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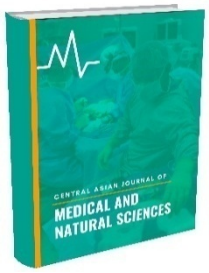

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Potential Genetic Target Changes in Height and Branches -A Short Review on Osmir535

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ABSTRACT: OsmiR535 is a member of the miR156/529/535 superfamily, which is one of the most conserved miRNA families in plants. OsmiR535 regulates the cold-stress response, modulates plant growth, and controls panicle design and grain length. However, the significance of OsmiR535 in plant drought and salinity responses is unknown. The miR535s identified in terrestrial plants were less conserved than miR156s during evolution, according to this study. Similar to OsmiR529 in rice, miR535 is expressed a very low level during vegetative growth but is significantly accumulated in early panicles. Overexpression of OsmiR535 in rice decreased plant height by reducing the length of the 1st and 2nd internodes. There was also a significant impact on panicle architecture due to the overexpression of OsmiR535. In addition, OsmiR535 overexpression inhibited the expression of OsSPL7/12/16, as well as OsDEP1, OsLOG, and OsSLR1. OsmiR535 regulates plant height, panicle architecture and grain form in rice in several ways, according to our findings

Keyword : OsmiR535; Oryza sativa; miRNA; panicle; grain form.

I. Introduction

This complex may identify and block the expression of target genes at the post-transcriptional level using miRNAs, which are a family of non-coding RNAs with a length of 18–24 nucleotides. MiRNAs appear to impact a variety of biological activities, including plant growth and development, stress response, and metabolic functions, according to accumulating scientific data. SQUAMOSA promoter-binding protein-like (SPLs) expression is modulated by the miR156/miR529/miR535 families, which has a high degree of sequence similarity. Plant growth and development are modulated by miR156 and its target SPL (SQUAMOSA promoter binding like) genes, which have been thoroughly characterized over the past decade. When it comes to leaf development, tiller growth, panicle branching, and grain productivity, rice's OsmiR156 and OsSPL14 play a critical role. Beyond OsSPL13 and OsSPL16, other important genes for grain output are OsSPL7 and OsSPL17.

Despite the fact that miR529 and miR156 have a 14-nt homology, miR156 is more conserved in plant lineages than miR529. Eudicots have a selective loss of MiR529. As with OsmiR156, OsmiR529

targets rice SPL genes OsSPL7, OsSPL14 and OsSPL17. OsmiR529a, and not OsmiR529b, regulates panicle morphology and grain yields, according to reports. These data show that although while miR529 is less genetically conserved than miR156, it nevertheless plays a vital function in plant growth despite its lower evolutionary conservation. According to our findings, OsmiR535 has various functions in rice, including controlling plant height, panicle architecture and grain form. There was also a change in expression of OsSPLs and panicle-related genes in the transgenic lines expressing osmiR535. OsmiR535 plays a critical role in rice development, and our findings will help guide future research into the coordinated regulation of the miR156/mir529/miR535 complex.

II. RESEARCH METHODOLOGY

A. Sequence analysis and target prediction of OsmiR535

Under different stress conditions, we investigated the expression of OsmiR535 and its putative target genes SPL2/7/11/14/16/18/19. Real-time qPCR assays indicated that dehydration, 200 mM NaCl, 10 M ABA, and 20 percent PEG may increase OsmiR535 expression, as shown in Figure 1. An higher OsmiR535 expression level, on the other hand, should suppress the expression of OsSPL2/7/11/14/16/18/19, whose coding sequence contains a targeting site for OsmiR535. However, dryness, 200 mM NaCl, and 20 percent PEG treatments increased the expression of OsSPL2 and OsSPL18 by 10 times. Under diverse drought-related conditions, OsSPL7/11/14/16 have not exhibited a downregulated pattern with higher OsmiR535 expression levels. When drought and salinity were present, only OsSPL19 was consistently downregulated among the seven genes targeted by OsmiR535 (Figure 1). As a result of these findings, OsmiR535 appears to have a role in rice's drought and salinity stress response.

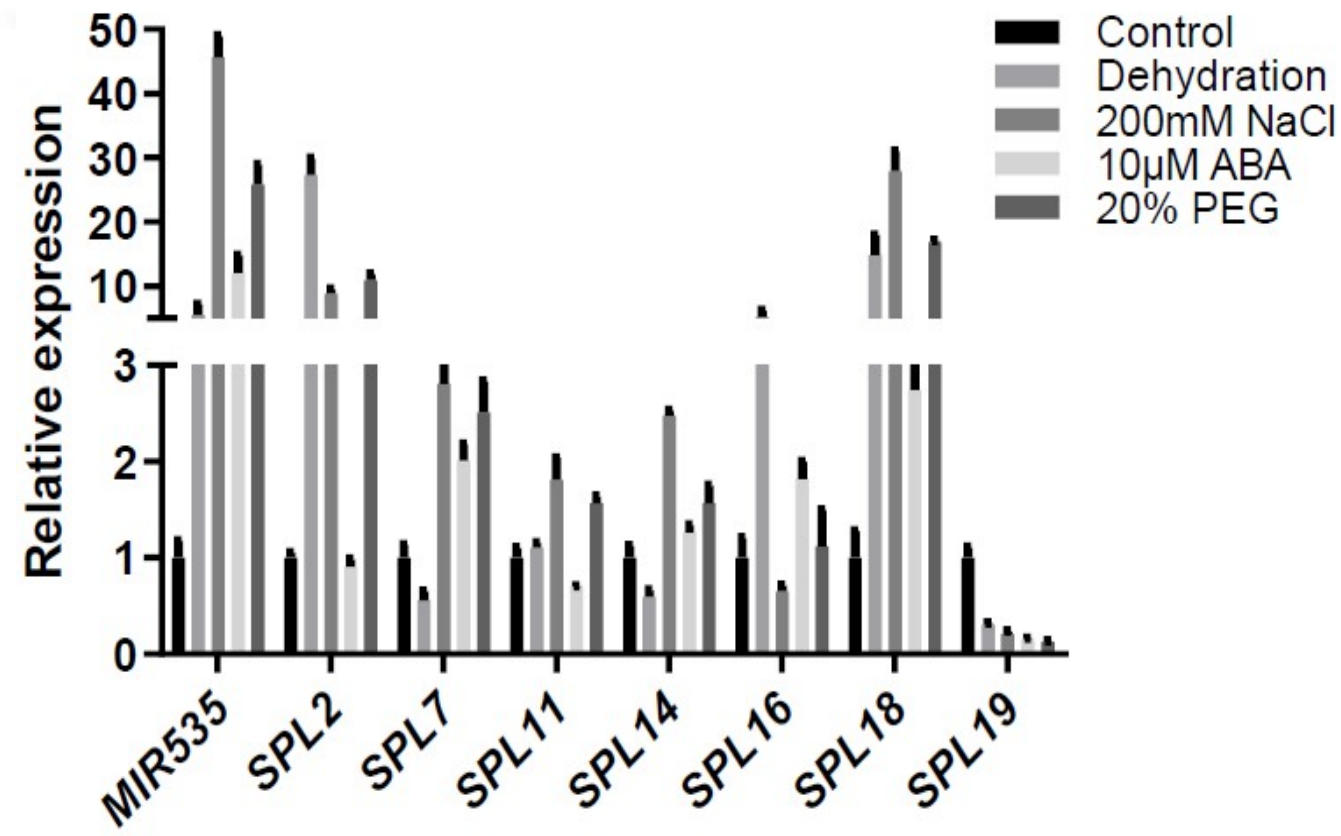

Figure №1: Under hormone and stress treatments, including dehydration, 200 mM NaCl, 10 μM ABA, and 20 percent PEG, OsmiR535 and OsSPL2/7/11/14/16/18/19 were expressed in seedlings.

B. Plant materials and growth conditions

The wild type (WT) utilised in this study is the rice cultivar Kongyu 131 (Oryza sativa ssp. japonica). In Heilongjiang province in northeast China, it is a cultivar of early maturity. Meanwhile, this cultivar possesses excellent tillering and lodging resistance, along with a high level of cold toleration. After being sterilised for 30 minutes with NaClO (10%), rice seeds were washed 3–5 times. They were then placed in a growth room at 28 °C and allowed to germinate. Greenhouse seedlings were transferred into soil or Yoshida solution with 16-hour light/night cycles. Plumules, leaves, and shoot apexes from vegetative seedlings as well as leaves, shoot apexes, and early panicles from reproductive plants were collected for tissue expression investigation of OsmiR535 accordingly. WT and transgenic plants were also examined for mRNA and miRNA expression alterations. The leaves, shoot apexes, and immature panicles were also sampled.

Transgenic line generation and molecular identification

It was PCR amplified using gene-specific primers (Table S1), and introduced into the pCAMBIA330035Su vector to overexpress pre-OsmiR535. Using the recombinant Agrobacterium cultures, the callus tissues of the rice cultivar Kongyu 131 were infected with the recombinant construct. Glufosinate-containing media was used to select the rice calli, and the white, actively developing calli were then transferred to regeneration medium, where they grew into plants. PCR analysis using Bar-specific primers was used to identify the regenerated plants. In order to produce T2 plants, the T1 seeds were collected from the individual PCR-positive plants and replanted. As a result of the lack of glufosinate resistant segregation, the T2 lines were deemed homozygotes. The expression levels of Pre-OsmiR535, OsmiR156, and OsmiR529 in homozygous lines were determined using semiquantitative RT-PCR and quantitative real-time PCR (qRT-PCR). In the following investigation, homozygous OsmiR535-OX lines of T3 generation were utilised. The RT-PCR and quantitative real-time PCR tests are semiquantitative and quantitative, respectively.

As part of this study, total RNA was extracted using the Trizol technique and cDNA was generated using the SuperScriptTM III Reverse Transcriptase kit (Invitrogen). On OsmiR156 and OsmiR529, we utilised oligodT primers (Table S1), whereas mRNAs and pre-OsmiR535 were reverse-transcribed with stem-loop specific primers. stemloop cannot successfully differentiate OsmiR535 from OsmiR156b-j because to their great sequence similarity (the only difference is that OsmiR535 has two extra nucleotides GC at the 3' terminus) (Fig. 1). Since only pre-OsmiR535 is expressed in this study, the results are limited.

The C1000 TouchTM fast PCR apparatus was used to perform the semi-quantitative RT-PCR utilising Transgen Biotech's EasyTaq PCR SuperMix (BioRad). TransStart Top Green qPCR SuperMix (Transgen Biotech) was used on the CFX96 TouchTM Real-Time PCR Detection System to conduct the qRT-PCR (BioRad). The relative expression levels of mRNAs and miRNAs were adjusted against OsElf1- and U6, respectively. It was carried out at least twice biologically and twice technically. Table S1 lists the primers that were utilized in this investigation.

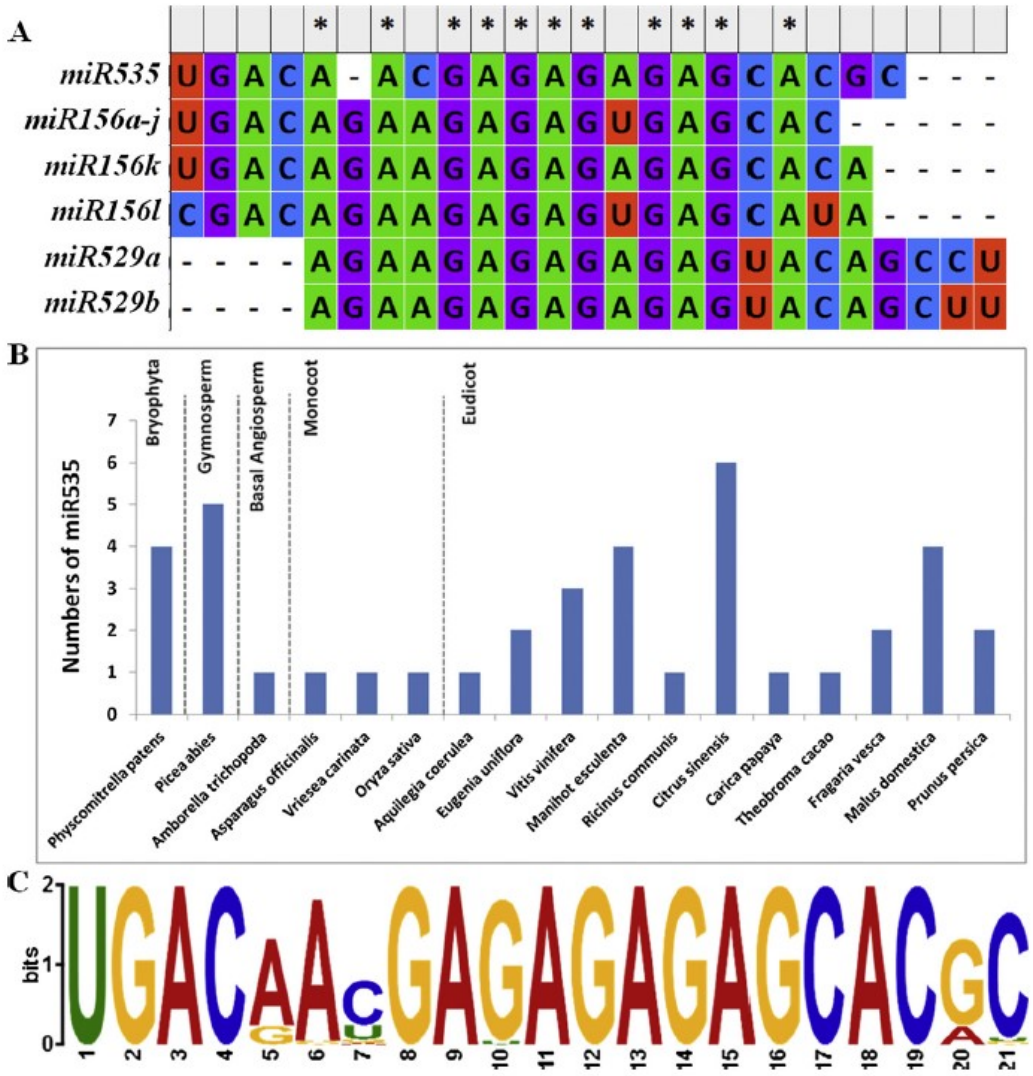

Figure 2: Analysis of miR535s in different plant species based on sequence analysis. (A)Rice miR156/miR529/535 superfamily sequence alignment. (B) Varying plant species have different numbers of miR535 gene copies. (C) sequence logo of the plant miR535s.

III. RESULTS AND DISCUSSION

In terrestrial plants, miR156, miR529, and miR535 are clustered together due to their significant sequence similarity. Only one miR535 is found in rice, but there are 12 miR156s and 2 miR529 (Fig. 2A). "G" deletion (6th), "A-to-C" substitution (8th), "A-to-G" substitution (21st) and an extra "G" at the 3' end were the only differences between OsmiR535 and OsmiR156k. In previous evolutionary investigations, miR156 has been found to be extremely conserved in plant lineages, while miR529 has been found to be less conserved through time. MiR535 has received far less investigation than miR156 and miR529. The evolution of miR535s in terrestrial plants was studied in this work.

Forty miR535 genes were found in 17 plant species including bryophytes, gymnosperms and angiosperms using the miRBase database (Fig. 2B), indicating the early appearance of miR535s in land plants. Physcomitrella patens, a bryophyte, has four copies of miR535; Picea abies, five; and basal angiosperms, one (Amborella trichopoda). Angiosperm monocots have just one copy, while dicots have anything from one to six copies (Citrus sinensis). Arabidopsis, soybean, and maize are examples of monocot and dicot species that lack miR535 expression. Compared to miR156, miR535 is less evolutionarily conserved in plants, according to these data.

MiR535s were also examined for sequence variation in various species. From bryophytes to angiosperms, miR535 sequences shared 15 identical nucleotides and diverged at the 5th, 6th, seventh, 10th and 21st nucleotide positions (Fig. 2C). Among these, the 7th position showed the greatest sequence divergence.

Transgenic rice with OsmiR535 overexpression

The agronomy characteristics of the WT and OsmiR535-OX lines were compared in order to determine the role of OsmiR535 in rice growth and development. It is evident from Figure 3A that the aboveground portions of the OsmiR535-OX lines are shorter than WT. This is further confirmed by the fact that OsmiR535-OX lines have a lower plant height than WT (Fig. 3B). WT and OsmiR535-OX plants were measured at the 1st, 2nd, and 3rd internodes, respectively. According to Figure 3C, the internodes of the OsmiR535-OX lines were considerably shorter than those of WT, which resulted in the OsmiR535-OX lines being shorter than WT.

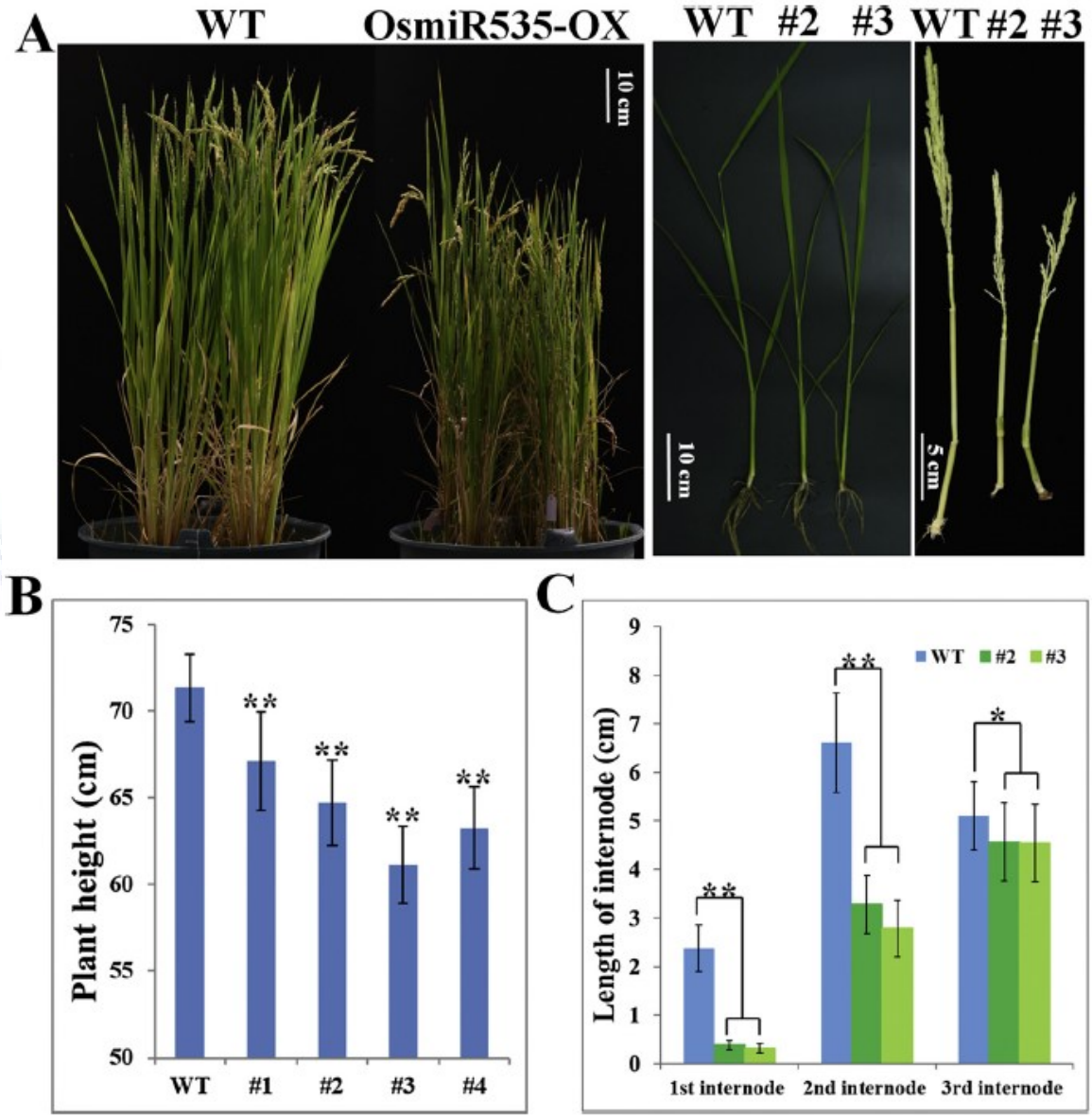

Figure 3: Height-related effects of OsmiR535 overexpression (A) Representative pictures of the WT and OsmiR535-OX lines demonstrating their plant heights. (B)WT and OsmiR535-OX lines have different plant heights. (C)This is the length of the OsmiR535-OX and WT interconnects.

IV. Conclusion

As a result of their relatively high degree of sequence similarity, the plants miR156, miR529, and miR535 families are referred to as the superfamily, which is composed of these three gene families. Toutes of them are directed at the SPL family genes. Plant miRNA function will be better understood via evolutionary studies of these three miRNA families. It appears that these three miRNA families are present in bryophytes, and that they share an embryophyte progenitor. Among terrestrial plants, the miR156 family is highly evolutionarily conserved, but the miR529 and miR535 families have restricted taxonomic distributions During plant evolution, miR156's copy number rose, whereas miR529's copy number dropped. The copy number of miR535 varies greatly throughout plant lineages. These data imply that these three miRNA families have diverse evolutionary processes. In contrast, miR529 and miR535 evolved more fast.

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