in cell to exhibit wilt stress tolerance mechanism in soybean.

Keywords: Circular RNAs, wilt stress, soybean, miRNAs, eTMs

Introduction

Soybean is one of the largest grown legume crops worldwide (Singh et al. 1992) [17] and is also important for oil extraction. The crop contains about 23% oil and is also rich in protein content to an extent of 42% (Dornbos et al. 1992) [5]. However, the productivity of the crop has been found to drastically reduce due to the effect of Fusarium blight or wilt. Studies on the regulatory roles of non-coding RNAs on wilt stress tolerance have been made in the past in various leguminous crops. Kohli et al. (2014) [8] identified and characterized wilt stress responsive miRNAs in chickpea and found that a number of miRNAs were highly up-regulated in response to fungal infection. It has also been observed that lncRNAs are important components of the antifungal networks in A. thaliana (Zhu et al. 2014) [21]. Several studies have been made in the recent past on the function of non-coding RNAs for a variety of stresses in soybean. Panda et al. (2018) [14] reported that circular RNAs might have played significant roles in regulating genes responsible for different stress tolerance mechanisms in plants. These circRNAs are a class of non-coding RNAs formed by a covalent linkage between the 5’ and 3’ ends of an RNA molecule. Ebbesen et al. (2016) [6] reported that circRNAs might have originated from exons, introns or intergenic regions. Chu et al. (2018) [3] found that the circRNAs may have a variable length ranging from 100 nucleotides to several kilobases and may act as sponges to miRNAs. This may prevent the miRNAs from regulating the miRNAs. Differentially expressed circRNAs may act as functional regulators to biological processes specific to stress responses in plants (Li et al. 2018) [12]. Zhao et al. (2017) [20] identified 5,367 circRNAs associated with resistance to defoliating insects in soybean leaves. However, studies are yet to be fully explored on characterization of circRNAs in soybean for wilt stress tolerance. Hence, in this study, the circRNAs were identified from wilt affected soybean crop using RNA-Seq data and their role as eTMs in regulating genes responsible for wilt stress tolerance was studied in silico through bioinformatic approaches.
Materials and Methods
RNA-seq data for wilt under control and wilt affected soybean were downloaded from https://www.ncbi.nlm.nih.gov/ having accession numbers: SRR4095539 (control), SRR4095541 (wilt affected)
Soybean whole genome data and miRNAs present in soybean were downloaded from http://plants.ensembl.org/index.html and http://www.mirbase.org/ respectively.

Data preparation
FastQC (Andrews, 2010) [1] was used for processing the raw data to get quality reads and Trimmomatic tool (Bolger et al. 2014) [2] was used to trim the poor quality segments of the reads. Finally quality reads were subjected to further downstream analysis.

Circular RNAs identification and differential expression pattern analysis
Mapping of the trimmed reads onto the reference genome was done using BWA MEM (Li, 2013) [9] and the resulted SAM file and reference genome were used for identifying circRNAs by using CircRNA Identifier (CIRI) (Gao et al. 2015) [10]. For the analysis of differential expression pattern of the identified circRNAs, expression levels were initially calculated using RSEM (Li and Dewey, 2011) [10] and finally the differentially expressed circRNAs were detected using the R package DESeq2 (Love et al. 2014) [13].

CircRNA-miRNA-mRNA interaction
Initially, prediction of miRNAs that target the differentially expressed circRNAs was done by Target Finder (http://www.bioit.org.cn/ao/targetfinder.html). Subsequently, these miRNAs were subjected to psRNA Target (http://plantgrn.noble.org/psRNATarget/) (Dai and Zhao 2018) [4] to identify their target genes or mRNA targets. These two steps indicate that miRNAs instead of targeting the mRNAs might target the circRNAs that may mimic the mRNAs and hence may not target the genes or mRNAs. Finally, annotation of the targeted miRNAs was done using BLAST2GO (https://www.blast2go.com/).

Results and Discussion
Identification of circular RNAs under wilt stress condition
A total of 48 circular RNAs were identified under control condition (CT), out of which 31 (64%), 15 (32%) and 2 (4%) were of intergenic type, exonic type and intronic type respectively (Fig 1a). Whereas, 75 circular RNAs were identified under wilt stress condition, out of which 53 (71%), 18 (24%) and 3 (4%) were found to be of intergenic, exonic and intronic types respectively (Fig 1b).

Expression pattern of circular RNAs under wilt stress condition
The expression patterns of circRNAs between control and wilt affected plants were compared in order to investigate whether they are expressed in specific manner under wilt stress condition. A total of 24 differentially expressed circular RNAs were detected between wilt stress and control conditions. Among them 12 were found to be downregulated (log2FoldChange<0) (Table 1), and 12 were found to be upregulated (log2FoldChange>0) (Table 2).

Table 1: List of downregulated circRNAs

| circRNA ID                  | Base Mean | log2FC | SE(log2FC) | Wald Statistic |
|-----------------------------|-----------|--------|------------|----------------|
| 11_dna:chromosome_chromosome: Glycine_max_v2:11:1:347666867:1 | 2666.467  | -0.26914 | 0.354095   | -0.76009       |
| 17_dna:chromosome_chromosome: Glycine_max_v2:17:1:446306461:1 | 709.3314  | -2.29063 | 1.350556   | -1.69606       |
| KZ847214_dna:supercontig_supercontig: Glycine_max_v2:1:KZ847214:1:64:745:1 | 38.78359  | -1.34509 | 1.109218   | -1.21264       |
| 11_dna:chromosome_chromosome: Glycine_max_v2:11:1:347666867:1 | 356.1966  | 0.87585  | 0.757436   | -1.15633       |
| 2_dna:chromosome_chromosome: Glycine_max_v2:12:1:48577505:1 | 710.5561  | -0.7436  | 0.670172   | -1.10956       |
| 9_dna:chromosome_chromosome: Glycine_max_v2:19:1:50189764:1 | 319.6854  | -0.57223 | 1.069754   | -0.53583       |
| 16_dna:chromosome_chromosome: Glycine_max_v2:16:1:37887014:1 | 130.0271  | -0.44501 | 0.531682   | -0.83699       |
| 18_dna:chromosome_chromosome: Glycine_max_v2:18:1:50081841:1 | 145.7446  | -0.40209 | 0.409557   | -0.8049        |
| 8_dna:chromosome_chromosome: Glycine_max_v2:8:1:78319740:1 | 52.8615   | -0.37559 | 0.580686   | -0.6468        |
| 7_dna:chromosome_chromosome: Glycine_max_v2:7:1:446306461:1 | 58.78775  | -0.23515 | 0.510527   | -0.45668       |
| 13_dna:chromosome_chromosome: Glycine_max_v2:13:1:45874162:1 | 42.2537   | -0.2012  | 0.559047   | -0.3599        |
| 2_dna:chromosome_chromosome: Glycine_max_v2:2:1:48577505:1 | 234.5386  | -0.0819  | 0.312221   | -0.26232       |
**Table 2:** List of upregulated circRNAs

| circRNA_ID | Base Mean | log2FC | SE (log2FC) | Wald Statistic |
|------------|-----------|--------|-------------|---------------|
| 12_dna:chromosome_chromosome: Glycine_max_v2.1:1:40091314:1 | 41.64133 | 5.29E-08 | 0.540154 | 9.79E-08 |
| 6_dna:chromosome_chromosome: Glycine_max_v2.1:6:1:51416486:1 | 48.37742 | 0.035332 | 0.511331 | 0.194195 |
| 4_dna:chromosome_chromosome: Glycine_max_v2.1:4:1:52389146:1 | 1509.702 | 0.071321 | 0.588745 | 0.305426 |
| 3_dna:chromosome_chromosome: Glycine_max_v2.1:3:1:45779781:1 | 34.49698 | 0.114331 | 0.588745 | 0.173271 |
| 14_dna:chromosome_chromosome: Glycine_max_v2.1:14:1:49042192:1 | 9.185587 | 0.167549 | 0.966981 | 0.173271 |
| 12_dna:chromosome_chromosome: Glycine_max_v2.1:12:1:40091314:1 | 445.8071 | 0.281126 | 0.382938 | 0.73413 |
| 14_dna:chromosome_chromosome: Glycine_max_v2.1:14:1:49042192:1 | 433.5597 | 0.368008 | 0.440086 | 0.836218 |
| 3_dna:chromosome_chromosome: Glycine_max_v2.1:3:1:45779781:1 | 82.46615 | 0.387959 | 0.536982 | 0.72248 |
| 6_dna:chromosome_chromosome: Glycine_max_v2.1:6:1:51416486:1 | 2620.342 | 0.511998 | 0.518077 | 0.988266 |
| 3_dna:chromosome_chromosome: Glycine_max_v2.1:3:1:45779781:1 | 740.1542 | 0.866811 | 0.736806 | 1.176443 |
| 8_dna:chromosome_chromosome: Glycine_max_v2.1:8:1:47837940:1 | 1900.396 | 1.843349 | 1.436851 | 1.282908 |

In our present study the expression patterns were found to be significantly different for 24 circRNAs suggesting that they might play a role in responding to the stress condition. Interestingly we found one down-regulated circRNA “7_dna:chromosome_chromosome: Glycine_max_v2.1:7:1:44630646:1” with significant p-value (<0.09). Thus to understand the potential roles of the predicted differentially expressed circRNAs, their probable binding with the miRNAs and their regulatory functions were detected.

### Circ RNAs-miRNAs-mRNAs interactions for wilt stress tolerance

The miRNAs targeting the differentially expressed circRNAs are given in Supplementary Table1, which shows that there are in total 10 unique miRNAs targeting 18 differentially expressed circRNAs. It was found that majority of the circular RNAs have more than one miRNA binding sites. The miRNAs that are regulated by the identified miRNAs are presented in Supplementary Table2 which shows that there are 275 genes regulated by the miRNAs. It was also found that the interaction between miRNA and mRNA are of types: translation inhibition and cleavage inhibition. The targeted miRNAs were subjected to BLAST2GO and the gene ontology (GO) functional categorization generated 14 annotations which are presented in Fig 2, Fig 3, Fig 4 and Fig 5.

**Fig 2:** Differentially expressed circRNAs of soybean under wilt stress condition in GO terms of biological processes, cellular components and molecular functions

**Fig 3:** Classification of biological processes for mRNAs
Prediction of eTMs

The circRNAs identified in this study have been found to be targeted by miRNAs of families like MIR1533, MIR166a-5p, MIR4415, etc (Supplementary Table1). Among these families MIR4415 (Kulcheski et al. 2013) [11] and MIR166-5p (Ramesh et al. 2019) [15] had been reported earlier to be involved in fungal infection in soybean indicating their chance of involvement in Fusarium wilt infection. It has also been found that MIR1533 is one of the largest miRNA families that are involved in plant stress responses (Ren et al. 2014) [16]. It was reported that MIR4415 remains downregulated in fungus affected soybean crops (Ramesh et al. 2019) [15], i.e., the miRNAs regulated by these miRNAs remain free which increase the tolerance of the crop against the fungus. Thus, when these miRNAs get bound to the circRNAs, they become unavailable to regulate the mRNAs which leaves the mRNAs free. This may be a possible reason for inducing the disease tolerance to the crop. Moreover, when the functions of the mRNAs were observed, it was found that they are mainly involved in structural constituent of cell wall, in polygalacturonase activity, carbohydrate metabolic process, MAP kinase activity, carbonate dehydratase activity etc. Thus, when the eTM circRNAs are targeted by the miRNAs, probably the genes mentioned above may help the plant in tolerating the stress condition due to their non-regulation by miRNAs. Hence, due to such interaction, the differentially expressed circRNAs may be indirectly involved in regulating the genes responsible for wilt stress tolerance in soybean and thus can be considered as probable eTMs of mRNAs with regard to miRNAs.

Table 1: Supplementary

| miRNA_ID     | circRNA_ID | Target_start | Target_end | miRNA_aligned_fragment | circRNA_aligned_fragment |
|--------------|------------|--------------|------------|------------------------|--------------------------|
| gma-miR4415a-3p | 11_dna:chromosome_chromosome:Glycine_max_v2:1:1:34766867:1 | 11402       | 11422      | UUGAUUCUCAGCAGCAUUAG    | UGAUGUGUCUGAUGAAUUU      |
| gma-miR4415b-3p | 11_dna:chromosome_chromosome:Glycine_max_v2:1:1:34766867:1 | 11402       | 11422      | UUGAUUCUCACACAGCAUUG    | UGAUGUCUGAUGAAUUUAA      |
| gma-miR10196  | 8_dna:chromosome_chromosome:Glycine_max_v2:1:8:1:47837940:1 | 12408       | 12429      | UGAUGUGUGGGAGAGCAUUUCAU | UCAAAGUGCCUCUCUUUACAAU   |
| gma-miR10407a | 3_dna:chromosome_chromosome:Glycine_max_v2:1:3:1:45797981:1 | 16858       | 16881      | AGUUAACGGAGAUGAUGAUAG   | GAUAAUUUAAUUAUUAUCAGU    |
Table 2: Supplementary

| miRNA_ID | mRNA_ID   | PFE | miRNA_st | miRNA_end | Target_st | Target_end | miRNA_aligned_fragment | Target_aligned_fragment | Inhibition |
|----------|-----------|-----|----------|-----------|-----------|------------|------------------------|------------------------|------------|
| gma-miR10407a | Glyma.11G18070 | 0.1 | -1 | 1 | 24 | 2605 | 2628 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407a | Glyma.11G18070 | 0.2 | -1 | 1 | 24 | 2624 | 2647 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407a | Glyma.11G18070 | 0.1 | -1 | 1 | 24 | 2669 | 2692 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407a | Glyma.11G18070 | 0.3 | -1 | 1 | 24 | 3258 | 3281 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407b | Glyma.11G18070 | 0.1 | -1 | 1 | 24 | 2605 | 2628 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407b | Glyma.11G18070 | 0.2 | -1 | 1 | 24 | 2624 | 2647 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407b | Glyma.11G18070 | 0.1 | -1 | 1 | 24 | 2669 | 2692 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR10407b | Glyma.11G18070 | 0.4 | -1 | 1 | 24 | 3258 | 3281 | AGUUACCGGAGAUAAGAAAU | UACCUUUAUCUUUCU | Cleavage |
| gma-miR1513a | Glyma.14G01660 | 0.1 | -1 | 1 | 19 | 267 | 285 | AUAAUAUAAUAUAUUGA | UACAUAAUAUAUAUAUAA | Cleavage |
| gma-miR1513a | Glyma.14G01660 | 0.2 | -1 | 1 | 19 | 267 | 285 | AUAAUAUAAUAUAUUGA | UACAUAAUAUAUAUAUAA | Cleavage |
| gma-miR1513a | Glyma.14G01660 | 0.4 | -1 | 1 | 19 | 267 | 285 | AUAAUAUAAUAUAUUGA | UACAUAAUAUAUAUAUAA | Cleavage |
| gma-miR1513a | Glyma.14G01660 | 0.3 | -1 | 1 | 19 | 267 | 285 | AUAAUAUAAUAUAUUGA | UACAUAAUAUAUAUAUAA | Cleavage |
| gma-miR1513a | Glyma.07G18720 | 0.1 | -1 | 1 | 21 | 104 | 124 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G18720 | 0.2 | -1 | 1 | 21 | 89 | 109 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G18720 | 0.3 | -1 | 1 | 21 | 104 | 124 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G1830 | 0.1 | -1 | 1 | 21 | 89 | 109 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G1830 | 0.2 | -1 | 1 | 21 | 104 | 124 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G1830 | 0.3 | -1 | 1 | 21 | 89 | 109 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G1830 | 0.4 | -1 | 1 | 21 | 104 | 124 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| gma-miR1513a | Glyma.07G1830 | 0.5 | -1 | 1 | 21 | 89 | 109 | UAGGAGAAAACCAUGACU | AC | Cleavage |
| miR | Glyma.01G23270 | -1 | I | 21 | 107 | 127 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
|-----|----------------|----|---|----|-----|-----|-----------------------|--------------------------|-----------|
| miR | Glyma.16G20260 | -1 | I | 21 | 107 | 127 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20260 | -1 | I | 21 | 107 | 127 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.07G19700 | -1 | I | 21 | 113 | 133 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G28740 | -1 | I | 19 | 19 | 37 | AUUAUUAAUAUAUAUAAG | UUGGUAUUUUUUUUAAUC | Cleavage |
| miR | Glyma.08G28740 | -1 | I | 19 | 19 | 37 | AUUAUUAAUAUAUAUAAG | UUGGUAUUUUUUUUAAUC | Cleavage |
| miR | Glyma.13G08330 | -1 | I | 19 | 2756 | 2774 | AUUAUUAAUAUAUAUAAG | UCAACAUUUUUUUUUAAUC | Cleavage |
| miR | Glyma.13G08330 | -1 | I | 19 | 2756 | 2774 | AUUAUUAAUAUAUAUAAG | UCAACAUUUUUUUUUAAUC | Cleavage |
| miR | Glyma.13G16170 | -1 | I | 19 | 162 | 180 | AUUAUUAAUAUAUAUAAG | UAAUUUUUUUUUUUUAAUC | Cleavage |
| miR | Glyma.17G01830 | -1 | I | 21 | 125 | 145 | UGAGAAGGCUAGCUUCCUCA | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.06G19710 | -1 | I | 21 | 92 | 112 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.06G20280 | -1 | I | 21 | 65 | 85 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20300 | -1 | I | 21 | 98 | 118 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20290 | -1 | I | 21 | 107 | 127 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.06G19720 | -1 | I | 21 | 86 | 106 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25180 | -1 | I | 21 | 89 | 109 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.17G01760 | -1 | I | 21 | 254 | 274 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25230 | -1 | I | 21 | 179 | 199 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25230 | -1 | I | 21 | 179 | 199 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25230 | -1 | I | 21 | 183 | 203 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25220 | -1 | I | 21 | 161 | 181 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25160 | -1 | I | 21 | 121 | 141 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.07G25560 | -1 | I | 21 | 231 | 251 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25230 | -1 | I | 21 | 183 | 203 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25230 | -1 | I | 21 | 179 | 199 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.17G01760 | -1 | I | 21 | 254 | 274 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.08G25180 | -1 | I | 21 | 89 | 109 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.06G19720 | -1 | I | 21 | 86 | 106 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20290 | -1 | I | 21 | 107 | 127 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20300 | -1 | I | 21 | 98 | 118 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.16G20290 | -1 | I | 21 | 65 | 85 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.07G25560 | -1 | I | 21 | 121 | 141 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.07G25560 | -1 | I | 21 | 161 | 181 | GGAACAGAGGCUAGACUUAC | GUAAGCUACUGGUUUCUCCUCAC | Cleavage |
| miR | Glyma.09G16800 | -1 | I | 19 | 84 | 102 | AUUAUUAAUAUAUAUAAG | UAAUUUUUUUUUUUUAAUC | Cleavage |
| miR | Glyma.09G16800 | -1 | I | 19 | 84 | 102 | AUUAUUAAUAUAUAUAAG | UAAUUUUUUUUUUUUAAUC | Cleavage |
|                   | miR4415b | miR6299 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 | miR1533 |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Glyma.04G23170    | 0.3      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.06G09320    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.09G67430    | 0.2      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.11G21270    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.10G15370    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.01G07110    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.17G13250    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.19G10640    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.15G19200    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.13G12480    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.13G12480    | 0.6      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.02G28000    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.07G15900    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.08G25680    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.20G05160    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.20G05190    | 0.2      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.08G32610    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.20G05190    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.08G32610    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.14G04130    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.13G07690    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.06G08560    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Glyma.06G20190    | 0.1      |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| 0.1 | Glyma.11G10980 0.4 | -1 | 1 | 19 | 262 | 280 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G10980 0.3 | -1 | 1 | 19 | 262 | 280 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.08G26960 0.1 | -1 | 1 | 19 | 368 | 386 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G2810 0.1 | -1 | 1 | 19 | 182 | 200 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G10110 0.1 | -1 | 1 | 19 | 4585 | 4603 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G10110 0.1 | -1 | 1 | 19 | 4606 | 4624 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G10110 0.1 | -1 | 1 | 19 | 4627 | 4645 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G10110 0.1 | -1 | 1 | 19 | 4648 | 4666 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.09G2810 0.2 | -1 | 1 | 19 | 182 | 200 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.17G19730 0.1 | -1 | 1 | 19 | 3839 | 3857 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.17G19730 0.1 | -1 | 1 | 19 | 3860 | 3878 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.17G19730 0.1 | -1 | 1 | 19 | 1892 | 1910 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.17G19730 0.2 | -1 | 1 | 19 | 3714 | 3732 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.17G19730 0.2 | -1 | 1 | 19 | 3735 | 3753 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.07G23350 0.1 | -1 | 1 | 19 | 3542 | 3560 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G07350 0.6 | -1 | 1 | 19 | 37 | 55 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G07350 0.6 | -1 | 1 | 19 | 58 | 76 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G2870 0.2 | -1 | 1 | 19 | 3979 | 3997 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.8 | -1 | 1 | 19 | 1709 | 1727 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.06G26700 0.1 | -1 | 1 | 19 | 234 | 252 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.5 | -1 | 1 | 19 | 1709 | 1727 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.11 | -1 | 1 | 19 | 1709 | 1727 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.12G13600 0.1 | -1 | 1 | 19 | 236 | 254 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.9 | -1 | 1 | 19 | 1709 | 1727 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.6 | -1 | 1 | 19 | 1709 | 1727 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G2870 0.1 | -1 | 1 | 19 | 3806 | 3824 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.11G25130 0.16 | -1 | 1 | 19 | 1892 | 1910 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G11460 0.3 | -1 | 1 | 19 | 3524 | 3542 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G11460 0.2 | -1 | 1 | 19 | 3521 | 3539 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G11460 0.1 | -1 | 1 | 19 | 3533 | 3551 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.13G11460 0.4 | -1 | 1 | 19 | 3530 | 3548 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.16G21780 0.1 | -1 | 1 | 19 | 3652 | 3670 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.06G05640 0.1 | -1 | 1 | 19 | 329 | 347 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.06G05640 0.3 | -1 | 1 | 19 | 329 | 347 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.04G12550 0.1 | -1 | 1 | 19 | 2785 | 2803 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.04G12550 0.1 | -1 | 1 | 19 | 2806 | 2824 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.04G12550 0.1 | -1 | 1 | 19 | 2827 | 2845 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.18G22580 0.1 | -1 | 1 | 19 | 2881 | 2899 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
| 0.1 | Glyma.20G01430 0.1 | -1 | 1 | 19 | 2935 | 2953 | AUAAUAAAAAAUAUAUGA | AUAAUAAAAAAUAUAUUAAUUAAU | Cleavage |
miR1533 Glyma.20G01430 0.1 -1 1 19 2956 2974 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.3 -1 1 19 214 232 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.3 -1 1 19 235 253 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.2 -1 1 19 214 232 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.2 -1 1 19 235 253 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.20G01430 0.2 -1 1 19 2950 2988 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.5 -1 1 19 214 232 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.5 -1 1 19 235 253 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.4 -1 1 19 235 253 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.1 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.06G05640 0.2 -1 1 19 329 347 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.12G01940 0.1 -1 1 19 2793 2811 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.10G24390 0.2 -1 1 19 2519 2537 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.19G22580 0.2 -1 1 19 2821 2839 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.1 -1 1 19 146 164 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

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miR1533 Glyma.13G03250 0.8 -1 1 19 146 164 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

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miR1533 Glyma.08G18340 0.8 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.6 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.16G11600 0.1 -1 1 19 261 279 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.16G11600 0.1 -1 1 19 282 300 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.2 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

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miR1533 Glyma.08G18340 0.10 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.16G22580 0.3 -1 1 19 2751 2769 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.7 -1 1 19 169 187 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.7 -1 1 19 190 208 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.6 -1 1 19 169 187 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.13G03250 0.6 -1 1 19 190 208 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.9 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.5 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.7 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.4 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.08G18340 0.3 -1 1 19 178 196 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.09G19510 0.2 -1 1 19 193 211 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.06G23040 0.1 -1 1 19 2998 3016 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

miR1533 Glyma.09G08520 -1 1 19 70 88 AUAAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU

AUAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU AUAAUAAAAUAUAUGA UUAUUUAUUUAUUUAUUU
| miR1533 | Glyma.08G16730 | 0.1 | -1 | 1 | 19 | 2114 | 2132 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G19510 | 0.1 | -1 | 1 | 19 | 193 | 211 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.07G13050 | 0.1 | -1 | 1 | 19 | 206 | 224 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G17310 | 0.1 | -1 | 1 | 19 | 194 | 212 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.13G27450 | 0.1 | -1 | 1 | 19 | 2797 | 2815 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.20G04780 | 0.5 | -1 | 1 | 19 | 112 | 130 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.15G26960 | 0.2 | -1 | 1 | 19 | 2618 | 2636 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.15G14520 | 0.1 | -1 | 1 | 19 | 130 | 148 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.06G29990 | 0.1 | -1 | 1 | 19 | 217 | 235 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G17310 | 0.2 | -1 | 1 | 19 | 194 | 212 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G13470 | 0.1 | -1 | 1 | 19 | 2523 | 2541 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G13470 | 0.1 | -1 | 1 | 19 | 2555 | 2573 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.15G26960 | 0.1 | -1 | 1 | 19 | 2517 | 2535 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G92720 | 0.1 | -1 | 1 | 19 | 52 | 70 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G92720 | 0.1 | -1 | 1 | 19 | 73 | 91 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.09G92720 | 0.1 | -1 | 1 | 19 | 94 | 112 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.10G15880 | 0.1 | -1 | 1 | 19 | 554 | 572 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.02G03940 | 0.1 | -1 | 1 | 19 | 118 | 136 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.15G21360 | 0.2 | -1 | 1 | 19 | 2696 | 2714 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.02G23700 | 0.1 | -1 | 1 | 19 | 30 | 48 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.08G94400 | 0.2 | -1 | 1 | 19 | 1730 | 1748 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.06G31600 | 0.1 | -1 | 1 | 19 | 503 | 521 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.04G11740 | 0.1 | -1 | 1 | 19 | 2611 | 2629 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.13G27450 | 0.2 | -1 | 1 | 19 | 2441 | 2459 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.13G27450 | 0.2 | -1 | 1 | 19 | 2462 | 2480 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.16G01710 | 0.1 | -1 | 1 | 19 | 2601 | 2619 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.02G29700 | 0.1 | -1 | 1 | 19 | 194 | 212 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.04G11740 | 0.4 | -1 | 1 | 19 | 2578 | 2596 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.04G11740 | 0.3 | -1 | 1 | 19 | 2575 | 2593 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR1533 | Glyma.16G00570 | 0.3 | -1 | 1 | 19 | 2375 | 2393 | AUAAUAAAAAUAAUGA | UUAUUAAUUUAAUAUAUAU |
| miR    | Glyma.16G00570 0.2 | Glyma.07G10770 0.1 | Glyma.04G11740 0.5 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.04G11740 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.04G11740 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.16G00570 0.2 | Glyma.04G11740 0.2 |
|--------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| gma-miR1533 | -1 I 19 2396 2414 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2531 2549 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2543 2561 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2363 2381 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2384 2402 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2542 2560 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2360 2378 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2381 2399 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2359 2377 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2380 2398 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 130 148 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 151 169 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2348 2366 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2369 2387 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 192 210 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2394 2412 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 172 190 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2084 2102 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2114 2132 AUAUUUAAUAUAUUGA AUCGUAAUAUAUUGA | -1 I 19 1160 1178 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2272 2290 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2293 2311 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2260 2278 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2281 2299 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 130 148 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 151 169 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 192 210 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 257 275 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 104 122 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2210 2228 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2231 2249 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2391 2409 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 162 180 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 183 201 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 204 222 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2377 2395 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 25 43 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 2367 2385 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | -1 I 19 1160 1178 AUAUUUAAUAUAUUGA AUAUUUAAUAUAUUGA | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage | Cleavage |
| miR   | Gene ID          | Exp. Ratio | Length | Seed Sequence |
|-------|------------------|------------|--------|---------------|
| gma-miR1533 | Glyma.16G01710   | -1         | 19     | AUAAUAAAAAAUAAUGA |
|       | Glyma.14G05920   | -1         | 19     | 2117          |
|       | Glyma.18G06520   | -1         | 19     | 2035          |
|       | Glyma.17G05130   | -1         | 19     | 2182          |
|       | Glyma.08G21870   | -1         | 19     | 466           |
|       | Glyma.20G02350   | -1         | 19     | 2222          |
|       | Glyma.01G11190   | -1         | 19     | 2232          |
|       | Glyma.14G05920   | -1         | 19     | 2034          |
|       | Glyma.16G01710   | -1         | 19     | 1601          |
|       | Glyma.16G13500   | -1         | 19     | 25            |
|       | Glyma.13G18450   | -1         | 19     | 255           |
|       | Glyma.01G08300   | -1         | 19     | 2131          |
|       | Glyma.10G20660   | -1         | 19     | 249           |
|       | Glyma.16G01710   | -1         | 19     | 249           |
|       | Glyma.16G01710   | -1         | 19     | 2140          |
|       | Glyma.16G01710   | -1         | 19     | 1854          |
|       | Glyma.13G18450   | -1         | 19     | 255           |
|       | Glyma.16G01710   | -1         | 19     | 55            |
|       | Glyma.12G21200   | -1         | 19     | 13            |
|       | Glyma.03G09010   | -1         | 19     | 68            |
|       | Glyma.09G19790   | -1         | 19     | 239           |
|       | Glyma.16G01710   | -1         | 19     | 2160          |
|       | Glyma.03G25600   | -1         | 19     | 2033          |
|       | Glyma.05G16820   | -1         | 19     | 168           |
|       | Glyma.14G05990   | -1         | 19     | 1758          |
|       | Glyma.16G01710   | -1         | 19     | 10            |
|       | Glyma.09G09830   | -1         | 19     | 1990          |
|       | Glyma.08G03530   | -1         | 19     | 90            |
|       | Glyma.02U18000   | -1         | 19     | 1714          |
**Conclusion**

The analysis carried out in the present paper on RNA Seq data of soybean under control and wilt stress conditions resulted in the prediction of circRNAs and their wilt stress specific expression patterns. Functional enrichment analysis of the circRNA-host genes also revealed the indirect involvement of the circRNAs in regulating stress condition by acting as sponges for miRNAs, which may fail to regulate the genes associated with wilt stress tolerance mechanism in soybean.

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**References**

1. Andrews S. Fast QC: A Quality Control Tool for High Throughput Sequence Data. 2010.

http://www.bioinformatics.babraham.ac.uk/projects/fastqc
2. Bolger AM, Lohse M, Usadel B. Trimmmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15):2114-2120.
3. Chu Q, Shen E, Ye CY, Fan L, Zhuo QH. Emerging roles of plant circular RNAs. J. Plant Cell Develop. 2018; 1(1):1-14.
4. Dai X, Zhuang Z, Zhao PX. psRNA Target: a plant small RNA target analysis server (2017 release). Nucleic Acids Res. 2018; 46(1):49-54.
5. Dornbos DL, Mullen RE. Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. Journal of the American Oil Chemists Society. 1992; 69(3):228-231.
6. Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: identification, biogenesis and function. Biochim. Biophys. Acta Gene Regul. Mech. 2016; 1859(1):163-168.
7. Gao Y, Wang J, Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. Genome Biol. 2015; 16(1):4-20.
8. Kohli D, Joshi G, Deokar AA, Bhardwaj AR, Agarwal M, Katiyar-Agarwal S et al. Identification and characterization of wilt and salt stress-responsive microRNAs in chickpea through high-throughput sequencing. PloS one. 2014; 9(10):e108851.
9. Kulcheski F, Manavella P, Weigel D, Margis R. The role of MIR4415 in soybean response to asian soybean rust infection. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology. 2013; 94(2).
10. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011; 12(1):323.
11. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. 2013; arXiv preprint arXiv: 13033997.
12. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. Mol. Cell. 2018; 71(3):428-442.
13. Love M, Anders S, Huber W. Differential analysis of count data—the DESeq2 package. Genome Biol. 2014; 15(550): 10-1186.
14. Panda AC, Gorospe M. Identifying intronic circRNAs: progress and challenges. Noncoding RNA Investig. 2018; 2:34-36.
15. Ramesh SV, Govindasamy V, Rajesh MK, Sabana AA, Praveen S. Stress-responsive miRNAome of Glycine max (L.) Merrill: molecular insights and way forward. Planta. 2019; 249(5):1267-1284.
16. Ren J, Zhou JJ, Duan WK, Song XM, Liu TK, Hou XL, et al. Copper stress induces the differential expression of microRNAs in non-heading Chinese cabbage. Biologia plantarum. 2014; 58(3):491-498.
17. Singh U, Singh B. Tropical grain legumes as important human foods. Econ. Bot. 1992; 46(3): 310-321.
18. Wang Y, Yang M, Wei S, Qin F, Zhao H, Suo B. Identification of circular RNAs and their targets in leaves of Triticum aestivum L. under dehydration stress. Front. Plant Sci. 2017; 7:2024-2034.
19. Ye CY, Chen L, Liu C, Zhu QH, Fan L. Widespread noncoding circular RNAs in plants. New Phytol. 2015; 208(1):88-95.
20. Zhao W, Zhang C, Shen X, Xiao L, Lu J, Zhang Y et al. Characterization of circRNAs associated with resistance to defoliating insects in soybean. Oil Crop Sci. 2017; 2:23-37.
21. Zhu QH, Stephen S, Taylor J, Helliwell CA, Wang MB. Long noncoding RNA s responsive to Fusarium oxysporum infection in Arabidopsis thaliana. New Phytologist. 2014; 201(2):574-584.
22. Zuo J, Wang Q, Zhu B, Luo Y, Gao L. Deciphering the roles of circRNAs on chilling injury in tomato. Biochem. Biophy. Res. Commun. 2016; 479(2):132-138.