Chemical Science Review and Letters ISSN 2278-6783

www.chesci.com Research Article

Elucidation of Single Nucleotide Polymorphisms in Starch Synthase, a Key Starch Metabolic Gene of *Musa*

Muhilan Mahendhiran* and Hoshiyar Singh

Faculty of Agriculture, Vivekananda Global University (VGU), Sector 36, NRI Colony Rd, V I T Campus, Jagatpura, Jaipur - 303012, Rajasthan, India

Abstract

Starch is a very important energy source for the human diet. In higher plants, the starch metabolism is under the control of a regulatory pathway. In this study, Starch synthase (SS) enzyme was studied, and this enzyme plays a major role in starch metabolism. The investigation has been done in the Single Nucleotide Polymorphisms (SNPs) and the variations in the partial sequences of starch metabolism gene SS. To understand the basis of phenotypic differences at the molecular level, the genomic DNA was isolated from 10 *Musa* accessions and amplicons of SS were cloned and sequenced representating wild ancestors, dessert, plantain and cooking/beer bananas. Amplicons of the length of 831 bp of SS were obtained by PCR reactions subsequently purified, and the gene fragments cloned and then sequenced. Cloned sequences for SS (109 clones) enzymes were obtained and analyzed. The bioinformatics analyses revealed that SNPs/Indels could be identified in both coding and non-coding regions of the gene fragments. A total of 73 SNPs giving 34 haplotypes were found in two SS alleles, from that 19 were found in exons and 54 in introns, which were correlated to the type of variants in this enzyme. The phylogenetic analysis showed that the relationships between the haplotypes and the out-groups (externals) of monocots and dicot species. These results concluded that, there is more than one member in *Musa* and SS gene families.

Keywords: Starch synthase, Banana, SNPs, Bioinformatics, Haplotypes, and *Musa*

***Correspondence**
Author: Muhilan Mahendhiran
Email: m.muhilan@vgu.ac.in

Introduction

Starch synthase

Starch synthase or SS (EC 2.4.1.21), catalyzes the transfer of a glucosyl unit from ADP Glucose to a growing polymer chain through α-(1-4) glycoside bond in the starch metabolism [1, 2]. ADP glucose is the product outcome of AGPase enzyme and it is polymerized into α (1, 4) linked chains by multiple isoforms of starch synthase.

Various SS isoforms have been identified through gene sequences in several plant genomes. In higher plants, there are 10 SS isoforms separated into five classes (GBSS, SSI, SSII SSIII and SSIV); two granule-bound starch synthase (GBSS) isoforms (GBSSI and GBSSII) in the GBSS type, one SSI isoform in the SSI type, three SSII isoforms (SSIIaor SSII-3), SSIIb (SSII-2) and SSIIc (SSII-1) in the SSII type, two SSII isoforms (SSIIIa or SSIII-2) and SSIIIb (SSIII-1) in the SSIII type, and two SSIV isoforms (SSIVa or SSIV-1) and (SSIVb or SSIV-2) in the SSIV type [3]. The isoforms within each of the major classes of SS genes are highly conserved in higher plants through the dicots and monocots [4]. The objective of this work was to investigate the Single Nucleotide Polymorphisms (SNPs) in these sequences in both coding and non-coding regions and analyse their evolutionary relationships. Further studies of these genes may provide valuable insights and outputs in bananas and plantains.

Materials and Methods

Sample collection

Young cigar leaves from different banana and plantain genotypes were collected from the INIFAP (Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias) research farm at Uxmal, Yucatan, Mexico. Ten representative accessiones were selected for getting amplicons from Starch synthase in starch metabolism including dessert, plantains, cooking bananas. Tissues were disinfected before further extraction of DNA from the plant cigar leaves.

DNA extraction

Plants used for this study were obtained from the Bioversity´s International TransitCentre. Total Genomic DNA from the ten *Musa* accessions was extracted from 100 mg of frozen young cigar leaves following the protocol from [5] with some modifications. DNA quality and concentration were determined using a spectrophotometer according to Stulnig and Amberger, 1994 [6].

Primer designing

Specific primer was designed for Starch synthase, associated with starch biosynthesis. In order to obtain the homologous sequences in banana, sequence information was obtained from the closely related monocotyledonous plants such as maize, wheat, rice and other species. The amino acid sequences retrived from the databases and check out the consensus sequences to make the specific primer for Starch synthase. The highly conserved regions were selected from the pairwise alignments and used to create a multiple sequence FASTA file. Subsequently the sequences were aligned using the software Bio-edit (12.5 version) and this software shows the regions of conserved sequences. After that these conserved regions were analyzed to identify identical sequences shared by homologous genes.

A pair of specific primers was designed for the gene encoding SS. Protein sequence information was obtained from the *Musa acuminata* accession (NCBI-GenBank ABF69970.1) corresponding to the cDNA. The homologous sequences of 10 closely related plant accessions (including *Musa)* for primer design are listed below: AAC14014.1- *Zea mays*; AAF87999.1- *Triticum aestivum*; AAF88000.1-Aegilops tauschii; AAS88893.1- *Ostreococcus tauri;* AAY42381.1- *Chlamydomonas reinhardtii*; ABF69970.1- *Musa acuminata*; CAA64173.1- *Solanum tuberosum*; CAB40374.1- *Vigna unguiculata*;NP_172637.1- *Arabidopsis thaliana*; NP_001053933- *Oryza sativa* Japonica group. The sequence of starch synthase, the forward primer sequence was 5'- ATGGAGCTGATTCTGCTGATGTGG-3' and the reverse primer sequence was 5'- GAGTTCCATATAGTCCAAAGCCG-3´ respectively. The optimal (T_m) annealing temperature for SS primer sets was 55°C and the expected size of the amplicon of this primer set was 831 bp.

Results

Amplification of SS fragments

The designed primers amplified a fragment of 831 bp of the SS, which extends from exons 6 to 7 of *Musa acuminata* (NCBI-GenBank ABF69970.1). The amplicons were isolated and purified for the next transformation and cloning experiments.

Analysis of starch synthase gene fragments

In order to analyse the variation from SS gene fragments at the molecular level, 6 *Musa* cultivars and 4 *Musa* wild ancestors were selected as representatives in this study (Table 1.1). For the amplification of SS, the designed primers amplified a fragment of 831 bp which extends from exon 6 to 7 of AFF69970.1, which constitutes two exon regions and one intron from the amplified DNA (**Figure 1**).

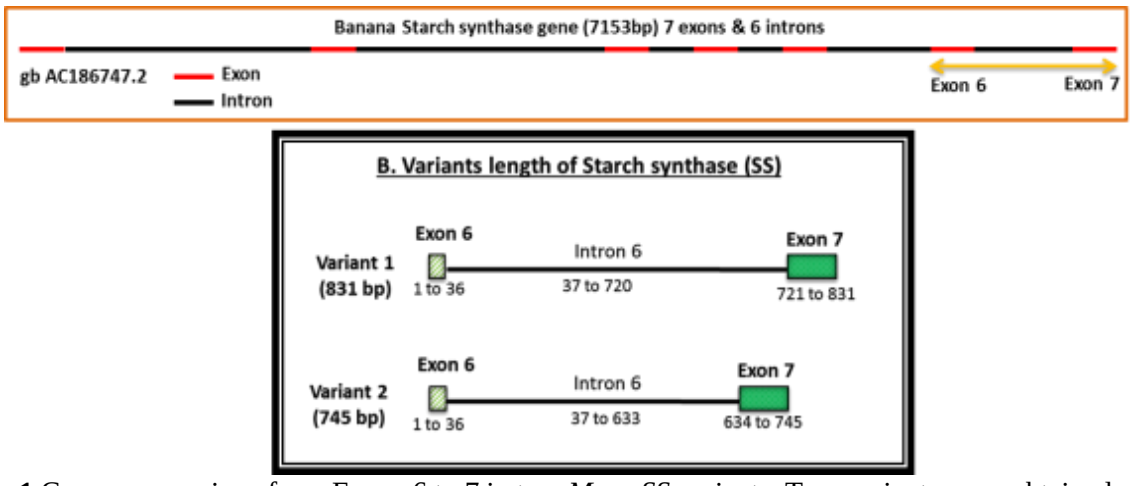


Figure 1 Consensus regions from Exons 6 to 7 in two *Musa SS* variants. Two variants were obtained and the consensus is shown in the figure

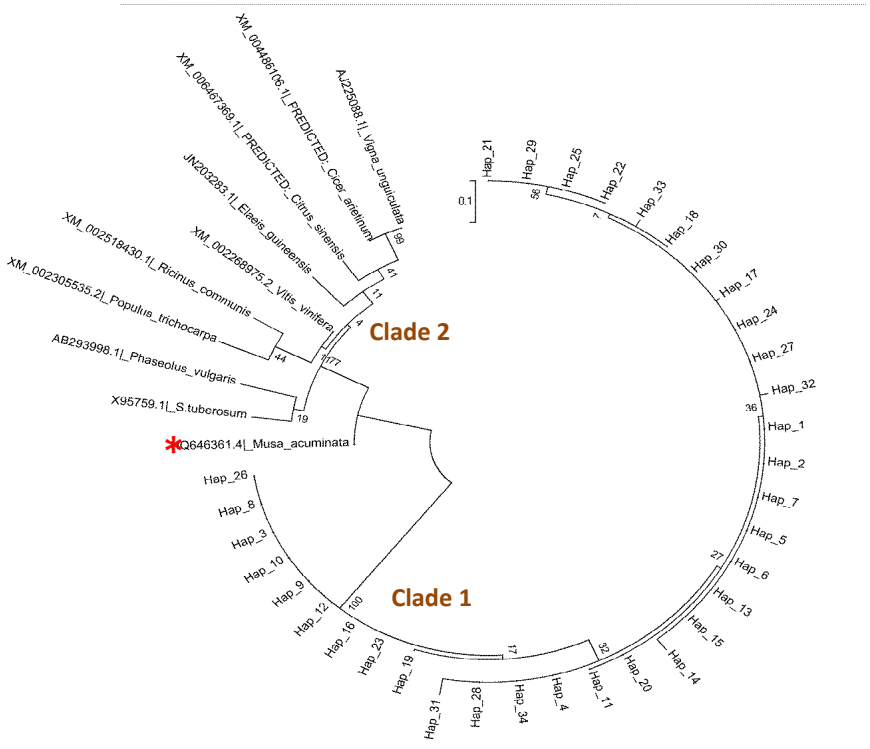
Table 1 Sequenced Clones & Variants distribution of SS gene fragments within 10 *Musa* accessions. The sequenced clones were distributed into 2 variants for SS enzyme

No	Accession Name	*ITC	Genome	Starch Synthase (SS)		
				Variant 1 (No. of clones)	Variant 2 (No. of clones)	Total No. of clones
1	*M. a.* banksii	0623	AA	8	3	11
2	*M. a.* malaccensis	1345	AA	10	2	12
3	*M. a.* cv. Pisang lilin	1121	AA	8	2	10
4	Safet velchi	0245	AB	10	0	10
5	*Musa balbisiana*	-	BB	9	3	12
6	Gros Michel	0484	AAA	9	1	10
7	Mbwzruimbe	0084	AAA	7	3	10
8	Bluggoe	0767	ABB	7	3	10
9	Popolou	0335	AAB	7	4	11
10	*Musa beccari*	1070	-	8	5	13
	Total clones	-	-	83	26	109

*ITC: International Transit Centre, Leuven, Belgium

Phylogenetic analysis and haplotype diversity of SS

One hundred and nine sequences were analyzed in the exon regions of SS, and 34haplotypes were obtained for the phylogenetic analysis (**Figure 2**). In SS, some sequences either have the 6th exon or the 7th exon of the amplicon. In order to find the SNPs based variations in the intron region of SS, the non-coding sequences were analyzed and a dendrogram was created. In order to avoid redundancy, haplotypes were created from the consensus sequences; analysis with DnaSP 5.0 detected 34 haplotypes out of 109 sequences in SS 6 and 7 exon regions for 10 *Musa* accessions from their nucleotide alignments. Ten outgroup plant species of monocots and dicots were included in the phylogenetic tree and they formed 2 clades (Figure 2).



* *Musa acuminata* sucrose synthase mRNA partial cds

Figure 2 Neighbor-joining tree showing the phylogenetic position of 34 haplotypes of SS sequences with outgroup plant species (both monocots and dicots). Scale bar represents 0.1 nucleotide substitution per site (1000 Bootstrap replications was used in the phylogeny test). Jukes-Cantor (JC) parameter model was used with 95% partial deletion data treatment in this tree. The external (monocots and dicots) plant species were used in thisphylogenetic tree apart from *Musa* (HQ646361.4: *Musa_acuminata* AAA starch synthase gene).

Clade 1 consists of all the 34 haplotypes of *Musa* accessions which were analysed in this study. Within this clade 1 there are 2 subclades: - subclade 2 contains haplotypes 3, 8, 9, 10, 12, 16, 19, 23 and 26; all the other haplotypes belong to subclade 1 in clade 1. In clade 2 contains outgroups including monocots plant *Elaeis guineensis*; and dicots such as *Solanum tuberosum*, *Phaseolus vulgaris*, *Populus trichocarpa*, *Ricinus communis*, *Vitis vinifera*, *Citrus sinensis*, *Cicer arietinum* and *Vigna unguiculata*. The dendrogram indicates the sequence arrangements of SS intron regions.

Discussion

At the time of designing the primers for SS obtain amplicons, the *Musa* genome was still not accessible and only limited sequence data from BAC clones and ESTs for *Musa* was available; so the primers were designed on conserved regions of homologous sequencesof related plant species. The use of multiple sequence alignments of the SS sequence withhomologous ones allowed the identification of the most conserved regions. Primers were designed over those regions but only from the existing *Musa* sequence (AFF69970.1). Although not-degenerate, the actual identity percentage of these primers to the *Musa* target genes was unknown, requiring a lot of testing to find optimal amplification conditions (primer/DNA ratio concentrations and Tm). Instead of directly sequencing the amplified PCR products, the extra steps of purifying and cloning the fragments were carried out. The reason for this was due to the fact that these enzymes belong to gene families containing more than one member.Without information about the exact number of copies or paralogous sequences for each enzyme, since the primers were designed on the most conserved regions, the amplification products are most likely mixes of fragments originating from each of the copies. For this reason, we were able to recover two different variants (probably alleles) forSS enzymes.

Previous studies in potato SS revealed, three isoforms [7]. From our data analysis, two alleles of SS also have been identified from chromosome 5, although harbor SS two chromosomes (5 and 11) in both the *Musa acuminata* and the *Musa balbisiana* genome database [8, 9]. Variant 1 (97% sequence identity) and variant 2 (90% sequence identity) of SS matched with chromosome 5, and these variants were considered as alleles of SS enzyme because both are locatedin same chromosome. It might be possible to detect further isoforms or alleles in studieson a large number of clones or accessions. [10] stated that GBSS showed variations of DNA polymorphisms in wild and cultivated *Musa* accessions.

SNPs molecular markers allow the analysis of the specific parts or selected genes of the genome [11]. Our result with the intronic variants of SS suggests that they are alleles of the same chromosome (same place in chromosome 5). This study reveals the alleles of SS gene fragments in starch metabolism in bananas and plantains. Genetic variation is the initial aspect of the breeding outcome, but in a vegetative propagated crop such as banana it is difficult, because of its sterility and polyploidy. Variation can be induced by hybridization and mutation [12]. Marker assisted selection may assist breeders in more efficiently selecting for improved breeding material or varieties.

Genomic research in banana has advanced in the last decades [13], and the banana genome A and B, genome sequences have been released [8, 9]. The human population will be estimated to reach up to 10 billion at 2050 AD [14]. New technologies may assist to increase the quantity of food with the same or less land area. *Musa* could be a model plant for polyploidy plant species in genomic aspects [11]. Apart from the human diet, industrial starch such as that from cassava (tapioca) and high amylopectin potato are used for industrial applications; banana starch could also be used at the industrial level. [15] stated that, in maize the *wx1* locus amylose and created high amylopectin maize by modern breeding. The variation of starch loci in maize was developed for use in creating high amylopectin cultivars for industrial purposes.

Conclusion

Marker assisted selection may assist breeders in more efficiently selecting for improved breeding material or varieties. In *Musa* it may also be used to increase the starch yield by the mutation breeding. The transcripts will be obtained and analyzed to find out the expression of the key metabolic starch genes. Further studies of these genes will lead to valuable insights of starch metabolism in bananas and plantains.

Acknowledgements

The authors would like to thank Vivekananda Global University, Jaipur, Rajasthan, India for their kind and continuous support.

References

[1] Davey, M.W., R. Gudimella., J.A. Harikrishna., L.W. Sin., N. Khalid and J. keulemans. A draft Musa balbisiana genome sequence for molecular genetics in polyploidy, inter- and intra-specif Musa hybrids. BMC Genomics, 2013: 14, 683.

[2] Dellaporta, S.L., J. Wood and J.B. Hicks. A plant DNA mini-preparation: version II, Plant Mol.Biol.Rep, 1983: 1, 19-21.

[3] D´Hont, A., Denoeud., J.M. Aury., F.C. Baurens., F. Carreel., O. Garsmeur., B. Noel., S. Bocs., G. Droc., M. Rouard., C. Da Silva., K. Jabbari., C. Cardi., J. Poulain., M. Souguet., K. Labadie., C. Jourda., J. Lengelle., M. Rodier-Goud., A. Alberti., M. Bernard., M. Correa., S. Ayyampalayam., M.R. Mckain., J. Leebens-Mack., D. Burgess., M. Freeling., AMD. Mbeguie., M. Chabannes., T. Wicker., O. Panaud., J. Barbosa., E. Hribova., P. Heslop-Harrison., R. Habas., R. Rivallan., P. Francois., C. Poiron., A. Kilian., D. Burthia., C. Jenny., F. Bakry., S. Brown., V. Guignon., G. Kema., M. Dita., C. Waalwijk., S. Joseph., A. Dievart., O. Jaillon., J. Leclercq., X. Argout., E. Lyons., A. Almeida., M. Jeridi., J. Dolezel., N. Roux., AM. Risterucci., J. Weissenbach., M. Ruiz., JC. Glaszmann., F. Quetier., N. Yahiaoui and P. Wincker. The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. Nature, 2012, 488: 213-217.

[4] Hirose, T and T. Terao. A comprehensive expression analysis of the starch synthase gene family in rice (Oryza sativa L.) Planta, 2004, 220: 9-16.

[5] Larsson, C.T., P. Hfvander., J. Khoshnoodi., L. Rask and H. Larsson. Three isoforms of starch synthase and two isoforms of branching enzymes are present in potato tuber starch. Plant Sci, 1996, 117: 9-16.

[6] Li, L F., H.Y. Wang., C. Zhang., X.F. Wang., F.X. Shi., W.N. Chen and X.J. Ge. Origins and Domestication of Cultivated Banana Inferred from Chloroplast and Nuclear Genes. Plos One, 2013, 8 (11): 1-11.

[7] Li, Z., G. Mouille., B. Kosar-Hashemi., S. Rahman., B. Clarke., K.R. Gale., R. Appels and M.K. Morell. The Structure and Expression of the Wheat Starch Synthase III Gene. Motifs in the Expressed Gene Define the Lineage of the Starch Synthase III Gene Family. Plant Physiology, 2000, 123: 613-624.

[8] Ortiz R, Swennen R. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol Adv. 2014, 32(1): 158-69.

[9] Pillay, M., G, Ude and C. Kole. Genetics, Genomics and Breeding of Bananas. CRC Press, Science Publishers. 2012, Chapter 1: p. 1-33.

[10] Pillay, M and A. Tenkouano. Banana Breeding Progress and Challenges. 2011, Chapter 17, p. 321-350. CRC Press, Taylor and Francis Group, LLC.

[11] Jain, S.M and R. Swennen. Banana Improvement: Cellular, Molecular Biology, and Induced Mutations. Science Publishers, Inc. Enfield (NH), USA. 2004, Chapter 3: pp. 23-32.

[12] Stulnig, T.M and A. Amberger. Exposing contaminating phenol in nucleic acid preparations. Bio Techniques, 1994: 16, 403-404.

[13] Tetlow, I.J., M.K. Morell and M.J. Emes. Recent developments in understanding the regulation of starch metabolism in higher plants. Journal of Experimental Botany, 2004, 55 (406): 2131-2145.

[14] Whitt, S.R., L.M. Wilson., M.I. Tenaillonn., B.S. Gaut and IV.E.S. Buckler. Genetic diversity and selection in the maize starch pathway. PNAS, 2002, 99 (20): 12959-12962.

[15] Zhang, X., N. Szydlowski., D. Delvalle., C. D´Hulst., M.G. James and M.A. Myers. Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in Arabidopsis. BMC Plant Biology, 2008: 8(96), 1-18.

Publication History
Received 07.06.2024
Revised 18.06.2024
Accepted 25.06.2024
Online 30.06.2024