J. Plant Biochem. Biotechnol. (January–March 2016) 25(1):1–2
DOI 10.1007/s13562-015-0326-6

EDITORIAL

RNA sequencing as a tool for understanding biological complexity of abiotic stress in plants

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Received: 23 June 2015 / Accepted: 23 August 2015 / Published online: 17 October 2015

The radical innovations, reduction in sequencing cost and speed is transforming plant research. Genomes of many plants are completely sequenced and many genome sequences are on the way. Plant scientists use state of the art sequencing tools to aid crop improvement by understanding plant Biochemistry and physiology. Instead of backbreaking purification and characterization of proteins, characterization of Cellular RNA transcripts is a gateway to get at hard to do plant biochemistry (Martin et al. 2013, Kellner et al. 2015).

The complete set of transcripts in a cell, both in relations of type and quantity are called as Transcriptome. The arrival of high-throughput sequencing based methods has placed the study of transcriptomes within reach. This involves direct sequencing of complementary DNAs (cDNAs) using high-throughput DNA sequencing technologies (Severin 2010).

The tools and technology for RNA sequencing (RNA-Seq) and transcriptome exploration are promptly moving ahead (Martin et al. 2013).The knowledge so gained could assist in appreciating the importance of erratic and altered expression of genes as they endorse complex traits such as abiotic stresses. Enquiry into genome-wide differential RNA expression under varying environments may yield intuitions into biological corridors, complexity and molecular mechanisms that police activity of genes involved in adaptation to abiotic stresses (Bhardwaj et al. 2015, Van Verk et al. 2013, Voytas 2013; Dolgin (2015). The technology may be used for detecting cellular pathway alterations and differential gene expression during abiotic stresses. RNA-seq may yield information about the affiliations between genes and their products and allow isolation of genes for traits whose biochemistry is difficult. Genes involved in abiotic stress such as drought tolerance in weeds whose genomes are not yet sequenced may be defined (5). Such genes may be codon optimized, chemically synthesized and expressed in crop plants to understand their impact on the overall physiological fitness of the transgenic plant (Kellner et al. 2015, Góngora-Castillo et al. 2012, Malik 2013). The synthetic genes can also be expressed in *Phichia Pastoris* or *Escherichia coli* to produce corresponding protein for biochemical studies. This technology could illuminate structural and regulatory gene networks to enlighten how plants reply to fast evolving hints and their environs (González-Ballester et al. 2010).

Fast analyses of plant's fluctuating transcriptomes through sequencing of their related complementary DNA populations allowing sequencing of the RNA transcripts in a cell including mRNA transcripts, small RNA, miRNA, tRNA is referred to as RNA-sequencing (RNA-seq). It can top score unique RNA existence and quantity from novel genomes of ferns, mosses, algae, weeds and other hardy plants adapted to stress at a precise point of development and physiological stage. Analysis of genome-wide differential RNA expression provides canvassers with greater insights into biological pathways and molecular mechanisms that regulate cell fate, development, and changes during and due to abiotic stresses

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(Duhoux et al. 2015). For mRNA analysis, the 3′ polyadenylated (poly(A) tail is hybridized with poly (T) oligos that are linked to magnetic beads. The non-poly(A) RNA does not bind to the beads, 90 % of which is ribosomal RNA and is removed by probe hybridization. Remaining RNA is non coding that is hydrolyzed into 200–300 nucleotides and the cDNA is further broken to the fragment length required by the sequencing system. The RNA is fractionated on size exclusion gels before addition of linkers to the 3' and 5' end used for sequencing.

Bioinformatics tool can then be used to analyze these sequences to stare at alternative gene spliced transcripts, post-transcriptional modifications, gene fusion, exon/intron boundaries, transcription start sites, gene boundaries, new splicing variants, and insight of the variable level of gene expression (3). RNA-seq avoids prejudices against low-abundance transcripts and enriched transcript termini. Analysis of the transcriptome recognized 42 % of intron-containing genes of Arabidopsis and 48 % of rice (*Oryza sativa*) genes as alternatively spliced. Observation of unbiased population of transcripts consents the documentation of novel transcripts, fusion transcripts and non- coding RNAs that go undetected with dissimilar technologies (Nagalakshmi et al. 2010).

Time course experiments involving RNA-Seq permit a far-reaching gestalt and exact account of the physiological changes happening over time. Quantifying mRNA levels could tell how the transcriptional machinery of the cell is affected by the changing abiotic stresses (4, &7). Post transcriptional gene regulation by RNA interference may intrude the correlation between the abundance of mRNA and the related proteins. RNA-seq is limited to transcribed regions. Differential regulation of the splice isoforms of the same gene may be marked to foretell their biological functions (9). The deep sampling used in RNA-seq may slice up the genetic substructures of precise phenotypes while searching for complex interactions within polyploid genomes (Ilut et al. 2012).

The study of transcriptome assemblies reduces the intricacies of ploidy, genome sizes with repetitive sequences, entire or partial genome duplication, heterozygosity and paralogy. It allows access of the gene space of unsequenced recalcitrant species. Technological perfections in sequencing platforms; software and smart algorithms will further improve management and mining of large, repetitive plant genomes leading to understanding of plant biochemistry and physiology for application to crop improvement (Lohse et al. 2012, Moerkercke et al. 2013).

Declaration of conflict of interest The author declares that he has no conflict of interest.

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