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Plant Noncoding RNAs Identification and Characterization - A Short Review

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Abstract

Advances in sequencing technologies have facilitated multitudinal research analysis. Ample number of genes, transcriptional factors, gene families and genes associated to agronomical traits has been discovered. Similarly, several noncoding RNA (ncRNA) transcripts have also been identified which are researchers interest these days. RNA molecules that are not translated to a protein molecule are marked as ncRNA. They are categorized based on their size to small and long non-coding RNAs (lncRNA). Several ncRNAs have been identified in plants and animals, however many among them are yet to be characterized or proven with any biological roles. In this review we discuss the advancement of ncRNA research in plants and focus in their identification, types and characterization with the available resources.

Keywords: Noncoding; Small Noncoding RNAs; Traits; Long Non-Coding RNAs

Introduction

RNA molecule that is not translated into a protein molecule is termed as ncRNAs. They are known to regulate genes while playing major roles in plant growth and stress tolerance [1,2]. NcRNAs are categorized into two groups based on their size into small and long non-coding RNAs; where microRNAs (miRNA), small interfering RNAs (siRNAs), form the first group with < 50 nucleotide (nt) length and long noncoding RNAs like long intergenic RNAs (lincRNAs), circular RNAs (circRNAs) forming the second group of regulatory ncRNAs with a length > 200nt [3]. Among the different classes of ncRNAs miRNAs and lncRNAs are researchers interest these days. MiRNAs are the widely known small ncRNA with 18–24 nucleotides (nt) length abundantly found in plants and animals. They are transcribed as pri-miRNAs in the nucleus by RNA polymerase II, processed by Dicer-like 1 into pre-miRNAs forming a hairpin structure. Later they are loaded into the Argonaute (AGO) protein complex to execute their functions [3]. MiRNAs regulate gene expression by complementary binding to specific mRNAs and function through post transcriptional gene silencing [3,4]. Subsequently, lncRNAs are potential biological regulators that have originated as the reason of transposable element insertions, chromosomal rearrangement or as a cause of mutations in coding genes [5]. A large number of ncRNAs have been identified and characterized in plants such as *Arabidopsis thaliana (A. thaliana), Zea mays (Z. mays) and Medicago truncatura (M. trunculata)* [5-7]. The availability of sequence information as expressed sequence tags (ESTs), complementary DNA (cDNA), microarray, whole genome sequence (WGS), and RNA sequencing (RNA seq) have enabled the identification of both the ncRNAs. Besides these, the advancement in sequencing technologies has provided a paradigm in ncRNA research.

Identification and Characterization of ncRNAs in Plants

Cloning the small RNA and comparative were the initial methods followed in identification of miRNAs. In 2002 Reinhart BJ, et al., used these techniques to identify 16 *Arabidopsis thaliana* (*A. thaliana*) miRNAs showing different expression pattern during different developmental stages. This method was overcome by their identification of miRNAs from ESTs in plants like *A. thaliana*, *O. sativa*, *B. rapa* [8-10]. The advent of NGS technologies like small RNA sequencing has been the widely used method recently for the identification of miRNAs. These methods have enabled several genome wide identifications which enriched our knowledge with growing number ncRNAs publicly available. Also, bioinformatics methods have enabled the target prediction for these miRNAs. The identified miRNAs are evidenced to involved in many developmental process, biotic and abiotic stress responses [11,12]. Several miRNAs have been proven to involve in floral development, leaf formation, stem development, root development, signal transduction, male and female reproductive development, and many other cellular processes in several plants [13-21].

Similarly, a large number of lncRNA have been identified and characterized in plants utilizing cDNA sequences, RNA sequencing and strand specific RNA sequencing technologies [6,22-24]. Besides their regulatory roles few lncRNAs are known to play roles in phosphate-starvation, nodulation and cold stress [25]. Although several lncRNAs have been identified, most of their functions yet remain unknown. A very small number of plant lncRNAs have been characterized thus far. The most well-known lncRNAs COOLAIR and COLDAIR identified in *Arabidopsis* relate to the flowering locus (FLC) and function during flowering time [26,27]. LncRNAs, GmRNAD40 and MtENOD40 identified in soybean and Arabidopsis mediate nodule formation [28,29]. Other lncRNAs functioning in phosphate uptake have also been reported in tomato, rice and barley [25,30,31]. In plants like maize more than 90% of lncRNAs are known to function as precursors of small RNAs [7].

Experimental Validation

Besides genome wide identification, validation of these miRNAs and lncRNAs using experimental procedures are also in the increasing phase. MiRNAs are generally validated using RT-QPCR (real time methods), however for few miRNAs their stem loop structure makes it difficult in their identification. Hence several array-based methods and northern blot techniques have become the major techniques used in the miRNA validation [32]. The next step after the miRNA validation is the target validation which helps major to understand the impact of the miRNA on the gene of interest. Rapid Amplification of cDNA ends (RACE) techniques and RLM-RACE are the widely used techniques for the target validation on small scale [33]. Degradome sequencing is an emerging technique for the miRNA-target validation on genome wide basis, however RLM-RACE (RNA Ligase Mediated RACE) is the most widely accepted validation method for the miRNA mediated cleavage. Similarly, lncRNA are generally validated using RT-PCR methods.

Recent Trend in ncRNA Identification

NcRNA identification keeps evolving with the advancing technologies. Recently miRNA sequencing (miRNA seq) and lncRNA sequencing (lncRNA seq) are the techniques designed to focus on the ncRNAs of interest only. Such studies have identified several new miRNAs and lncRNAs that have not been identified earlier [34]. However, these methods seem highly expensive techniques, RNA seq experiments have been the most widely used and cost-effective method in the identification of these ncRNAs.

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