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Expression Profiling of Yield Related Genes in Banana

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Abstract: **Banana (Musa spp., family Musaceae) is one of the principle fruit crop of the world, which is cultivated and consumed in many countries. The gene expression profiling of the six subtractive suppression cDNA clones SSh1c03, SSh1f02, SSh1g06, SSh3e08, SSh2a02 and SSh2b11of the transcripts of zinc finger A20 and AN1 domain-containing stress-associated protein, putative nuclease HARBI1, E3 ubiquitin-protein ligase UPL1-like, thioredoxin H1- like, 9S-lipoxygenase 4 and nematode resistance protein-like HSPRO2 of banana (Musa acuminata subsp. malaccensis) respectively was studied in flower and leaf of Grand Naine (Control) and four local genotypes (BAT, DRG, BHM and GA) of banana by semi- quantitative reverse transcriptase PCR approach. The genes belongs to four cDNA clones SSh1c03, SSh1f02, SSh1g06 and SSh3e08 among the selected transcripts were showed up- regulation in floral and leaf tissues of all the selected genotypes of banana may contribute in yield. The local genotypes of banana did not show any significant difference by comparing with the control in their expression.**

Keywords**: Banana, yield related genes, RT-PCR, expression profiling.**

I. INTRODUCTION

Banana (Musa spp., family Musaceae) is the principle fruit crop of the world, which is cultivated and consumed with vast majority. It is a key crop across the humid subtropics with a global production of about 100 million tonnes, providing subsistence to the farmers and small holders with both nutrition and income. Even under unfavourable conditions, bananas give a beneficial crop and contribute to food security. An increase in the yield of banana may resolve the serious problems such as poverty, malnutrition and starvation of many developing counties. There is lake of information of the genetics of banana. Since the nature of the crop as a parthenocarpic, mostly triploid, sterile plant represents many facets of breeding and selection that have not applied yet on banana, which are possible to other crops. The genomic studies on banana have been developed rapidly since 2000 [1]. The Global Musa Genomics Consortium aims to assure the sustainability of banana as a staple food crop by developing an integrated genetic and genomic understanding of the crop, allowing targeted breeding, transformation and more efficient use of Musa biodiversity. Many resources are available: large-insert genomic BAC libraries made from DNA of *Musa acuminata* 'Calcutta 4' and *Musa balbisiana* 'Pisang Klutuk Wulung' [2], and DNA sequence information, with annotation and curation on the Musa genomics website. [3] have analyzed 3 million bases of Musa DNA sequence from BAC ends and shown many features of its organisation and genetic structure, while characterization for repetitive DNA component of the Musa genome has been carried out to understand the genomics of the species [4] and [5], which represents a substantial part of the 550 Mbp of DNA [6]. Expressed sequence tags (ESTs) have been published, including by [7]. Genome expression profiling has been carried out in recent past [8], along with transcriptional profiling [9]. Microarrays can be used to examine changes in gene expression [4], and advances are being made in generating protein profiles from banana [10]. Banana genomics researchers can exploit similarities between banana and other species to identify gene functions. Analysis of the banana transcriptome led to identification of over 842 genes that were not annotated by the Musa genome project, so there is need of the study function, expression and annotation of the genes of banana. Yield is the most important character of any crop. Higher the

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yield of banana may contribute to the economy of the developing countries of the world. The yield of banana can enhance by plant tissue culture, breeding and genomics approaches. If we identify the genes responsible to contribute the yield, by the up-regulation and enhanced expression of those genes we can increase the yield and production of banana. Therefore, identification and expression profiling of those genes in the banana may provide us information about the up-regulation and down-regulation during flowering and fruiting, this may useful for creation of variation in such a way to increase the yield and production.

II. MATERIALS AND METHODS

The cultivars of banana grown in the farms and greenhouses of the "Aditya Biotech Lab and Research Pvt. Ltd., Raipur (Chhattisgarh) were selected as experimental material for the present study. An elite cultivars namely Grand Naine (from Israel) and the different local genotypes namely DRG, BAT, BHM and GA were selected. The Grand Naine was assumed as control to compare with other selected local cultivars. The methods and procedural steps of the present study were as follows:

Retrieving cDNA clone sequences of the concerned genes and their primer designing:

The concerned gene specific sequences were retrieved from the Subtraction suppression hybridization (SSH) cDNA library of NCBI of the banana [11]. The genes responsible for flowering and fruiting were selected for study. The BLASTn were performed for retrieved cDNA sequences to check the identity and similarity with banana genome. The cDNA sequences which showed the highest similarity were selected for the study. These selected cDNA sequences were undergone to the primer designing by a primer designing tool Batch Primer-3. In this tool the generic primers were designed for the sequences with required settings. The primers and their sequences were enlisted in Table 1.

RNA isolation and cDNA synthesis of the banana samples:

RNA was isolated by Trizol method (Department of Agronomy, Iowa State University, USA, 2009):

Table 1: List of primers used for amplification of cDNA copies of the transcripts of selected banana cultivars

Sl. No.	Name of cDNA clone	Primer sequence	No. of Nucleotides	Tm (˚C)
1.	Actin	**F**-GAGAAGATACAGTGTCTGGA	20	49.7
		R- ATTACCATCGAAATATTAAAA	22	43.7
2.	SSh1c03	**F**-TCAGCGAGAGGAGAAGG	20	59.4
		R-GCATGTCTGGCACATGTTGT	20	57.3
3.	SSh1f02	**F**-CACGCCTTCAACGAGAAGAT	20	57.3
		R-AAGGCCATGGTGTAGCAGAT	19	57.3
4.	SSh2b11	**F**- CTGCACGATCCAACAGATCC	20	53.8
		R- GTCCATCAGAAGGTGGAGGTC	20	56.3
5.	SSh1g06	**F**- CATGGAAATATGCGGTTCGT	20	55.3
		R-AGATCTCACTCGGCAGCACT	20	59.4
6.	SSh3e08	**F**-CTCATGTGGAGCTCAATCTTCTT	23	53.5
		R-ATAAGTTCACCGATGCCATCTT	22	51.1
7.	SSh2a02	**F**-ACAGTATTCGGTCACCCATGTAT	23	53.5
		R-CCGTCATCTACAGAAACTGGAAC	23	55.3

Synthesis of cDNA or Reverse Transcriptase Enzyme Reaction:

i. The first strand cDNA synthesis kit of New England Bio Labs, Proto Script II (E65605) was used to synthesize cDNA from isolated RNA templates of different cultivars of banana

ii. The kit components were thaw on ice and mix by inverting several times.

iii. Denaturation of RNA template were done by mixing components as Table 2.

Table 2: Components of denaturation mixture for template RNA

Sl.No.	Component	Volume
a)	Total RNA	2 µl
b)	$d(T)_2$, VN (50mM)	2 µl
c)	Nuclease free water	To a total volume of 8 µl

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iv. The RNA templates/ $d(T)_2$ VN were denatured for 5 min at 65˚C inside the thermal cycler, then spun briefly and put promptly on ice.

v. Reverse Transcriptase enzyme reaction was organised by mixing the components according to Table 3.

Table 3: Components of cDNA synthesis reaction

Sl.No.	Component	Volume
a)	Denatured sample	8 µl
b)	ProtoScript II Reaction Mix (2X)	10 µl
c)	Protoscript II Enzyme Mix (10X)	2 µl
	Total volume of cDNA synthesis reaction	20 µl

vi. The 20 µl of cDNA synthesis reactions were incubated at 42˚C for one hour inside the thermal cycler

vii. The enzyme was inactivated by incubating samples at 80 ˚C for 5 minutes inside the thermal cycler.

viii. The cDNA products were kept into the -20˚C freezer to preserve.

Semi-quantitative PCR amplification of synthesized cDNAs:

The amplification of cDNA was carried out using standard protocol of PCR adjusted to laboratory conditions with seven pairs of forward and reverse expressed sequence tag (EST) primers to the coding sequences of the genes distributed among. The panel of primers was based on the ability of the EST primers to differentiate the expression banana varieties.

The *in vitro* amplification of cDNA was carried out in a thermo-cycler using 20 µl master mixtures prepared by varying components involved in a definite fraction of the mixture (Table 4) in the case of each primer pair used in the study (Table 1). Negative and positive controls were used in amplification conducted to verify the absence of contamination and the efficiency of amplification reaction.

Table 4: Requirements for the PCR amplification

Sl.No.	Name of Reagent	Quantity of reagent
1.	Template cDNA	2 µl
2.	10X Taq Buffer with 15mM $MgCl_2$	2 µl
4.	dNTP mix (2mM of each)	2 µl
5.	Primer F (1 µM)	1 µl
	Primer R (1µM)	1 µl
6.	Taq DNA Polymerase (5u/ µl)	0.3 µl
7.	Deionized water	12 µl
	Total	**20 µl**

Table 5: Profile for relative PCR amplification of cDNA copies of the transcripts

Sl. No.	Steps	Temperature of thermal cycler	Duration
1.	Initial denaturation	95 ˚C	5 min
2.	35 cycles of		
	a) Denaturation	94 ˚C	1 min
	b) Annealing	48-60 ˚C	1 min
	c) Extension	72 ˚C	2 min
3.	Final extension	72 ˚C	7 min

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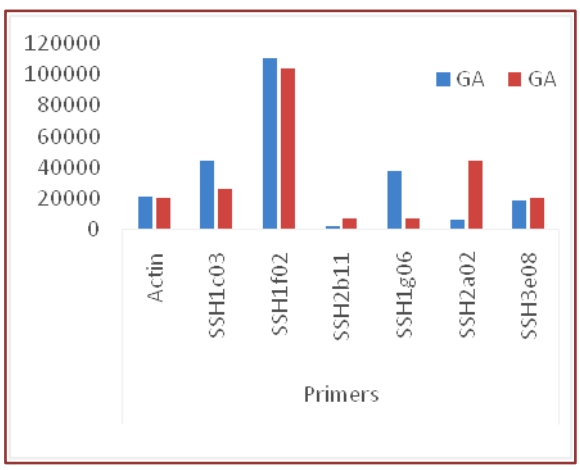
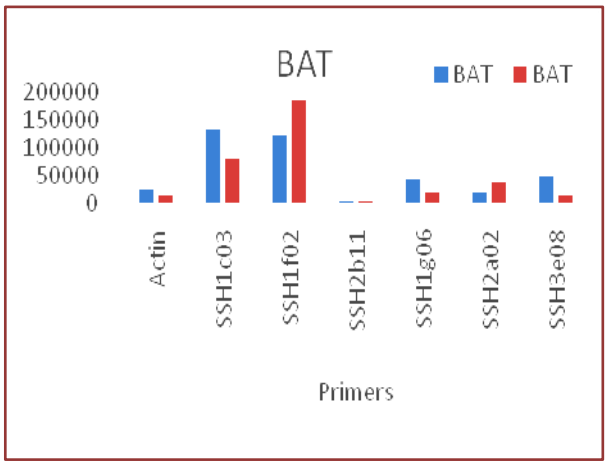
III. RESULTS AND DISCUSSION

The transcripts corresponded to the concerned genes of selected genotypes of banana were undergone to Semi-quantitative RT (Reverse Transcriptase) PCR and their cDNA copies were amplified. The expression of the genes were studied in floral and somatic (leaf) tissues of Grand Naine (Control) genotype compared to other selected four local genotypes. The expression in the form of volume intensity of the bands of amplified cDNA copies of the selected transcript were compared to the amplified cDNA copies of the transcript of the Actin (a housekeeping gene), which was used as control. Similar study was conducted to determine tissue specific expression of banana *CYCD2; 1* [12]. The expression was up-regulated maximum for SSh1c03 followed by SSh1f02, SSh1g06 and SSh3e08, while SSh2b11 down-regulated by comparing with volume intensities of amplified cDNA copies Table 6 and Figure 1. Two clones of transcriptsSSh1c03 and SSh1f02 were showed the up-regulation from five to six folds among the selected transcripts in both floral and leaf tissues for all the genotypes with respect to Actin, while one clone SSh2b11 showed the down-regulation from one to two folds. Another research conducted [11] reports that banana fruit (cv Cavendish) grown under pedoclimatical conditions undergoes changes in ethylene responsiveness between 40 (immature fruit unable to respond to ethylene), 60 and 90 DAF (days after flowering; early and late mature fruit able to respond to ethylene, respectively). In contrast to the above the BLASTn results showed that the selected subtractive suppression hybridization (SSH) clones SSh1c03, SSh1f02, SSh1g06 and SSh3e08 have similarity to zinc finger A20 and AN1 domain-containing stress-associated protein, putative nuclease HARBI1, , E3 ubiquitin-protein ligase UPL1-like and thioredoxin H1-like of banana ((*Musa acuminata* subsp. *malaccensis*) respectively. The clones SSh2a02 have similarity with linoleate 9S-lipoxygenase 4 and clone SSh2b11 have similarity with nematode resistance protein-like HSPRO2banana of banana (*Musa acuminata* subsp. *malaccensis*). All the selected genotypes not showed any significant difference among them by comparing with the control (genotype Grand Naine).

Table 6: Volume intensities of amplified cDNA copies of the transcript in different selected genotypes of banana

Sl. No.	Banana Cultivars		Primers						
			Actin	SSh1c03	SSh1f02	SSh2b11	SSh1g06	SSh2a02	SSh3e08
1.	Grand Naine (Control)	Flower	21860.6	268151.0	134287.9	3402.70	99306.2	30831.2	24060.0
		Leaf	17537.3	59355.20	132034.7	18475.5	7692.80	43557.8	10974.0
2.	BAT	Flower	24825.2	132160.0	122716.8	5481.80	43849.9	22014.2	48978.6
		Leaf	16322.2	80603.00	184270.7	5148.20	20791.8	39839.8	15358.2
3.	DRG	Flower	20969.5	73215.30	104416.9	8445.70	35379.8	56306.1	21973.9
		Leaf	23123.1	121259.2	110963.6	6035.20	3988.70	57270.6	10409.5
4.	BHM	Flower	24825.2	90850.00	157029.4	4511.80	38288.7	77307.2	34805.8
		Leaf	16322.2	43046.00	126989.0	5288.00	11379.1	45916.5	22036.0
5.	GA	Flower	20437.2	43989.00	110494.7	1994.70	37633.5	5506.60	18355.1
		Leaf	19731.6	26117.50	104042.6	6444.20	6604.00	44496.0	19854.9





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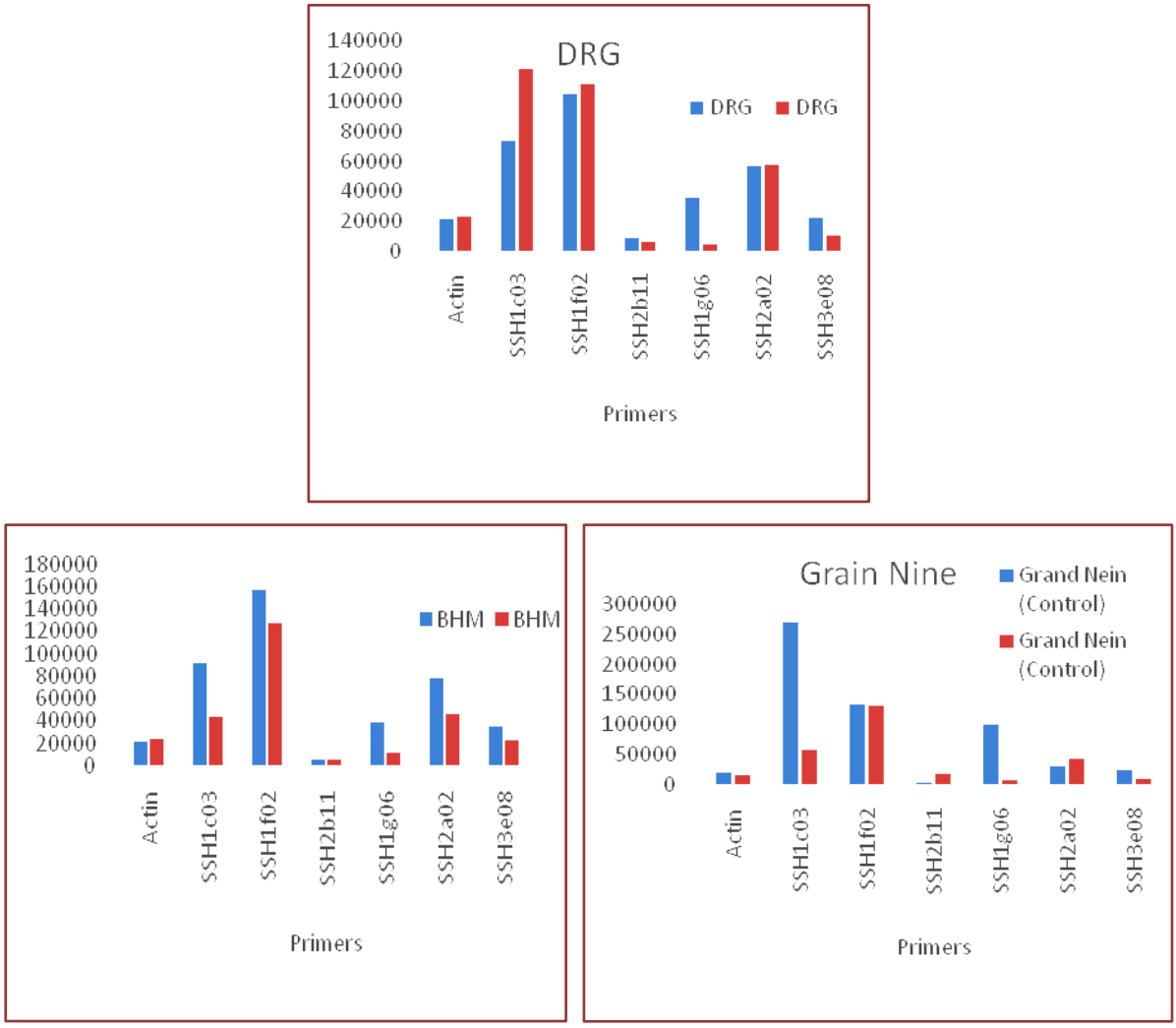

Fig 1: RT-PCR based transcript expression analysis of yield related genes

IV. CONCLUSION

- The genes belongs to fourcDNA clones SSh1c03, SSh1f02, SSh1g06 and SSh3e08 among the selected transcripts were showed up-regulation in floral and leaf tissues of all the selected genotypes of banana may contribute to reproductive growth and yield.
- The studied genotypes of banana did not show any significant difference in their expression profile of the cDNA copies of selected transcripts.

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