J. Hortic. Sci.
Vol. 20(2), 2025

https://doi.org/10.24154/jhs.v20i2.4097

Original Research Paper

In silico mining of banana circular RNAs in response to biotic and abiotic stress: Classification and their distribution on genome

Elangovan D., Malarvizhi M., Laxman R.H. and Ravishankar K.V.*

Division of Basic Sciences, ICAR Indian Institute of Horticultural Research, Bengaluru - 560 089, India

*Corresponding author Email: kv_ravishankar@yahoo.co.in

ABSTRACT

Banana (*Musa* spp.) is highly susceptible to a range of abiotic and biotic stresses, leading to significant reductions in yield and productivity. Recent advances in molecular biology have highlighted the critical roles of non-coding RNAs in the regulation of gene expression and stress adaptation in plants. Among these, circular RNAs (circRNAs)—a class of covalently closed, stable RNA molecules—have emerged as important regulators of diverse biological processes, including plant stress responses. In the present study, circRNAs were systematically identified from transcriptome datasets of *Musa acuminata* subjected to various abiotic (cold, salt, osmotic and drought) and biotic (*Mycosphaerella* sp. and *Fusarium* sp.) stress conditions. A total of 1,114 circRNAs were identified under abiotic stress and 497 circRNAs under biotic stress. Notably, a high proportion of these circRNAs originated from intergenic regions, accounting for 80.7% (900) and 90.74% (451) of total circRNAs under abiotic and biotic stress, respectively. Chromosomal distribution analysis of abiotic and biotic circRNAs showed statistically significant variation across the 11 chromosomes of banana as determined by the Kolmogorov–Smirnov (K–S) test (0.75). These findings provide a foundational resource for understanding the landscape of stress-responsive circRNAs in banana. Further functional characterization and validation studies are warranted to elucidate their precise regulatory roles in stress tolerance mechanisms.

Keywords: Banana, biotic and abiotic stress, circular RNA, non-coding RNAs, transcriptome

INTRODUCTION

Banana (*Musa* spp.), a staple food for millions and a critical export commodity, is the most important commercial fruit crop in tropical and sub-tropical regions, with global production exceeding 120 million metric tons annually. This monocotyledonous perennial herb is not only vital for nutrition but also serves as a primary source of income for smallholder farmers. However, its production is severely constrained by a complex interplay of environmental pressures. Abiotic stresses such as drought, salinity, sub-optimal temperatures (both cold and heat), and oxidative stress disrupt cellular homeostasis, leading to reduced photosynthetic efficiency, impaired growth, and significant yield loss. Concurrently, devastating fungal, bacterial, and viral diseases like *Fusarium* wilt (Panama disease), black Sigatoka leaf spot, *Xanthomonas* wilt (BXW), and banana bunchy top virus (BBTV) create persistent biotic pressures that compromise plant health and can decimate entire plantations (Kotari et al., 2016). These combined challenges are exacerbated by climate change and the monocultural nature of commercial plantations, threatening both food security and economic stability in producer nations. To address these threats, breeding programs increasingly aim to develop stress-resilient cultivars. However, conventional breeding is hindered by the sterile, triploid nature of most commercial bananas and the complex polygenic nature of stress tolerance. Consequently, there is a pressing need to elucidate the sophisticated molecular and regulatory mechanisms, particularly the roles of non-coding genetic elements, which govern stress adaptation in banana.

The post-genomic era, powered by advances in next-generation sequencing (NGS) and sophisticated bioinformatic algorithms, has revolutionized our understanding of genome regulation, moving beyond the protein-centric view. It is now well-established that non-coding RNAs (ncRNAs) are central orchestrators of gene expression, development, and environmental responses in plants. The repertoire of regulatory ncRNAs extends beyond the well-characterized microRNAs (miRNAs) and small interfering RNAs

(siRNAs) to include long non-coding RNAs (lncRNAs) and, more recently, circular RNAs (circRNAs) (Ijaz et al., 2020). Among these, circRNAs have emerged as a fascinating and stable class of regulatory molecules with significant, yet largely unexplored, potential in plant stress biology.

Circular RNAs are a distinct class of endogenous, covalently closed single-stranded RNA molecules formed through a non-canonical "back-splicing" event, where a downstream 5' splice donor is ligated to an upstream 3' splice acceptor (Jeck et al., 2013). This unique biogenesis results in a circular topology lacking terminal 5' caps and 3' polyadenylated tails. This closed-loop structure confers extraordinary stability, making circRNAs highly resistant to degradation by ubiquitous exonucleases like RNase R, unlike their linear mRNA counterparts (Suzuki et al., 2014). In plants, circRNAs are typically between 200 and 600 nucleotides in length, though longer transcripts have been reported, and they originate from diverse genomic loci, including exons (exonic circRNAs), introns (intronic circRNAs), and intergenic regions (intergenic circRNAs) (Jeck & Sharpless, 2014; Zhang et al., 2020a). While the precise molecular mechanisms driving plant circRNA biogenesis are still being unraveled, evidence indicates that canonical *cis*-elements and splicing machinery, including specific complementary intronic sequences facilitating back-splice site pairing, are essential (Starke et al., 2015).

Functionally, circRNAs are versatile regulators implicated in a myriad of biological processes. They can modulate transcription and alternative splicing, bind and sequester RNA-binding proteins, and, most notably, act as competitive endogenous RNAs (ceRNAs) or "miRNA sponges". By harboring miRNA response elements (MREs), circRNAs can adsorb specific miRNAs, thereby preventing them from repressing their natural mRNA targets and fine-tuning gene expression networks critical for stress acclimation (Zhang et al., 2020a). Crucially, circRNA expression is highly spatiotemporally dynamic and responsive to environmental cues, including pathogen attack and abiotic stress, positioning them as key components of plant stress signaling and adaptive responses (Salzman et al., 2013; Hong et al., 2020).

The proliferation of strand-specific, ribosomal RNA-depleted RNA-seq protocols and dedicated computational tools (e.g., CIRI2) has enabled the systematic discovery of thousands of circRNAs across diverse plant species. Resources like CircFunBase (Meng et al, 2019), PlantcircBase (Chu et al, 2020), GreenCircRNA (Zhang et al., 2020b) have been established to catalogue these discoveries, providing platforms for comparative analysis. However, research in most non-model crops, including banana, lags significantly. While foundational studies have profiled miRNAs and lncRNAs in banana under stress conditions (Sampangi-Ramaiah et al., 2019), the landscape of banana circRNAs remains a *terra incognita*. No comprehensive, genome-wide identification or characterization of circRNAs in response to the major biotic and abiotic stresses affecting banana cultivation has been reported. This represents a critical knowledge gap, as understanding this layer of regulation could unveil novel molecular markers and genetic targets for breeding programs.

Therefore, to address this deficiency, the present study undertakes a systematic *in silico* mining of stress-responsive circRNAs in banana (*Musa acuminata*). We harness publicly available RNA-seq datasets from the NCBI SRA database, representing a spectrum of key stresses: abiotic (heat, cold, salt, osmotic, drought) and biotic (infection by *Fusarium oxysporum* f. sp. *cubense* and *Mycosphaerella fijiensis*). Using a robust computational pipeline based on the reference DH-Pahang v2 genome and the CIRI2 detection algorithm, we aimed to: (1) identify and catalogue high-confidence circRNAs expressed under each stress condition, (2) classify them based on their genomic origin (exonic, intronic, intergenic), (3) perform a comparative analysis of circRNA abundance and class distribution between biotic and abiotic stress responses, and (4) map the genomic distribution of these circRNAs across the banana chromosomes to identify potential stress-associated genomic hotspots. Our work provides the first extensive atlas of banana circRNAs, establishing a foundational resource for future functional genomics studies aimed at leveraging these stable regulatory RNAs for enhancing banana resilience.

MATERIALS AND METHODS

Data acquisition and processing

To ensure a comprehensive analysis, transcriptomic data from 12 different *Musa acuminata* tissue samples subjected to distinct stress conditions were retrieved

Table 1 : Details of banana transcriptome data used for mining of CircRNAs

NCBI accession	Project details	Stress type	Reference
SRX1746167	Banana heat stress induction (3 samples)	Abiotic (heat)	Vidhya et al. (2018)
PRJNA343716	Transcriptome of banana cultivars under cold, salt, and osmotic stresses (6 samples)	Abiotic (cold, salt, osmotic)	Hu et al. (2017)
PRJNA341326	Transcriptome analysis of drought-tolerant and sensitive banana cultivars (2 samples)	Abiotic (drought)	Muthusamy et al. (2016)
PRJNA323552	Study of *Mycosphaerella fijiensis* gene expression during infection of banana (3 samples; host reads analyzed)	Biotic (black sigatoka)	Noar & Daub (2016)
SRX181885	Comparative transcriptomics of *Fusarium oxysporum* f. sp. *cubense* races during banana infection (3 samples; host reads analyzed)	Biotic (*Fusarium* wilt)	Guo et al. (2014)

from the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA). The selected datasets encompassed a targeted range of environmental challenges: heat stress, cold stress, salt stress, osmotic/drought stress, and infections by the fungal pathogens *Fusarium oxysporum* (*Fusarium* wilt) and *Mycosphaerella fijiensis* (Black Sigatoka). Details of the SRA/Project accessions and experimental designs are provided in Table 1. Raw sequence reads in FASTQ format were downloaded using the SRA Toolkit (v2.10.8). Adapter sequences and low-quality bases (Phred score < 20) were trimmed from all reads using Trimmomatic (v0.39) with parameters: ILLUMINACLIP:TruSeq3-PE.fa:2:30:10, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:20, MINLEN:36.

Reference genome and annotation

The high-quality, chromosome-level genome assembly of *Musa acuminata* subsp. *malaccensis* cv. DH-Pahang (v2) was downloaded from the Banana Genome Hub. This reference genome, comprising 11 chromosomes, is recognized for its high contiguity and accurate annotation. The corresponding gene annotation file (GTF format) was also retrieved to guide read mapping and downstream analysis.

Identification of circular RNAs

The identification of circRNAs relies on detecting unique back-splice junction (BSJ) reads in RNA-seq data. Our analysis pipeline was implemented as follows:

1. **Read alignment:** The cleaned paired-end reads from each sample were independently aligned to the DH-Pahang v2 reference genome using the Burrows-Wheeler Aligner (BWA-MEM, v0.7.17) with default parameters. This step produced sequence alignment map (SAM) files for each library.

2. **CircRNA detection:** The resulting SAM files, along with the genome annotation file, were used as input for CIRI2 (v2.0.6), a widely used and accurate algorithm for *de novo* circRNA identification. CIRI2 employs a multiple-segment splitting and realignment strategy to accurately pinpoint BSJ sites. A circRNA was considered confidently identified if it was supported by at least two unique BSJ reads (the default threshold), ensuring high-confidence calls and minimizing false positives from template switching or mis-splicing events.

3. **Classification and merging:** The identified circRNAs from all samples were categorized into three classes based on their genomic coordinates relative to the annotation: exonic (fully within exons), intronic (fully within introns), and intergenic (located in regions between annotated genes). Redundant circRNA calls from different samples (identical chromosome, start, and end coordinates of the BSJ) were consolidated into a non-redundant master list.

Statistical and genomic distribution analysis

To compare the abundance and class distribution of circRNAs identified under biotic versus abiotic stress conditions, descriptive statistics were calculated. A two-sample Kolmogorov-Smirnov (K-S) test, a non-

parametric statistical test sensitive to differences in the shape of distributions, was employed to determine if the chromosomal distribution of circRNAs (i.e. the count per chromosome as a proportion of the total) differed significantly between the two major stress categories. The analysis was performed using custom R scripts (v4.1.0). The chromosomal locations of all high-confidence circRNAs were visualized using the R package *karyoploteR* to generate a genome-wide distribution map.

RESULTS AND DISCUSSION

Genome-wide identification and classification of stress-responsive banana circRNAs

Our *in silico* mining pipeline successfully identified a total of 1,611 unique circRNAs from the analyzed banana transcriptomes under various stress conditions. When segregated by stress type, a notable quantitative difference was observed. Under abiotic stress conditions (heat, cold, salt, osmotic, drought), we detected a total of 1,114 circRNAs. In contrast, a lower number, 497 circRNAs, were identified in the datasets representing biotic stress responses (*F. oxysporum* and *M. fijiensis* infections). This discrepancy in abundance could reflect fundamental differences in the transcriptional reprogramming triggered by these stress types. Abiotic stresses often induce broad, systemic physiological changes affecting many cellular processes, potentially activating a wider array of regulatory circuits, including circRNA production. Biotic stresses, while severe, may engage more specific pathogen-response pathways.

Classification based on genomic origin revealed a striking and consistent pattern across both stress categories (Table 2). The vast majority of identified circRNAs were intergenic in origin, constituting 80.7% (900/1114) of abiotic stress-associated circRNAs and an even higher 90.74% (451/497) of biotic stress-associated circRNAs. Exonic circRNAs were the second most abundant class, representing 18% (202) and 8.4% (42) under abiotic and biotic stress, respectively. Intronic circRNAs were relatively rare, making up only 1.97% (22) and 0.8% (4) of the totals. This overwhelming prevalence of intergenic circRNAs in banana under stress is a significant finding and aligns with observations in some other plant species. For instance, a similar dominance of intergenic circRNAs (65.99%) was reported in apple, and 48.5% were found in tomato during *Phytophthora* infection (Hong et al., 2020; Wang et al., 2022). This suggests that a large fraction of plant circRNAs, particularly those induced under stress, may originate from unannotated genomic regions, potentially representing novel, stress-responsive non-coding transcriptional units. Their regulatory functions, whether as miRNA sponges, protein decoys, or scaffolds, warrant future investigation. It is important to note that this distribution contrasts with studies in *Arabidopsis*, maize, and tomato under drought or cold, where exonic circRNAs were more prevalent (Zhang et al., 2019; Yang et al., 2020), indicating potential species or stress-specific biogenesis preferences.

An intriguing result from our comparative analysis was the complete lack of overlap between the sets of circRNAs identified under biotic and abiotic stress. No common circRNA was found to be expressed across both major stress categories in the analyzed datasets. This suggests a high degree of specificity in circRNA induction, where distinct suites of circRNAs are transcribed in response to different environmental signals. This specificity aligns with the concept of circRNAs as fine-tuners of specific regulatory pathways rather than general stress responders, potentially offering highly precise biomarkers for diagnosing the type of stress a plant is experiencing.

Chromosomal distribution of circRNAs and statistical analysis

The 1,611 identified circRNAs were mapped across all 11 chromosomes of the *M. acuminata* genome (Fig. 1) with a non-uniform distribution. Under both biotic and abiotic stress conditions, chromosomes 4 and 9 consistently harboured the highest density of

Table 2 : Classification and abundance of identified banana circrnas under biotic and abiotic stress

Stress category	Total circ RNAs	Exonic circ RNAs (count, %)	Intronic circ RNAs (count, %)	Intergenic circ RNAs (count, %)
Abiotic Stress	1,114	202 (18.13%)	22 (1.97%)	900 (80.79%)
Biotic Stress	497	42 (8.45%)	4 (0.80%)	451 (90.74%)

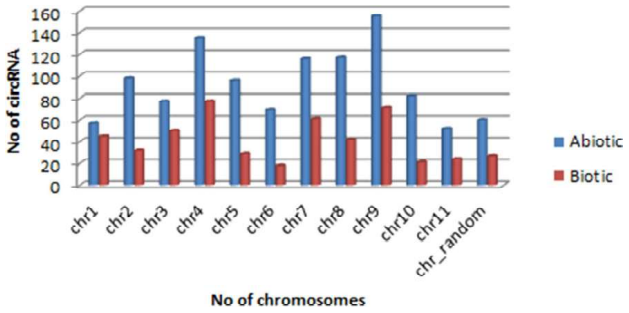

Fig. 1 : Chromosomal distribution of identified circRNAs under biotic and abiotic stress in banana

circRNAs. This clustering may indicate that these chromosomal regions are enriched with genomic features conducive to circRNA biogenesis, such as specific repeat elements, gene density, or chromatin architecture that facilitates back-splicing. Conversely, the lowest number of circRNAs was found on chromosome 6 for biotic stress and chromosome 11 for abiotic stress.

To objectively determine if the pattern of chromosomal distribution differed between biotic and abiotic stress responses, we performed a two-sample Kolmogorov-Smirnov test. The analysis yielded a test statistic (D) of 0.75 with a significant *p*-value of 0.0023. This statistically significant result confirms that the genomic distribution of stress-responsive circRNAs is not random and, importantly, varies depending on the nature of the stress. This finding reinforces the stress-specific nature of the circRNA response and suggests that different stresses may activate or silence back-splicing events from distinct genomic loci.

CONCLUSION

This study presents the first comprehensive, genome-wide catalogue of circRNAs in banana (*Musa acuminata*) under major biotic and abiotic stress conditions. Our *in silico* analysis reveals that banana produces a diverse population of stress-responsive circRNAs, predominantly originating from intergenic regions, with distinct sets induced by pathogenic infection versus environmental adversity. The non-random, stress-type-specific chromosomal distribution of these molecules further underscores their potential as specialized components of stress-responsive networks.

These findings open several promising avenues for future research. First, the candidate circRNAs identified here, particularly those from high-density chromosomal hotspots, require experimental validation through techniques like RT-PCR. Second, functional characterization is paramount. Predicting miRNA binding sites within the sequenced circRNAs can suggest their roles as ceRNAs. Identifying their interacting protein partners and correlating their expression with putative parent genes will shed light on their regulatory mechanisms. Third, exploring the conservation of these circRNAs across different banana cultivars and species (*M. balbisiana*) could reveal evolutionarily conserved stress modules. Ultimately, this foundational work paves the way for leveraging circRNAs as novel tools in molecular breeding—either as diagnostic markers for stress tolerance or, through biotechnology, as synthetic sponges to modulate key miRNA-regulated pathways for enhancing banana resilience.

ACKNOWLEDGEMENT

The authors acknowledge ICAR-NICRA project for providing financial support and the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru for providing necessary facilities to carry out this research work.

REFERENCES

Chu, Q., Bai, P., Zhu, X., Zhang, X., Mao, L., Zhu, Q. H., Fan, L., & Ye, C. Y. (2020). Characteristics of plant circular RNAs. *Briefings in bioinformatics*, *21*(1), 135-143.

Guo, L., Han, L., Yang, L., Zeng, H., Fan, D., Zhu, Y. … & Huang, J. (2014). Genome and transcriptome analysis of the fungal pathogen *Fusarium oxysporum* f. sp. cubense causing banana vascular wilt disease. *Plos one*, *9*(4), e95543.

Hong, Y. H., Meng, J., Zhang, M., & Luan, Y. S. (2020). Identification of tomato circular RNAs responsive to *Phytophthora infestans*. *Gene*, *746*, 144652.

Hu, W., Ding, Z., Tie, W., Yan, Y., Liu, Y., Wu, C. ... & Jin, Z. (2017). Comparative physiological and transcriptomic analyses provide integrated insight into osmotic, cold, and salt stress tolerance mechanisms in banana. *Scientific Reports*, *7*(1), 43007.

Ijaz, U., Ali, M. A., Nadeem, H., Tan, L., & Azeem, F. (2020). RNA world and heat stress tolerance in plants. In: Wani, S.H., Kumar V. (Eds.), Heat stress tolerance in plants: Physiological, molecular and genetic perspectives (pp. 167-187). Wiley. https://doi.org/10.1002/9781119432401.ch8.

Jeck, W. R., & Sharpless, N. E. (2014). Detecting and characterizing circular RNAs. *Nature Biotechnology*, *32*, 453–461.

Jeck, W. R., Sorrentino, J. A., Wang, K., Slevin, M. K., Burd, C. E., Liu, J., Marzluff, W. F., & Sharpless, N. E. (2013). Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA*, *19*(2), 141-157.

Kotari, P., Swarupa, V., & Ravishankar, K. V. (2016). Genomics of biotic stress tolerance in banana. In: Mohandas, S., Ravishankar, K. (Eds.), Banana: genomics and transgenic approaches for genetic improvement, (pp. 61-75). Springer, Singapore. https://doi.org/10.1007/978-981-10-1585-4_5.

Meng, X., Hu, D., Zhang, P., Chen, Q., & Chen, M. (2019). CircFunBase: a database for functional circular RNAs. *Database*.

Muthusamy, M., Uma, S., Backiyarani, S., Saraswathi, M. S., & Chandrasekar, A. (2016). Transcriptomic changes of drought-tolerant and sensitive banana cultivars exposed to drought stress. *Frontiers in Plant Science*, *7*, 1609. https://doi: 10.3389/fpls.2016.01609

Noar, R. D., & Daub, M. E. (2016). Transcriptome sequencing of *Mycosphaerella fijiensis* during association with *Musa acuminata* reveals candidate pathogenicity genes. *BMC Genomics*, *17*(1), 690. https://doi: 10.1186/s12864-016-3031-5.

Salzman, J., Chen, R. E., Olsen, M. N., Wang, P. L., & Brown, P. O. (2013). Cell-type specific features of circular RNA expression. *PLoS genetics*, *9*(9), e1003777.

Sampangi-Ramaiah, M. H., Ravishankar, K. V., & Rekha, A. (2019). Long non-coding RNAs in banana: prediction, mapping and their comparative studies using *Musa balbisiana* and *Musa acuminata* transcriptome. *Trees*, *33*(2), 359-369.

Starke, S., Jost, I., Rossbach, O., Schneider, T., Schreiner, S., Hung, L. H., & Bindereif, A. (2015). Exon circularization requires canonical splice signals. *Cell Reports*, *10*(1), 103-111.

Suzuki, H., & Tsukahara T. (2014). A view of pre-mRNA splicing from RNase R resistant RNAs. *International Journal of Molecular Sciences*, *15*, 9331–9342.

Vidya, S. M., Kumar, H. V., Bhatt, R. M., Laxman, R. H., & Ravishankar, K. V. (2018). Transcriptional profiling and genes involved in acquired thermotolerance in banana: A non-model crop. *Scientific Reports*, *8*(1), 10683.

Wang, D., Gao, Y., Sun, S., Li, L., & Wang, K. (2022). Expression characteristics in roots, phloem, leaves, flowers and fruits of apple circRNA. *Genes*, *13*(4), 712.

Yang, X., Liu, Y., Zhang, H., Wang, J., Zinta, G., Xie, S., ... & Nie, W. F. (2020). Genome-wide identification of circular RNAs in response to low-temperature stress in tomato leaves. *Frontiers in Genetics*, *11*, 591806.

Zhang, J., Hao, Z., Yin, S., & Li, G (2020b). GreenCircRNA: a database for plant circRNAs that act as miRNA decoys. *Database*.

Zhang, P., Fan, Y., Sun, X., Chen, L., Terzaghi, W., Bucher, E., Li, L., & Dai, M. (2019). A large scale circular RNA profiling reveals universal molecular mechanisms responsive to drought stress in maize and Arabidopsis. *The Plant Journal*, *98*(4), 697-713.

Zhang, P., Li, S., & Chen, M. (2020a). Characterization and function of circular RNAs in plants. *Frontiers in Molecular Biosciences*, *7*, 91.

(***Received : 5.3.2025; Revised : 4.7.2025; Accepted : 15.7.2025)***