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Next Generation Sequencing for Better Understanding Alternative Splicing: Way Ahead for Model and Non-Model Plants

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Understanding and resolving plant protein diversity and complexity is still a convoluted and variegated issue that needs to be addressed keeping pace with advances in the complex sequencing chemistry. One of the critical phenomena that can improve understanding of the evolution of plant complex patterns is alternative splicing. Alternative splicing (AS) has been well reviewed previously [1] and landscape patterns for model species have been widely illustrated. Functionally, the splice-isoform pattern has been classified into four major categories: exon skipping (ExonS), alternative donor (AltD) or alternative acceptor (AltA) site, and intron retention (IntronR) [2].

Unraveling the pattern of alternative splicing is critical to accurate transcript mapping onto the well annotated reference genomes. However, lack of availability of the sufficient transcript catalog is the most rate-limiting step in efficient prediction of the complex splice patterns in the plants. Next generation sequencing (RNAseq or Transcriptomics) provide a cost effective solution with bloated amount of reads to effectively generate a reference catalog of transcripts for model and non-model species with much reduced cost and high breadth of coverage. Thus suitable applications of the RNA-seq can demonstrate the patterns of AS events in the model as well as non-model plant species [3]. Alternative splicing variants has been widely associated to several key genes involved in the physiological and the biochemical machinery of the plants [1,4]. Recently, in addition to the AS, constitutive splicing patterns have been shown to widely affect the intron containing genes [1].

Presently, many algorithms and effective tools are available for efficient read mapping and detection of AS isoforms, these includes MISO [5], Diffsplice [6], AltAnalyze [7], Splice Grapher [8], Splicing Compass [9], and MATS [10]. However, in case of non-model genomes, revealing the transcript splicing diversity is still a daunting task. A recently published approach implements the construction of De Bruijn graph from the RNA-seq reads to perform the local assembly of the polymorphic regions to predict *de novo* splice variants (Kissplice), which could potentially pave the way for non-model organism where the reference genome is still lacking. AS Finder also could also be potentially used for identifying transcript isoforms either by iterative mapping of the assembled RNA-seq transcripts onto reference genome or *de novo* identification from transcripts based on BLAS Tn homology without a reference genome [11].

Physiological and functional implications of AS patterns have a wide and intricate role in understanding stress transcriptomics and metabolic fluxes, which can layout the landscape genomics and adaptive trait evolution in model and the non-model species. Recently, exon-skipped transcript abundance has been shown to be the dominant form of AS involved in the responses to heat and drought stress, utilizing RNA-seq. This clearly indicates that broader coverage of RNA-seq can reveal the dominance of particular AS events underlying the studied effect [12]. AS also plays an important role in understanding floral responses and regulation of key flowering genes and pollen machinery, which is of wide importance in the global warming era [3,13]. Recently, RNA-seq assisted Integrated Genome Browsing (IGB) uncovered the role of the polypyrimidine tract binding protein homologs (PTBs) *PTB1* and *PTB2* in stimulating the splicing events specifically explaining that the cause of AS pattern changes in *PHYTOCHROME INTERACTING FACTOR6* is potentially due to PTB misexpression [3]. A coherent IGB assisted browsing revealed 1,908 high-confidence new splicing events, which could be of potential importance in understanding the pollen mechanism and the seed maturation in model plants [13]. Metabolic flux pattern analysis using NGS read assisted transcriptomics revealed an abundance of stress related gene ontology categories in the alternatively spliced genes in Iron (Fe^{+2}) deficiency suggesting a nutrient specific splicing pattern hypothesis [14].

Realizing the importance of the aforementioned studies, it can be concluded that there is a long way ahead to understand the expansion of AS events. High-throughput RNA-seq may provide the most effective and plausible answer to origin, distribution and contrasting patterns of variation in protein-coding diversity in plant species.

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