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Genome-wide identification, evolutionary relationship and expression analysis of *AGO*, *DCL* and *RDR* family genes in tea

Debasish B. Krishnatreya[1], Pooja Moni Baruah[1], Bhaskar Dowarah[1], Soni Chowrasia[2], Tapan Kumar Mondal[2] & Niraj Agarwala[1]✉

Three gene families in plants viz. Argonaute (*AGOs*), Dicer-like (*DCLs*) and RNA dependent RNA polymerase (*RDRs*) constitute the core components of small RNA mediated gene silencing machinery. The present study endeavours to identify members of these gene families in tea and to investigate their expression patterns in different tissues and various stress regimes. Using genome-wide analysis, we have identified 18 *AGOs*, 5 *DCLs* and 9 *RDRs* in tea, and analyzed their phylogenetic relationship with orthologs of *Arabidopsis thaliana*. Gene expression analysis revealed constitutive expression of *CsAGO1* in all the studied tissues and stress conditions, whereas *CsAGO10c* showed most variable expression among all the genes. *CsAGO10c* gene was found to be upregulated in tissues undergoing high meristematic activity such as buds and roots, as well as in *Exobasidium vexans* infected samples. *CsRDR2* and two paralogs of *CsAGO4*, which are known to participate in biogenesis of hc-siRNAs, showed similarities in their expression levels in most of the tea plant tissues. This report provides first ever insight into the important gene families involved in biogenesis of small RNAs in tea. The comprehensive knowledge of these small RNA biogenesis purveyors can be utilized for tea crop improvement aimed at stress tolerance and quality enhancement.

Gene regulation in eukaryotes depends on post-transcriptional RNA interference mechanisms which is mediated by the action of the small RNAs (sRNAs). Gene silencing molecules like miRNAs and siRNAs are not only responsible for endogenous regulation of gene expression but are also involved in cross-kingdom mutualistic relations and interaction networks[1]. The use of RNAi technology by involving artificial miRNAs has also been an effective control measure against various biotic threats to plants[1,2]. Since RNA silencing mechanism is important for various regulatory aspects of plants, so a comprehensive understanding of the components of this machinery is needed. The RNA dependent RNA polymerases (RDRs) and Dicer-like proteins (DCLs) are directly involved in small RNA biogenesis, whereas Argonaute (AGO) constitutes a significant component of the RNA induced silencing complex (RISC)[3]. RDRs are responsible for the synthesis of dsRNAs using an RNA template, whereas DCLs are responsible for cleavage of the dsRNAs to form 21–24 nucleotide long functional small RNAs. These sRNAs, either miRNAs or any class of siRNAs, get incorporated into the RISC to drive the gene silencing machinery[4]. The sRNAs bind to specific AGO proteins and then guide the RISC to their corresponding target genes through complementary base pairing between target mRNA and the guide strand of the sRNA. This mode of gene regulation may be mediated by two approaches, viz. target mRNA cleavage or translational inhibition[5].

The AGO proteins of plants and animals can be grouped into three types based on the nature of small RNAs with which they are associated. The first category of AGO proteins is known to interact predominantly with miRNAs and siRNAs, whereas the second category known as the PIWI proteins are exclusively found in animals which interact with PIWI-interacting RNAs (piRNAs). A third category of AGO proteins, which bind to secondary siRNAs, was reported in worms[6]. Several studies have suggested the presence of four typical domains in AGO proteins viz. N terminal domain (Argo-N), PAZ domain, MID domain and PIWI domain[7]. PAZ domain contains a nucleotide-binding pocket that anchors the two nucleotide 3′ overhangs of the small RNAs generated after RNase III-like activity of DCLs[8]. The PIWI domain exhibits extensive functional homology to RNase H and is known to impart 'slicer' activity of the AGO proteins[9]. The MID domain is known to bind the 5′ phosphates of small RNAs and anchors small RNAs onto the AGO proteins[10]. The Argo-N domain may facilitate the separation

[1]Department of Botany, Gauhati University, Jalukbari, Guwahati, Assam 781014, India. [2]ICAR-National Institute for Plant Biotechnology, IARI, LBS Building, Pusa, New Delhi 110012, India. ✉email: niraj_botany@gauhati.ac.in