Drought Stress-Modulated Alternative Splicing Landscapes in Drought-tolerant and -Sensitive Banana Cultivars

Muthusamy Muthusamy*, Uma Subbaraya , Backiyarani Suthanthiram, Saraswathi Marimuthu Somasundaram, Chandrasekar Arumugam

ICAR-National Research Centre for Banana, Thogamalai Road, Thayanur (Post), Tiruchirapalli-620 102, Tamil Nadu, India

Abstract:

Splice variants have major impact in plant response to drought stress. Alternative splicing a major post-transcriptional modification and its differential sensitivity to drought stress is of paramount importance to resolve complex molecular response of drought stress and to develop drought-resilient crops. In the present study, we analyzed the alternative splicing pattern of drought-tolerant (DT) and -sensitive banana (DS) cultivars under drought conditions and found that the number of spliced transcripts in DS (AAA genome) has increased to about 4.32 folds, while 0.19 fold among all the splicing events were reduced in DT (ABB genome). Categorization of drought -modulated alternative splicing (AS) events revealed that intron retention is the most abundant (42.5%) process, followed by alternative splice acceptor (22.6%), alternative splice donor (12.2%), and exon skipping (4.86%) in DS. Only 40-44% of intron retained transcripts have the protein coding capacity, indicating that their occurrence would participate in drought stress response as modified yet functional proteins. We observed that retained introns were slightly higher in GC content than constitutively spliced introns. The results reveal that the genotype dependent AS pattern may play an important role in drought tolerance in banana. This study will help in deciphering the molecular basis underlying phenotypic differences among tolerant and sensitive banana cultivars.

Publication History: Received: 6 June 2018 | Revised: 17 July 2018 |Accepted: 20 July 2018

Keywords:
Drought stress; Banana transcriptome; Intron retention; long non-coding RNA; RNA seq; exon skipping; mRNA decay pathway.

INTRODUCTION

Bananas are one of the important fruit crops in subtropical countries including India and because of its nutritive, medicinal value, affordability, varietal range and taste it is being consumed in almost all countries. Banana is sensitive to soil water deficit stress [1] and stress induced yield losses was calculated as high as 65% for some of the AAA genome bananas [2]. This dictates the greater urgency to produce drought stress-resilient banana varieties. Identification of molecular basis for drought tolerant phenotype is one of the prerequisites for development of new stress tolerant commercial varieties. The molecular cues comprising of several layers of regulatory mechanisms (signal transduction-transcription factors and kinases, stress responsive gene expression, post-transcriptional modifications and post translational modifications) for drought tolerance is continuously being investigated in variety of plants including banana [3-6]. Plants may acquire tolerance to drought stress via transcriptome reprogramming at transcriptional level, implicating anatomical adaptations, such as stomatal conductance [7]. AS, a major post transcriptional modification in eukaryotic genomes plays an important role in drought stress adaptation through transcriptional reprogramming which in turn responsible for transcriptome plasticity and protein isoforms [8-10] in addition to other post transcriptional RNA processing (biogenesis of microRNA and other non-coding RNAs) [11-13]. The recent uses of deep sequencing technologies in large scale gene expression profiling under stress conditions showed the importance of AS resultant isoforms and their differential role in stress tolerance or sensitive mechanisms in model and non-model plants [14, 13, 6, 15]. Moreover, it was shown that 40-50 % of stress responsive splice variants depends upon plant species and their genotypes, encoding for modified proteins hence not all splice variants undergo non-sense decay pathway [16, 17]. Therefore, deeper understanding of AS regulatory mechanisms, types and characterization of AS products for both control and stress conditions is very essential to find out molecular cues responsible for stress tolerance.

*Address correspondence to this author at the ICAR-National Research Centre for Banana, Thogamalai Road, Thayanur (Post), Tiruchirapalli-620 102, Tamil Nadu, India;
E-mail: biotech.muthu@gmail.com

Mesford Publisher Inc
Office Address: Suite 2205, 350 WEBB DRIVE, MISSISSAUGA, Ontario L5B3W4, Canada; T: +1 (647) 7109849 |E: cab@mesford.ca, contact@mesford.ca https://mesford.ca/journals/cab/

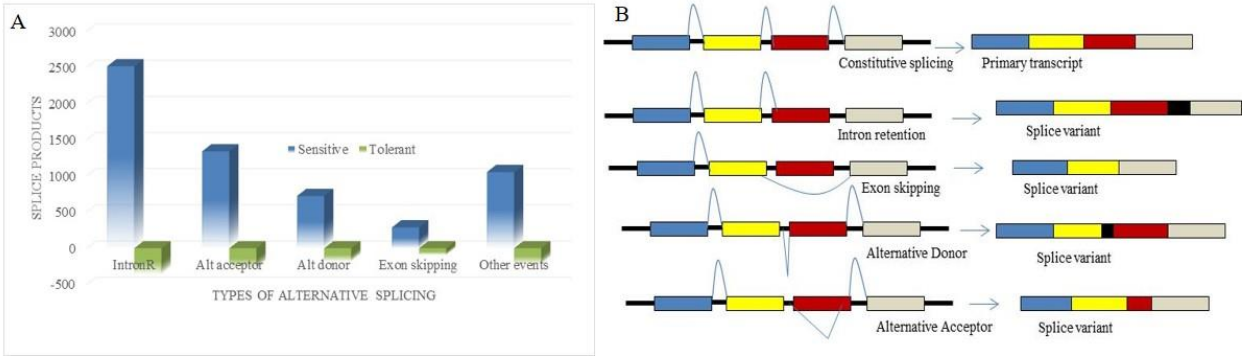

Fig. (**1A**). Major types of alternative splicing observed in drought-tolerant and sensitive banana cultivars under drought stress conditions. (**B**). Representative images showing the major types of alternative splicing in plants.

Till now four major types of splicing (Fig. **1B**) viz., intron retention (IR), alternative donor sites (AD), alternative acceptor sites (AA), and exon skipping (ES) have been identified in plants [18, 19, 13]. In addition, some complex splicing was also reported under stress conditions. These splicing events generate new proteins that might differ in structure, function and cellular localization from original proteins, which in turn expected to help plants thwart adverse conditions [20-23]. Generations of splice variants are modulated by many factors including splicing factors, tissue type, stress conditions, developmental stages and genotypes of plants [13, 24, 25]. IR, a predominant among all the AS event was widely studied in plant stress responses and also in developmental process [26, 16]. IR constitutes more than 50% of total splicing events in Arabidopsis and rice [16]. Unlike animals, exon skipping in plants is a least occurring splicing event but their contribution for transcriptome plasticity [27] has significant part in stress response. ES lead to rearrangements of domains in proteins which often results in altered binding properties, activity, stability, and sub-cellular localizations [20].

There are evidences that IR might increase the number of protein structures through genome rearrangements and exon shuffling which are likely responsible for drought and other stress tolerance [8, 22, 28, 29]. The potential role of splice variants (DRM1 and DRM2) was already demonstrated in stress response of Arabidopsis [23]. Hence, identification of drought-modulated AS is a priority to resolve their differential role in plant response. Comparing the differences of AS products between drought-tolerant and sensitive cultivars will reveal the broad molecular basis underlying phenotypic differences [30]. Therefore in this study, drought- modulated AS events of drought-tolerant and sensitive banana cultivars were investigated from their respective leaf transcriptome data both under control and drought stress.

MATERIALS AND METHODS

Drought stressed and control (irrigated) banana leaf transcriptome data (NCBI SRA-SRP087441) of drought-tolerant cultivar, Saba (ABB genome) and drought-sensitive cultivar, Grand Naine (AAA genome) generated in our previous study was used [6]. A total of 193772 transcriptional units (TUs) of drought tolerant (51026-control (CT), 48376-drought stressed (DT) and sensitive (43949-control (CS), 50421-drought stressed (DS)) cultivars were blasted with 11 chromosomes of banana reference genome (*Musa acuminata*) using perl scripts based ASFinder tool [31]. The ASFinder outputs were fed in ASTALAVISTA tool [2] to better visualize AS landscapes of CT, DT, CS and DS libraries. To analyze the impact of major splicing event, IR-transcripts were isolated and their coding potential was assessed using Coding Potential calculator [32]. Moreover, functional classification of IR-transcripts was done using the online tool Web Gene Ontology Annotation Plotting (BGI WEGO). The Algorithm predicted IR, and ES events were further confirmed randomly by multiple sequence alignment with original full length CDS and its respective chromosomal DNA. The ES resultant splice variants were annotated using Blast2Go tool to assess the impact on proteins due to exon rearrangement.

Moreover to understand the splicing regulatory mechanisms, all splicing associated transcriptional units present in digital gene expression data of DS and DT were extracted from transcriptome data and discussed.

RESULTS & DISCUSSION

The extent of genome-wide AS changes that occur during drought stress in banana is largely unknown. To understand the role of drought induced AS which is likely to be responsible for the phenotypic differences in drought tolerant and sensitive banana cultivars, transcriptome data of CT, CS and DT and DS (NCBI SRA-SRP087441) [6] were comprehensively analyzed. The results revealed that splice variants constitute approx. 18% of the total transcriptome data. The comparative analysis showed that drought induced splice variants has increased to 4.32 folds in DS than CS while, 0.19 fold of splice variants were found to be reduced in DT. In other words, drought has induced alternative splicing in AAA genotypes while reduced AS in ABB genotypes. Almost all major types of AS such as IR, AA, AD and ES were identified and it comprises of 80% of the AS observed in drought stress-responsive transcripts in both tolerant and sensitive banana cultivars (Fig. **1A**). Categorization of drought induced AS changes revealed that IR is the most abundant (42.5%) process, followed by AA (22.6%), AD (12.2%), and ES (4.86%). There was also a considerable portion of other unclassified AS events (17.79%) in DS. Similar AS pattern to drought stress was

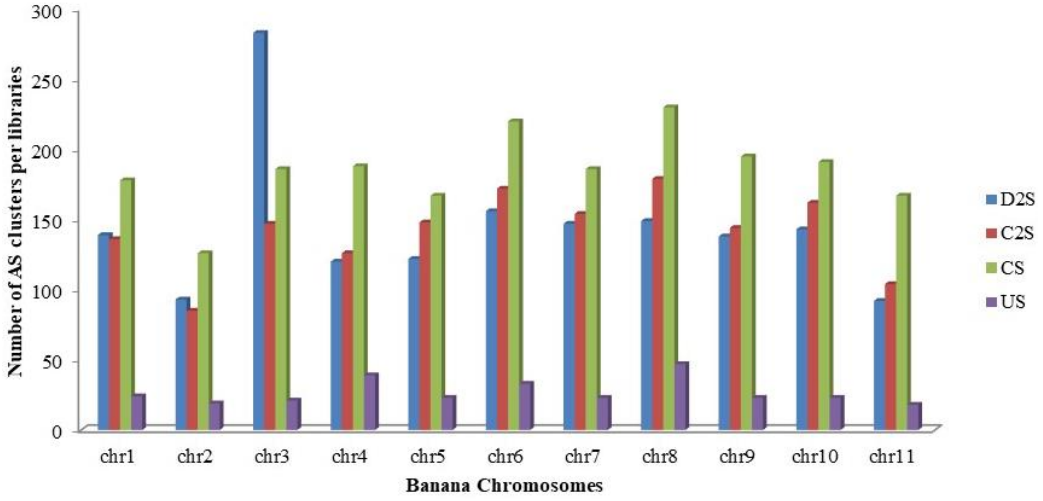

Fig. (2). Number of Alternative splicing clusters from four different libraries in banana chromosomes.

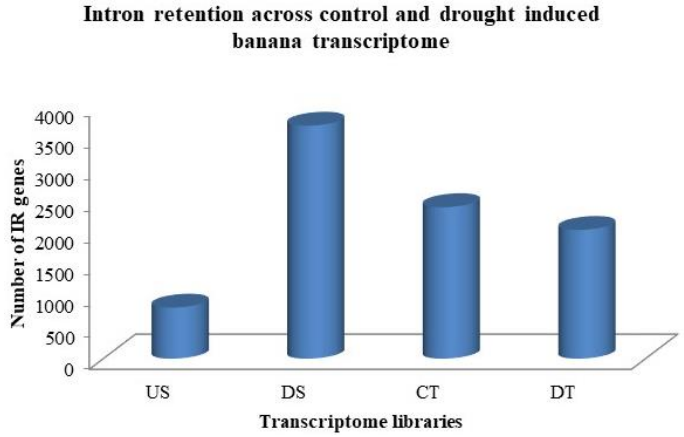

Fig. (3). Analysis of intron retention, a major splicing event in banana during normal and drought conditions of drought-tolerant and sensitive cultivars.

recently reported in maize (Miao *et al.* 2017). In this study, around 54-55% of the spliced products of DS and DT were directed to non-sense decay pathway. Similar results were observed in Arabidopsis and rice where more than 50% spliced products were directed to non-sense decay pathway [16, 17] indicating close evolutionary relationships in monocot plant species. Drought induced splice variants were more prevalent in AAA genotypes (DS) than ABB genotypes (DT). The prevalence of splice variants and presence of non-sense decay pathway could be explained to drought stress sensitive response of banana cv. Grand Naine, if clear mechanism were identified.

A total of 5952 (CT), 4815 (DT), 1558 (CS) and 8302 (DS) genes exhibiting AS was identified in this study and these AS pattern is expected to contribute for 23,096 and 23,079 unigenes respectively in tolerant and sensitive [6]. Interestingly, more AS changes was observed in drought-sensitive banana cultivars in response to drought stress than their tolerant counterparts. As per previous reports [12, 24], differential pattern of AS changes in tolerant (ABB) and sensitive cultivars (AAA) to drought stress might have regulated many factors including genomic constitutions. This genotype dependent AS changes to drought stress can also be evident from the recent findings in wheat [8]. However, the identification of changes in AS pattern responsible for drought tolerance seems to be complex and not clear. Among drought modulated AS changes, Intron retention, a prominent AS type accounts for 42.5%, which is almost in line with recent findings of [29], who noted 42% intron retention among observed AS in rice. In DT library, all splicing events were reduced and IR is topping the list which is closely followed by AA event and the least affected event is ES. It is clear that under normal conditions, CT largely undergo AS in comparison to CS. However, under drought stress, high number of alternatively spliced transcripts was found in DS which is highly different from its equivalent control (CS) while less changes for number of genes exhibiting AS were found in DT except chromosome 3 (Fig. **2**). Although, the impact of abruptly spliced transcripts of DS on drought stress response is yet to be drawn, previous reports suggested that around 50% of alternatively spliced transcripts choose to undergo mRNA degradation through non-sense decay pathway [17]. IR (Fig. **3**) and ES events are relatively found to be high in DS than DT. As reported by [33] failure in recognition of splicing signal would lead to exon skipping events which is predominantly found in animals. As of now, the splicing regulatory elements and their way of functioning in banana is not clearly known and warrants further research to understand exon skipping events.

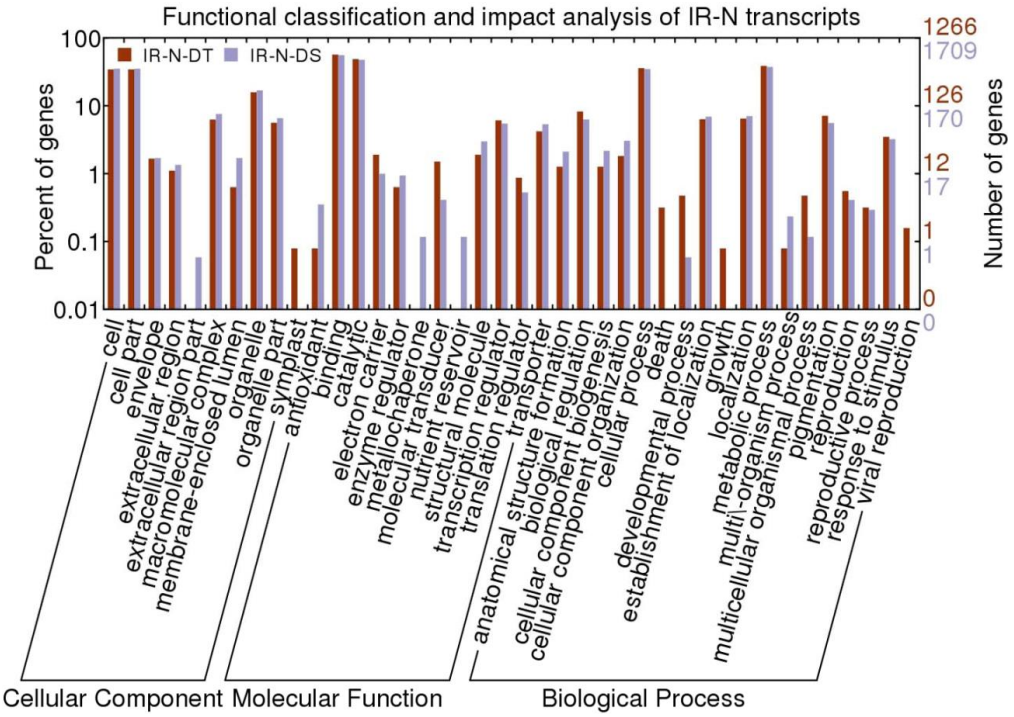

Fig. (4). The impact of intron retained no-protein coding transcripts on cellular, molecular and biological process under drought stress conditions.

Interestingly it was observed that all the splice variants identified are present in coding region (ORF) and it is expected to impact the functions of proteins. To examine the impact on known cellular, molecular and biological process, the gene ontology (GO) terms of all the splice variants and also the GO terms of intron-retained transcripts' were enriched and compared between sensitive and tolerant libraries. Gene ontology analysis of all the IR-transcripts in both DS and DT libraries has shown at least 10% of the transcripts participating either in the synthesis of cellular component (cell, cell part, and organelle), molecular components (catalytic and binding) or biological processes (metabolic and cellular process) retained the introns. Approximately 1% of stress-responsive genes also retained the introns indicating intron retention events altogether changing regulatory, developmental and metabolic process under drought conditions. Of which, most of them were expected to undergo non-sense degradation pathway (Fig. **4**), while a small fraction predicted to act as intronic regulators of other protein coding genes.

In eukaryotic genomes, AS are responsible for changes in major regulatory and functional capacity [34]. Our study is re-emphasizes that epigenetic responses to drought might be regulated through AS mediated gene expression. Identification of sub- cellular localization of spliced transcripts may indicate the altered functions of splice variants. However, the sub-cellular localization of most of the spliced transcripts could not be predicted. A small fraction of splice variants with known cellular localization are identified to be nuclei originated indicating occurrence of regulatory changes for transcriptional reprogramming during stress.

Among banana chromosomes, transcripts originated from chromosome (chr)-8 of both cultivars under normal condition tend to undergo more AS than other chromosomes (Fig. **2**). However, under drought conditions only chr-8 of drought sensitive banana cultivar is producing the most number of AS clusters while chr-3 has maximum AS clusters for tolerant cultivar. Chromosomes-8 of DS and chr-3 of DT participates in biogenesis of large number of long non-coding RNAs indicating most of the AS products in these chromosome likely are to become non-coding RNAs thus avoiding translation [6]. This finding further suggests that drought has differentially regulated AS in banana chromosomes which is depend upon to genomic constitution.

DIFFERENTIAL REGULATION OF INTRON RETENTION IN DROUGHT TOLERANT AND SENSITIVE BANANA CULTIVARS

Of the 3689 predicted IR events, 44% of the transcripts predicted to retain protein coding capacity in DS, as indicated by their coding potential calculator score (CPC). The other IR transcripts (55%) either becomes non-protein coding genes due to interruption of coding sequences thus prepared to undergo non-sense mRNA decay pathway or processed as intronic regulators of gene expression. Similarly, DT has shown 2038 IR events and only 40% of them expected to encode proteins under drought stress. In both libraries, 55-59% of the intron retained transcripts become impotent for protein coding and might have undergone mRNA degradation pathway. The intron length ranges from as small as 11 nt to 4228 nt, however, average length of retained intron in DT library is around 210.29 nt. In DS library, the average length of the retained introns (RIs) is 313 nt which is relatively higher than RIs of DT. This led to the conclusion that banana introns are greater in size than Arabidopsis introns which is about 146 nt [35]. The sequence comparison of randomly selected retained introns (RIs) with constitutively spliced introns revealed that RI has relatively high GC content (36.01%) than constitutively

spliced introns. In other words, the constitutively spliced introns were slightly richer in A, T (65.5%) than retained introns which have 63.98% of A, T bases.

In both DS and DT libraries, IR-transcripts showed that they are characteristically rich in A&T (57.06 DS, 56.43 DT) than G and C bases (42.93 DS, 43.56 DT) respectively. AT richness in IR transcripts partially might be due to retained introns because of characteristic features of plant introns which are rich in A and T than G and C bases. However, this sequence characteristic of IR transcripts is clearly distinct from usually GC rich banana genomes [36]. In the present study, the average size of the IR transcripts with protein coding capacity is 2605 bps which is slightly lesser in size than non-coding IR transcripts which averages about 2805 bps. There are evidences that intron retention functionally contribute and enhance protein expression. For instance, TU known as CUFF 13285.2 is coding for ABC transporter D family member 1 while its intron retained (128 nt) splice variant, CUFF.13285.3 is coding for ABC transporter D family member 2. ABC D2 localized at chloroplast while ABC D1 destined to peroxisome membrane indicating the critical role of IR in gene expression. Hence, it is concluded that IR splice products can code for different proteins. Our result corroborates the findings of previous studies [17, 37] that showed that splice variants differ in function and that would change the cellular localization of the original isoform.

Introns through AS regulate complex gene regulation either as an expression enhancers through IME (intron-mediated enhancement of gene expression) or as negative regulators through the generation of intronic microRNA. Therefore, to identify the possibility of intron retained transcripts that are likely directed to undergo mRNA degradation pathway as functional lncRNAs, those TUs were searched against banana long non-coding RNA (lncRNA) database [6]. The results revealed that not all transcripts but some of the non-coding intron retained transcripts of both DT and CS are processed into lncRNAs. Six IR transcripts of DT shown to produce 8 lncRNAs like MUSA-T-NC71, 315, 475, 480, 535, 591, 597, and MUSA-T-NC280. Of which, MUSA-T-535 can act as decoy for microRNA miRf10649-npr. Similarly, 4 lncRNA (MUSA-S-NC56, MUSA-S-NC147, and MUSA-S-NC388) possibly arises from non-coding IR transcripts of DS. The results indicate that intron retention which turns coding into non coding transcripts still can regulate gene expression through biogenesis of long non-coding RNAs in addition to the earlier findings that reported IR can modulate recycling of transcripts through degradation of transcripts which are no more useful for a particular physiological condition[34].

IMPACT OF ALTERNATIVE ACCEPTORS AND DONORS ON DROUGHT STRESS RESPONSE

Alternative acceptor (AA) is a second most predominant AS type observed in banana during normal and drought stressed condition. Around 27-30% of AS changes in banana cultivars under drought conditions is being contributed by AA type. AA (NAGNAG, N being any nucleotide) results in gain or loss of three nucleotides in the spliced mRNA [38]. The resultant splice variants may have altered functions [39, 40]. The coding potential assessment of these transcripts showed that 51 % of DT and 55% of DS can produce protein informs, however the functional importance to drought stress response in banana cultivars is not known. Similarly, 57, 51 % AD splice products of DT and DS respectively were found to be encoding for proteins. In comparison to IR, AA and AD relatively had better efficiency to code for functional protein isoforms as it is least altering the reading frame of coding sequences. The functional importance of AA resultant isoforms of methylation-sensitive DDT-TRANSCRIPTION FACTOR-6 was recently described in maize [24]. The impact of AA resultant splice variants in drought tolerance may be identified once functional characterization of splice variants is completed.

DROUGHT REGULATED EXON SKIPPING AND ITS IMPACT ON PROTEIN FUNCTIONS IN BANANA TRANSCRIPTOME

Exon skipping usually led to functional domain rearrangement in protein sequences which can either positively or negatively impact the functions or properties of the protein molecules [27]. Although, exon skipping is least abundant in banana, around 61-68% of the exon skipped transcripts results in protein isoforms which is highest for protein coding among other AS types observed in this study. A total of 195, 303 exon skipping events were reported in DT and DS libraries. Of the 195 TUs, 119 TUs were still coding for functional proteins with different isoforms. As indicated by CPC score, 76 TUs with skipped exon become non-coding transcripts possible due to functional domain rearrangement or completely lost during exon skipping. Similarly in DS, 94 out of 303 TUs become non-coding transcripts after exon skipping events.

In DS, 187 TUs could not find sequence similarity (threshold < 80%) to any of the annotated genes in database. However, its exon skipped corresponding transcripts had perfect or higher sequence similarity to known and uncharacterized Musa proteins. These results suggest that ES can help transcripts to attain its functional completeness under drought stress conditions. A total of 6 TUs annotated as misc_RNA such as chloroplast envelope membrane-like (LOC104000161) transcript variant, eukaryotic translation initiation factor 3 subunit G-like (LOC103990153) transcript variant, FAR1-RELATED SEQUENCE 5-like (LOC103969705) transcript variant, SUPPRESSOR OF FRI 4 (LOC103971769) transcript variant, transcription factor MYB36-like (LOC103996332) transcript variant and uncharacterized LOC103983436 (LOC103983436) transcript variant shed its representative exon under drought conditions to produce translatable mRNAs. Our study reinforces that ES in coding sequences either can abolish the function [27] or restore the reading frame and its functions/enhanced functions in plants [8]. Like DS, 194 original and its exon skipped TUs counterparts were found in DT library. Of which 25 TUs after exon skipping restored its sequence completeness. However, 99 TUs even after exon skipping failed to produce more than 80% sequence homology to known gene sequences thus indicating little impact of ES during drought stress in tolerant genotypes. Moreover, exon skipped variants of 27 TUs which includes

Table 1. Splicing regulatory elements of banana and their significant expression in drought-tolerant and –sensitive banana cultivars under drought stress.

Unique Gene ID	Gene Locus	Protein Names	CS vs DS	CT vs DT
GSMUA_Achr3G16860_001	3:18085536-18089448	Chloroplastic group IIA intron splicing facilitator CRS1, chloroplastic	2.0066	-
GSMUA_Achr5G23710_001	5:25355582-25359309	Chloroplastic group IIB intron splicing facilitator CRS2, chloroplastic	-1.84469	-
GSMUA_Achr5G02110_001	5:1333483-1353417	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase	2.29628	1.8E+308
GSMUA_Achr6G17860_001	6:12035013-12069941	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase	1.88714	-
GSMUA_Achr8G12550_001	8:9363366-9369203	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase	1.81538	-
GSMUA_Achr11G12280_001	11:11288454-11294320	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase	1.88565	-
GSMUA_Achr4G11020_001	4:7930880-7941256	Splicing factor U2af large subunit A	-	1.04343
GSMUA_Achr11G26080_001	11:24980061-24983970	Splicing factor U2af small subunit A	-	1.29166

ABC transporter I family member chloroplastic (LOC103992012) transcript variant, alpha, alpha-trehalose-phosphate synthase, F-box LRR-repeat At3g48880-like, programmed cell death 2 (LOC103987008) transcript variant mRNA and nuclear transcription factor Y subunit A-1-like were found to have no blast hits with database (sequence similarity set to 80%). Only 46 exon skipped TUs under drought conditions expected to code drought stress responsive proteins, thus warranting further in depth analysis to understand the impact of exon removal in drought stress response.

More importantly, transcription factor A-2b-like (LOC103983794) mRNA undergo exon skipping in DT and produced functional transcription factor A-2b-like transcripts for expression. Although literature suggests that drought stress can regulate AS of HSF genes in plants, HSFA2b classes and its variants were not reported for drought stress. The HSFA2b is known for its importance in pollination and fertilization at least in apple [41]. For e.g. TU (CUFF.2244000.4) has skipped an exon (70 nucleotide size) to produce splice variant (CUFF.2244000.5) which has higher sequence similarity to HSFA2b indicating exon skipping is essential to form fully functional HSFA2b under drought stress.

DROUGHT SPECIFIC AS PATTERN IN TOLERANT AND SENSITIVE BANANA CULTIVARS

Around 1452 out of 1558 AS products of CS were also present in DS libraries indicating that it could possibly regulate developmental process. Similarly, 3392 AS products of CT (5952) were also present in DT (4815). Around 83.71 % of AS changes that occurred in sensitive cultivars are due to drought stress response while, drought has regulated only about 29.55% of AS in tolerant cultivars. This genotype dependent AS pattern can be responsible for the phenotypic difference that exists in drought-tolerant cv. Saba (ABB) and sensitive cv. Grand Naine (AAA). In total, 709, 1963 unigenes were common between CS and DS library of sensitive and CT and DT library of tolerant genotypes respectively. Expression of most of the splice variants of DS either neutral or downregulated during drought stress indicating AS in sensitive cultivars would play negative regulation mostly to drought stress. However comparison of expression pattern of AS of DS and DT would not be drawn as expression profile of most of AS products of DT is not available from digital gene expression data or neglected due to its less significance in change of expression.

EXPRESSION OF SPLICING REGULATORS UNDER DROUGHT STRESS

Splicing regulatory elements play a critical role in AS events in plant response to drought stress [24]. Around 9 of 32 splicing regulators observed in transcriptome data was found to have significant changes in expression between tolerant and sensitive libraries during drought stress. Of which, 3, 6 splicing factors (Table **1**) were specific to tolerant and sensitive libraries respectively. Four TUs annotated as Probable pre-mRNA-splicing factor ATP-dependent RNA helicase from different loci (Table **1**) and two group II intron splicing facilitator (CRS1 and CRS2) of chloroplast transcripts were reported in DS. Under drought, expression of group IIA intron splicing facilitator CRS1 was induced while expression of group IIB intron splicing facilitator CRS2 was reduced. Their functional importance in drought stress response is not clear.

In DT, 2 TUs representing 2 subunits of plant conserved pre-mRNA splicing factor U2AF and 1 TUs encoding for Probable pre-mRNA-splicing factor ATP-dependent RNA helicase were found to be slightly upregulated in comparison to CT. Generally, pre-mRNA splicing factor known as U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) guides splice site selection during the formation of spliceosomal complex [16, 42]. The Musa U2AF subunits were found to have high homology with Arabidopsis, Oryza sativa subsp. Japonica (rice), and Nicotiana plumbaginifolia (tobacco) indicating that pre-mRNA splicing factor U2AF were evolutionarily conserved widely among plant species. The U2AF participates in splicing of group II introns from organellar genomes like chloroplast and mitochondria of plants. Group II introns are particularly abundant and diverse in the organellar genomes of plants, which can act as ribozymes [23].

To conclude, comparison of the differences of AS products between drought-tolerant and sensitive banana cultivars reveal

that drought has differentially regulated AS partly due to its genotype dependence. Drought induced AS significantly in drought-sensitive banana cultivars with AAA genotype (DS), in contrary it is being reduced in ABB genome containing tolerant banana cultivars. Under drought stress, the most predominant splicing type is IR and the intron retained transcripts found to be rich in G, C bases. Not all intron retention events in banana causes its degradation, approximately 40-44% of the intron retained transcripts encode proteins with altered properties. Moreover intron retained, no protein coding transcripts can be processed into long non-coding RNAs. AS products of AA and AD have better efficiency to form functional proteins than IR under drought stress. Exon skipping is the least abundant AS type in banana, nevertheless, its significance is inevitable as 61-68% of the exon skipped transcripts either help transcripts to attain its functional completeness or correcting the reading frame of the transcripts to form one or more targeted proteins. This study confirmed that epigenetic factors like drought can regulate gene expression effectively through AS in banana.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported, in part, by the Global Crop Diversity Trust of the Food and Agriculture Organization (FAO), Rome, Italy, and the NPTC-Functional Genomics component of the Indian Council for Agricultural Research (ICAR), India. The authors thank Director of the National Research Centre for Banana for providing infrastructure.

REFERENCES

[1]. Ravi I, Uma S, Vaganan MM, Mustaffa MM. Phenotyping bananas for drought resistance. Frontiers in physiology 2013; 4: 9.

[2]. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS. Genome-wide screening for novel, drought stress-responsive long non-coding RNAs in drought-stressed leaf transcriptome of drought-tolerant and -susceptible banana (Musa spp) cultivars using Illumina high-throughput sequencing. Plant Biotechnology Reports. 2015; 9:279-286.

[3]. Davey MW, Graham NS, Vanholme B, Swennen R, May ST, Keulemans J. Heterologous oligonucleotide microarrays for transcriptomics in a non-model species; a proof-of-concept study of drought stress in Musa. BMC genomics 2009; 10: 436.

[4]. Dong Y, Fan G, Deng M, Xu E, Zhao Z. Genome-wide expression profiling of the transcriptomes of four Paulownia tomentosa accessions in response to drought Genomics 2014; 104(4): 295–305.

[5]. Tripathi P, Rabara RC, Shen QJ, Rushton PJ. Transcriptomics analyses of soybean leaf and root samples during water-deficit. Genomics Data 2015; 5: 164–166.

[6]. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS, Chandrasekar A. Transcriptomic changes of drought-tolerant and sensitive banana cultivars exposed to drought stress. Frontiers in Plant Science 2016; 7: 1609.

[7]. Konstantinos PA, Imene T, Panagiotis MN, Roubelakis-Angelakis KA. ABA-dependent amine oxidases-derived H2O2 affects stomata conductance. Plant signaling & behavior 2010; 5:1153-1156

[8]. Mastrangelo AM, Marone D, Laidò G, De Leonardis AM, De Vita P. Alternative splicing: Enhancing ability to cope with stress via transcriptome plasticity. Plant Science 2012; 185: 40–9.

[9]. Liu Z, Qin J, Tian X, et al. Global profiling of alternative splicing landscape responsive to drought, heat and their combination in wheat (Triticum aestivum L.). Plant Biotechnology Journal 2017; 1–13.

[10]. Shang X, Cao Y, Ma L. Alternative splicing in plant genes: A means of regulating the environmental fitness of plants. International Journal of Molecular Sciences 2017; 18(2).

[11]. Mazzucotelli E, Mastrangelo AM, Crosatti C, Guerra D, Stanca AM, Cattivelli L. Abiotic stress response in plants : When post-transcriptional and post-translational regulations control transcription. Plant Science 2008; 174: 420–431.

[12]. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS. Computational prediction identification and expression profiling of microRNAs in banana. Journal of Horticultural Science and Biotechnology 2014; 89: 208–214.

[13]. Vitulo N, Forcato C, Carpinelli EC, et al. A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. BMC plant biology 2014; 14(1): 99.

[14]. Ding F, Cui P, Wang Z, Zhang S, Ali S, Xiong L. Genome-wide analysis of alternative splicing of pre-mRNA under salt stress in Arabidopsis. BMC genomics 2014; 15(1): 431.

[15]. Zhang H, Lin C, Gu L. Light regulation of alternative pre-mRNA splicing in plants. Photochemistry and Photobiology 2017; 93(1): 159–165.

[16]. Wang B.B, Brendel V. Genomewide comparative analysis of alternative splicing in plants. Proceedings of the National Academy of Sciences of the United States of America 2006; 103(18): 7175–80.

[17]. Syed NH, Kalyna M, Marquez Y, Barta A, Brown JW. Alternative splicing in plants – coming of age. Trends in Plant Science 2012; 17: 616–623.

[18]. Severing EI, van Dijk AD, Stiekema WJ, van Ham RC. Comparative analysis indicates that alternative splicing in plants has a limited role in functional expansion of the proteome. BMC genomics 2009; 10: 154.

[19]. Kalyna M, Simpson CG, Syed NH, et al. Alternative splicing and nonsense-mediated decay modulate expression of important regulatory genes in Arabidopsis. Nucleic acids research 2011; 40(6): 2454–69.

[20]. Dubrovina AS, Kiselev KV, Zhuravlev YN. The role of canonical and noncanonical pre-mRNA splicing in plant stress responses. BioMed research international 2013; 2013.

[21]. Remy E, Cabrito TR, Baster P, et al. A major facilitator superfamily transporter plays a dual role in polar auxin transport and drought stress tolerance in Arabidopsis. The Plant cell 2013; 25(3): 901–26.

[22]. Remy E, Cabrito TR, Batista RA, et al. Intron retention in the 5'UTR of the novel ZIF2 transporter enhances translation to promote zinc tolerance in Arabidopsis. PLoS Genetics 2014; 10(5): 15–19.

[23]. Rae GM, Uversky VN, David K, Wood M. DRM1 and DRM2 expression regulation: potential role of splice variants in response to stress and environmental factors in Arabidopsis. Molecular genetics and genomics : MGG 2014; 289(3): 317–332.

[24]. Thatcher SR, Danilevskaya ON, Meng X, et al. Genome-wide analysis of alternative splicing during development and drought stress in maize. Plant Physiology 2016; 170(1): 586–599.

[25]. Wong JJ, Gao D, Nguyen TV, et al. Intron retention is regulated by altered MeCP2-mediated splicing factor recruitment. Nature Communications 2017; 8: 15134.

[26]. Ner-Gaon H, Halachmi R, Savaldi-Goldstein S, Rubin E, Ophir R, Fluhr R. Intron retention is a major phenomenon in alternative splicing in Arabidopsis. Plant Journal 2004; 39(6): 877–885.

[27]. Bournay AS, Hedley PE, Maddison A, Waugh R, Machray GC. Exon skipping induced by cold stress in a potato invertase gene transcript. Nucleic Acids Research 1996; 24(12): 2347–2351.

[28]. Guerra D, Crosatti C, Khoshro HH, Mastrangelo AM, Mica E, Mazzucotelli E. Post-transcriptional and post-translational regulations of drought and heat response in plants: a spider's web of mechanisms. Frontiers in plant science 2015; 6: 57.

[29]. Wei H, Lou Q, Xu K, et al. Alternative splicing complexity contributes to genetic improvement of drought resistance in the rice maintainer HuHan2B. Scientific Reports 2017; 7(1): 11686.

[30]. Sammeth M, Foissac S, Guigó R. A general definition and nomenclature for alternative splicing events. PLoS Computational Biology 2008; 4(8): e1000147.

[31]. Min XJ. ASFinder: a tool for genome-wide identification of alternatively splicing transcripts from EST-derived sequences. International journal of bioinformatics research and applications 2013; 9(3): 221–226.

[32]. Kong L, Zhang Y, Ye ZQ, Liu XQ, Zhao SQ, Wei L, Gao G. CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic acids research 2007; 35: W345-9.

[33]. Morello L, Breviario D. Plant spliceosomal introns: not only cut and paste. Current genomics 2008; 9(4): 227–38.

[34]. Braunschweig U, Barbosa-Morais NL, Pan Q, et al. Widespread intron retention in mammals functionally tunes transcriptomes. Genome Research 2014; 24(11): 1774–1786.

[35]. Hebsgaard SM, Korning PG, Tolstrup N, Engelbrecht J, Rouzé P, Brunak S. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. Nucleic acids research 1996; 24(17): 3439–52.

[36]. D'Hont A, Denoeud F, Aury JM, et al. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature 2012; 488(7410):213-217.

[37]. Staiger D, Brown JWS. Alternative splicing at the intersection of biological timing development and stress responses. The Plant cell 2013; 25(10): 3640–56.

[38]. Bradley RK, Merkin J, Lambert NJ, Burge CB. Alternative splicing of RNA triplets is often regulated and accelerates proteome evolution. PLOS Biology 2012; 10(1): e1001229.

[39]. Black DL. Mechanisms of alternative pre-messenger RNA splicing. Annual review of biochemistry 2003; 72: 291–336.

[40]. Stamm S, Ben-Ari S, Rafalska I, et al. Function of alternative splicing. Gene 2005; 344: 1–20.

[41]. Giorno F, Guerriero G, Baric S, Mariani C. Heat shock transcriptional factors in Malus domestica: identification, classification and expression analysis. BMC Genomics 2012; 13(1): 639.

[42]. Sugliani M, Brambilla V, Clerkx EJ, Koornneef M, Soppe WJ. The conserved splicing factor sua controls alternative splicing of the developmental regulator ABI3 in Arabidopsis. The Plant Cell 2010; 22(6): 1936–1946.