Genome-wide DNA polymorphisms in Kavuni, a traditional rice cultivar with nutritional and therapeutic properties

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Genome-wide DNA polymorphisms in Kavuni, a traditional rice cultivar with nutritional and therapeutic properties

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Abstract

Although rice genome was sequenced in the year 2002, efforts in resequencing the large number of available accessions, landraces, traditional cultivars, and improved varieties of this important food crop are limited. We have initiated resequencing of the traditional cultivars from India. Kavuni is an important traditional rice cultivar from South India that attracts premium price for its nutritional and therapeutic properties. Whole genome sequencing of Kavuni using Illumina platform and SNPs analysis using Nipponbare reference genome identified 1,150,711 SNPs of which 377,381 SNPs were located in the genic regions. Non-synonymous SNPs (62,708) were distributed in 19,251 genes, and their number varied between 1 and 115 per gene. Large-effect DNA polymorphisms (7,769) were present in 3,475 genes. Pathway mapping of these polymorphisms revealed the involvement of genes related to carbohydrate metabolism, translation, protein-folding and cell death. Analysis of the starch biosynthesis related genes revealed that the granule-bound starch synthase I gene had T/G SNPs at the first intron/exon junction and a two-nucleotide combination, which were reported to favour high amylose content and low glycemic index. The present study provided a valuable genomics resource to study the rice varieties with nutritional and medicinal properties.

Keyword: Kavuni, SNPs, medicinal rice, whole genome sequencing

Introduction

Rice is a major staple food for half of the world's population providing up to 75% of the daily dietary energy intake (Khush, 2005). Besides commercial high yielding rice varieties, a large number of naturally occurring landraces and traditional varieties are cultivated in several parts of the world. Kavuni is an important traditional rice cultivar from South India, which is cultivated for its nutritional and therapeutic properties. The grains of Kavuni are black in colour, and rich in dietary fibre, carotenoids, minerals, and anti-oxidants. Grains of Kavuni contain 95% higher lutein compared to the improved rice varieties such as IR 64, CO 50 and White Ponni (Valarmathi et al. 2014). Lutein is predominantly found in the macular region of the retina, and consumption of lutein-rich food greatly reduces the risk of developing age-related macular degeneration (AMD) that leads to blindness (SanGiovanni and Emily, 2007). Methanol extract of Kavuni grains possess alpha-amylase and alpha-glucosidase inhibitory compounds to be useful in the management of type 2 diabetes (Valarmathi et al. 2014). These nutritional and therapeutic properties of Kavuni attracts premium price for farmers. However, the photosensitive nature and the low yield potential of Kavuni limits its large scale cultivation. Therefore, efforts are being made to understand the genetic basis of the desirable traits of Kavuni for introgression into improved varieties through marker assisted breeding. Here we report, for the first time, a genome-wide DNA polymorphism study in Kavuni using Illumina's pair-end sequencing. Detailed analysis of the starch biosynthesis related genes (SSRGs) revealed the presence of SNPs that are related to high amylose content and low glycemic index.

Materials and Methods

Seeds of *Oryza sativa* L. cv. Kavuni were collected from Kulithalai, Karur district, Tamil Nadu, India. The plants were grown in greenhouse and genomic DNA was extracted from the

young leaves of a single plant using cetyl trimethyl ammonium bromide (CTAB) method (Murray & Thompson, 1980). A paired-end genomic library was prepared according to the manufacturer's protocol (Illumina Inc., USA). Adapters were ligated to the ends of the DNA, and DNA clusters were prepared using cBOT and TruSeq PE Cluster kit v3-cBot-HS (Illumina Inc., USA). The DNA clusters were sequenced using Illumina's paired-end sequencing technology on Hiseq 2500 systems (Illumina Inc., USA) to generate 101bp paired-end reads. The reads were extracted as paired-end reads in FASTQ format for further downstream analysis. The adapters in the paired-end reads were trimmed using 'cutadapt' (<https://code.google.com/p/cutadapt/>). The trimmed reads were filtered using sickle master (<https://github.com/najoshi/sickle>) by retaining the bases with minimum Phred quality score of 30. The filtered paired-end reads were mapped to the *Oryza sativa* L. cv. Nipponbare reference genome (Os-Nipponbare-Reference-IRGSP-1.0) (Kawahara et al. 2013) using Burrows Wheeler alignment (BWA) tool with default parameters (Li and Durbin, 2009). The aligned reads in the SAM file were sorted using SortSam of Picard tool V1.118, and the sorted SAM file was converted to BAM file for variant calling using SAMtools (v0.1.19). The 'mpileup' of SAMtools was used for the identification of SNPs and InDel (Li et al. 2009). The identified DNA polymorphisms were filtered using the following stringent criteria, (1) number of reads per base between 5 and 75, (2) base quality ≥ 30 , (3) mapping quality ≥ 60 , (4) variant quality ≥ 90 , and (5) distance of adjacent variant ≥ 5 . Genomic distribution of DNA polymorphisms was determined by calculating their frequency in 100 kb interval on each chromosome. The chromosome regions were defined as SNP rich regions if SNPs/kb > 5 , and SNP poor regions if SNPs/kb < 0.1 . SnpEff V3.6 tool (Cingolani et al. 2012) was used for annotation of the filtered DNA polymorphisms with rice7 gene model database (http://sourceforge.net/projects/snpeff/files/databases/v3_6/snpEff_v3_6_rice7.zip). Genes harbouring DNA polymorphisms that affected the translation

of the encoded proteins were identified in the Kyoto Encyclopaedia of Genes and Genomes database (KEGG; <http://www.genome.jp/kegg>) and mapped to pathways. Further, gene ontology enrichment analysis was performed using BiNGO tool with p-value cut-off of ≤ 0.05 (Maere et al. 2005). The unmapped reads were extracted into a BAM file using SAMtools and the reads in the BAM file were converted to FASTQ format using BAMtools V2.3.0. The unmapped FASTQ reads were assembled into contigs by *de novo* using Velvet V1.2.10 (Zerbino and Birney, 2008). The assembly was optimized using different Kmer sizes, and all the contigs were used for further analysis. The *de novo* assembled contigs were subjected to Gene Ontology (GO) analysis using TAIR (<https://www.arabidopsis.org/tools/bulk/go/index.jsp>).

Results and Discussion

Whole genome sequencing of Kavuni rice variety yielded 42×10^6 paired-end reads, which were reference assembled to *Oryza sativa* L. cv. Nipponbare reference genome (IRGSP1.0.) (Fig. S1). In total, 91.0% of the reads were mapped to the reference genome, which included 77.7% uniquely mapped reads that covered 83.3% of the genome (Table S1). The BAM files generated for this study were submitted to the sequence read archive (SRA) at NCBI with the accession number PRJNA296443. Analysis of the uniquely mapped reads under stringent filtering conditions showed the presence of 1,150,711 SNPs and 104,984 InDels. The average number of DNA polymorphisms per chromosome was 104,641 with chromosomes 1 and 9 having the highest (151,294) and lowest (77,029) number of polymorphisms, respectively. The frequency of DNA polymorphisms were determined for every 100 kb of the genome (Fig. S2), and 344 SNP rich regions and 164 SNP poor regions were identified (Table S2). All the chromosomes were found to have SNP rich and SNP poor regions, and the longest SNP rich region was identified on chromosome 11 between 18.2 and 18.5 Mb (0.4

Mb). The longest SNP poor region was identified on chromosome 5 between 9.6 and 13.0 Mb (3.4Mb) in a similar region reported in other rice varieties (Hu et al. 2014; Jain et al. 2014; Wang et al. 2009). Such contiguous regions with abnormally low SNPs may represent adaptive gene selection during domestication and crop improvement of rice (Krishnan et al. 2014). A higher frequency of transition over transversion SNPs were observed in Kavuni with an average transition to transversion SNP ratio of 2.2. Such transition bias was previously reported in rice, and the ratio varied between 2.0 and 2.53 (Mace et al. 2013; Xu et al. 2012).

Annotation of DNA polymorphisms revealed the presence of 377,381 SNPs in the genic regions of the Kavuni genome (Table 1). Coding regions contained 107,938 SNPs, which included 62,708 non-synonymous SNPs that were distributed in 19,251 genes. The number of non-synonymous SNPs per gene varied between 1 and 115. The ratio between non-synonymous to synonymous SNP in the Kavuni genome was 1.39, which was reported to vary between 1.14 and 1.39 in other rice varieties (Arai-Kichise et al. 2011; Jeong et al. 2013; Rathinasabapathi et al. 2015; Subbaiyan et al. 2012). The Kavuni genome contained 2,451 large effect SNPs (loss of start codon, introduction of premature stop codon, and loss of stop codon) and 5,318 frame shift mutations in 3,475 genes that may affect the protein functions. KEGG pathway mapping identified 317 genes that were related to different biological functions such as signal transduction pathway, carbohydrate metabolism, protein folding and cell death (Fig. S3). Gene ontology (GO) enrichment analysis showed that the genes were enriched for the biological processes such as oligo-peptide transport, amino acid phosphorylation and localization. At the molecular function level, the genes for serine/threonine kinase activity, ATP binding, and metallo-endopeptidase activity were found to be enriched (Fig. S4).

High amylose content in rice grains result in low glycemic index (GI), which is desirable for the management of postprandial hyperglycemia in diabetes patients (Fitzgerald et al. 2011; Silva, et al. 2015). Therefore, starch biosynthesis pathway that favours amylose over amylopectin accumulation is a desirable health benefit. Starch biosynthesis in rice involves seven enzymes encoded by 18 starch biosynthesis related genes (SSRGs) (Tian et al. 2009). The SSRGs in the Kavuni genome contained 355 SNPs including 29 non-synonymous SNPs and 45 InDels (Table 2). The granule-bound starch synthase I gene (GBSSI) in particular contained T/G non-coding SNPs at the intron/exon 1 junction site, and a two-nucleotide combination ('A' at position 2,386 and 'C' at position 3,378), which were reported to be associated with highest amylose content in rice varieties and breeding populations (Kharabian Masouleh, 2013; Kharabian-Masouleh et al. 2011; Larkin and Park, 1999; Rathinasabapathi et al. 2015). A significant portion of the *indica* genome does not map to the *japonica* reference genome due to the natural genetic divergence between the two sub-species (Jain et al. 2014; Subbaiyan et al. 2012; Tang et al. 2006). Therefore, the unmapped reads of Kavuni (9.07%) were *de novo* assembled into contigs. The results showed 797 contigs that ranged in size between 120 and 6,188 bp. These contigs were subjected to annotation and Gene Ontology (GO) analysis in terms of cellular, biological and molecular functions. More number of contigs were related to transferase activity, macromolecular binding, and kinase activity (molecular processes) or cellular and metabolic processes (biological processes) or nucleus, cytoplasmic, intracellular and membrane components (cellular components) (Fig. S5).

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Table 1: Annotation of SNPs and InDels that were identified in Kavuni.

	SNPs	InDels
Total	1,150,711	104,984
Intergenic	773,330	61,690
Genic	377,381	49,294
Intron and regulatory sequences	223,886	21,483
UTRs	45,557	18,229
Coding region	107,938	3,582
Synonymous SNPs	45,230	
Non-synonymous SNPs	62,708	

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Table 2: Details of the SNPs and InDels that were identified in the starch biosynthesis related genes (SSRG) of Kavuni.

S.No	Gene Name	Non-Coding SNPs	Non-Synonymous SNPs	Synonymous SNPs	Non-coding InDels	Coding InDels
1	ADP- glucose pyrophosphorylase (small unit)	16	0	3	1	0
2	Alpha 1,4- glucanphosphorylase	0	0	0	1	0
3	Glucose 6-phosphate-translocator	10	1	3	3	0
4	Granule-bound starch synthase I	1	0	1	1	0
5	Granule-bound starch synthase II	67	11	2	7	0
6	Starch synthase I	60	2	7	5	0
7	Starch synthase IIa	17	1	3	1	0
8	Starch synthase IIb	8	2	1	3	0
9	Starch synthase IIIa	19	2	4	3	0
10	Starch synthase IIIb	8	4	1	1	0
11	Starch synthase IVa	9	2	1	1	0
12	Starch synthase IVb	17	0	4	1	0
13	Branching enzyme I	9	0	1	2	0
14	Branching enzyme IIa	0	0	0	0	0
15	Branching enzyme IIb	24	2	3	3	0
16	Debranching enzyme -isoamylase 1	11	1	3	1	0
17	Debranching enzyme -isoamylase 2	1	1	1	0	0
18	Debranching enzyme -Pullulanase	214	0	22	11	0
Total/ average		266	29	60	45	0

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Fig. S1. Classification of the Kavuni reads mapped on to the Nipponbare reference genome. The total number reads in the centre circle. Number of reads mapped on the nuclear genome and unmapped reads in the middle circle. The outer circle represents the uniquely and multiple mapped reads.

Fig. S2. Frequency of SNPs detected in Kavuni in each chromosome. The x-axis represented the physical distance along each chromosome, split into 100 kb windows. The total size of each chromosome was shown in brackets. The y-axis indicated the number of SNPs. The total number of SNPs in each chromosome was mentioned.

Fig. S3. Pathway mapping of large effect DNA polymorphism in rice using Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

A)

B)

Fig. S4. Analysis of gene ontology term enrichment of the genes detected with large-effect DNA polymorphisms. Node size is proportional to the number of transcripts in each category and colours shaded according to the significance level (white – no significant difference; Yellow scale – significantly enriched). A) Biological process B) Molecular function.

Fig. S5. Functional categorization of the contigs assembled from the unmapped reads of Kavuni into gene ontology (GO) terms: biological processes, cellular components, molecular functions.

Table S1: Mapping and coverage of the reads from Kavuni to the Nipponbare reference genome IRGSP1.0

Chromosome	Nipponbare genome (bp)	Uniquely Mapped Reads	Uniquely covered bases	Coverage (%)
1	43270923	3440333	37422819	86.5
2	35937250	2919632	31416258	87.4
3	36413819	3054972	32789741	90.0
4	35502694	2710415	28335645	79.8
5	29958434	2491118	26353056	88.0
6	31248787	2454017	25798844	82.6
7	29697621	2278588	24296820	81.8
8	28443022	2282285	23599317	83.0
9	23012720	1793538	19216723	83.5
10	23207287	2022948	19042193	82.1
11	29021106	2077217	22352387	77.0
12	27531856	2134555	21306714	77.4
Total/Avg.	373245519	29659618	311930517	83.3

Table S2: Number and densities of SNPs and InDels in the genome of Kavuni.

Chromosome	SNPs		InDels	
	Nos.	Density	Nos.	Density
1	138,240	318.8	13,054	29.9
2	118,921	329.8	11,195	30.8
3	114,013	312.0	11,058	30.0
4	91,143	255.6	7,721	21.5
5	79,421	265.2	7,550	25.1
6	99,483	317.4	9,103	28.8
7	93,812	315.4	8,677	28.9
8	87,212	305.6	8,018	27.9
9	70,845	307.5	6,184	26.7
10	78,721	338.7	6,878	29.4
11	100,056	343.1	8,597	29.2
12	78,844	285.2	6,949	24.9
Total/Avg.	1,150,711	307.9	104,984	27.7