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Identification of Novel Members of miR166 family in Barley

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Abstract: MicroRNAs (miRNAs) are important classes of small RNAs with nearly 22 nt in length that modulate gene expression at the transcriptional and posttranscriptional levels [1]. MiR166 plays crucial roles during seed development and flowering [2]. In rice and Arabidopsis with well-characterized whole genome, 13 and 7 members of miR166 have been reported, respectively, while only 3 members of this miRNA have been identified in barley. This research was conducted to identify putative novel members of miR166 in barley using *insilico* approaches. The precursor sequences of all mi166 memebers in rice and Arabidopsis were retrieved from miRBase (http://www.mirbase.org/index.shtml), and then used to find homologs against barley genome. The protein-encoding and other types of noncoding RNAs sequences were eliminated using BLAST against NCBI. The remaining sequences were assessed for secondary structure using RNAfold (http://rna.tbi.univie.ac.at/cgibin/RNAWebSuite/RNAfold.cgi) database. The candidates were then further evaluated based on the plant miRNAs criteria [3] including high identity of mature miRNA to known miR166s in other plants, the position of mature miRNA on the hairpin, and proper secondary structure with low negative minimal folding energy (MFE). As a result, seven putitive novel members of miR166 were identified in barley. Moreover, precursor sequences of all barley miR166s were subjected to a phylogenetic analysis. Consequently, the sequences of novel and known memebrs of miR166 in barley were aligned by using ClustalW and phylogenetic trees were constructed by using maximum likelihood method in MEGA 7, and the bootstrap value was calculated with 1000 replicates [3]. The five newly identified members were grouped together with known hvu-miR166b, while hvu-miR166g and hvu-miR166i were grouped with hvu-miR166a and hvu-miR166c, respectively. This study provides a promisisng insilico approach to identify putative novel members of miRNAs that enhance valuable information for further analysis on miRNAs in various plants.

Keywords: Barley genome; Evolutionary; Gene Regulation; miR166 fanmily

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