ABSTRACT

Plant respond to abiotic stresses by precisely regulating expression of stress responsive genes through several mechanisms such as transcriptional, post-transcriptional, and posttranslational regulations at different levels. MicroRNAs (miRNAs) are single-stranded non-coding RNAs that play critical roles in regulating gene expression at the post-transcriptional level by repressing translation or by enhancing degradation of specific target mRNAs. A large number of miRNA sequences are evolutionarily conserved across species boundaries and have near perfect complementarities with their specific targets which are messenger RNAs (mRNA). Regulation of gene expression through sequence specific interaction between miRNAs and their target mRNAs offers an accurate and inheritable mechanism for plant’s response to environmental stimuli.

_Hevea brasiliensis_ which is the major commercial source of natural rubber performs well in Kerala and Kanyakumari District of Tamil Nadu which experience favourable weather parameters like optimum sun shine hours, rain fall, humidity, etc. Due to the increasing demand for natural rubber coupled with non-availability of land in traditional rubber growing regions, cultivation of natural rubber is being extended to non-traditional regions which experience adverse climatic conditions which limit the growth and productivity of rubber. So it is highly imperative to identify or develop clones that can withstand such extreme weather factors. As miRNAs are known to be involved in regulating the abiotic stress responsive gene expression, their level of expression may vary in stress tolerant/susceptible clones of _Hevea_. If the miRNAs that are involved in regulating the stress tolerant genes can be identified, it would enable the plant breeders to identify or develop clones with improved stress tolerance. Hence the present work on identification and expression analysis of abiotic stress responsive miRNAs of _H. brasiliensis_ was
conducted to identify miRNAs associated with drought/ cold tolerance in *Hevea brasiliensis*.

In this study, attempts were made to identify drought and cold responsive miRNAs from *H. brasiliensis* through both conventional as well as by next generation sequencing method. Both drought and cold responsive miRNAs were identified from which differentially expressed miRNAs were selected for further validation. Expression of miRNAs was analyzed in various clones of *H. brasiliensis* with contrasting levels of drought and cold tolerance which led to the identification of miRNAs that are strongly associated with drought/cold tolerance. Further, their expression was validated in various germplasm accessions with different levels of tolerance in order to confirm their association with tolerance. In addition to this, targets of both known and novel miRNAs were predicted followed by expression analysis of selected miRNAs and their putative targets in order to evaluate their relationship.

From this study, miRNAs such HbmiRn_63, HbmiRn_42, miR168 and miR160 were found strongly associated with drought tolerance in *H. brasiliensis*. This study also revealed miR169, miR482 and miR159 to have strong association with cold tolerance. This study indicates the possibility of using these miRNAs as markers for drought/cold tolerance in *H. brasiliensis*. These miRNAs can be further utilized in the crop improvement programmes by the breeders to identify or develop drought/cold tolerant genotypes of *H. brasiliensis*.

Key words: *Hevea brasiliensis*, miRNAs, drought tolerance, cold tolerance, expression analysis, qPCR