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In Silico Identification and Characterization of Fruit-Trait-Related Genic-SSRs in Jackfruit

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Abstract

Jackfruit (*Artocarpus heterophyllus*), an economically valuable and nutritionally important fruit, contains various health-promoting ingredients that could supplement the nutritional needs of the world's rapidly growing population. Jackfruits are well-known for their varying fruit morphology, but little is known about their genetic and molecular basis. We identified 273,577 perfect SSRs using the jackfruit reference genome sequence. Out of 273,577 SSRs, 19,934 were genic, whereas 253,643 were non-genic. Annotation of the SSR-containing genes revealed that 10,445 and 9,489 SSRs were present in the annotated and non-annotated gene sequences. Out of 10,445 SSRs in annotated gene sequences, 683 were present in fruit-trait-related genes. The fruit-trait-related genic-SSRs identified in the present study may serve as functional markers for specific fruit-related traits.

***Key words*:** Fruit shape, Transcription factor, Fruit size, Jackfruit, SSRs, Annotation

Jackfruit (*Artocarpus heterophyllus*) is a tropical climacteric fruit tree often cultivated in home gardens [1]. It is an underused fruit since most fruits are wasted owing to ignorance, lack of post-harvest technologies, and supply chain system flaws. Jackfruit is rich in vitamins, minerals, proteins, and carbohydrates [2-5]. Numerous medical applications exist for jackfruit, including as an antioxidant, anti-inflammatory, antibacterial, anti-cancer, and anti-fungal agent. Fruit is the principal economic part of the jackfruit tree, with a huge variability in its color, shape, and size. These variations may be attributed to genetic, climatic, soil conditions, etc. Fruit size, shape, and color are the most visible manifestations of quality traits. They have become one of the criteria used by breeders to select high-fruit-quality fruit cultivars. Jackfruit possesses various medicinal properties, making it a promising candidate for numerous medical applications. It has been reported to exhibit antioxidant, anti-inflammatory, antibacterial, anti-cancer, and anti-fungal activities. These properties make it a valuable resource in traditional medicine and also hold potential for further exploration in modern pharmacology [6]. The economic significance of jackfruit primarily lies in its fruit, which constitutes the principal economic part of the tree. The fruit exhibits considerable variability in terms of color, shape, and size, influenced by factors such as genetics, climate, and soil conditions. These variations serve as important indicators of fruit quality and are often considered by breeders in the selection of high-quality fruit cultivars. Efforts to enhance the utilization and commercialization of jackfruit involve addressing challenges in production, post-harvest handling, and marketing [7]. Adoption of appropriate post-harvest technologies, education on the nutritional and medicinal benefits of jackfruit, and improvements in supply chain systems can all contribute to unlocking its full potential as a valuable crop. Moreover, continued research and breeding efforts aimed at enhancing fruit quality and addressing specific consumer preferences can further promote the cultivation and consumption of jackfruit [8-9].

Marker-assisted breeding (MAB) involves using molecular markers, such as SSRs, to select desired traits in plant breeding programs. SSR markers associated with fruit traits can be used for marker-assisted selection (MAS) to efficiently breed jackfruit varieties with improved fruit characteristics. Marker-based association studies involve correlating genetic markers with phenotypic traits across a diverse set of individuals or populations [10]. Fruit-trait-related genic SSRs identified in this study can serve as markers for conducting association studies to uncover the genetic basis of specific fruit traits in jackfruit. The ultimate goal of identifying fruit-trait-related genic SSRs is to facilitate the development of improved jackfruit varieties with desirable fruit traits such as flavor, size, texture, and nutritional quality [11]. By incorporating molecular markers into breeding programs, breeders can expedite the selection process and develop superior jackfruit cultivars that meet consumer preferences and market demands. The in-silico identification and characterization of fruit-trait-related genic SSRs in jackfruit can provide valuable insights into the genetic basis of important fruit traits and facilitate marker-assisted breeding efforts aimed at improving jackfruit varieties with desired characteristics [12]. The present study was aimed at identifying fruit-trait-related genic-SSRs in jackfruit. These SSRs would be highly useful for marker-based association studies for fruit-related traits in jackfruit.

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MATERIALS AND METHODS

Genomic data acquisition and identification of SSRs

The *A. heterophyllus* reference genome sequence (FASTA) and gene annotation (GFF) were downloaded from the 'Online Resource for Community Annotation of Eukaryotes' (https://bioinformatics.psb.ugent.be/orcae/aocc/overview/Arthe). The jackfruit whole genome sequence was subjected to the identification of perfect SSRs using Krait v.1.1.0 software [13] using the default parameters concerning the frequency of repeat motifs viz., 10, 7, 5, 4, 4, and 4 for mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats respectively. We did not include mononucleotide repeats in the study. The identified SSRs were categorized into genic and non-genic using genome annotation information (Arthe_gff_LATEST.tar) available at https://bioinformatics.psb.ugent.be/orcae/aocc/overview/Arthe. We chose only gene-based SSRs for further investigation. The primers for the genic-SSRs were designed using the BatchPrimer3 v.1.0 software (included with Krait v.1.3.3). The primers were designed using the following criteria: primer length = 18-27 bases (optimal of 20 bases), GC content = 30-80% with primer GC clamp 2, annealing temperature =58-65 °C (optimal 60°C), and product size = 100-300 bp.

Annotation of SSR-containing genes

The SSR-containing genes were annotated based on BLASTx analysis, InterProScan, and GO analysis using OmicsBox v.2.2.4 software (https://www.biobam.com/).

Identification of fruit-trait-related genes and fruit-trait-gene-based SSRs

We identified the characterized fruit-trait-related genes based on the literature survey. The orthologs of the fruit-trait-related genes identified in different crops were identified in jackfruit based on the annotation information.

RESULTS AND DISCUSSION

A total of 985.63 Mb of jackfruit genome is sequenced. These sequences harbor 41,997 protein-coding genes. Using the publicly available jackfruit genome sequence, we identified 273,577 perfect SSRs using the Krait v.1.1.0 software. These SSRs were classified into 19,934 genic and 253,643 non-genic SSRs. The annotation of the SSR-containing genes revealed that out of 19,934 genic-SSRs, 10,445 were present in the annotated gene sequences, whereas 9,489 SSRs were present in non-annotated gene sequences. Out of 10,445 SSRs in annotated gene sequences, 683 were present in fruit-trait-related genes. The SSRs present in the fruit-trait-related genes was characterized based on SSR motif, SSR number, number of reiterations, average length (bp), and distribution frequency of SSRs (Table 1). 683 of these annotated SSRs were found within genes associated with fruit traits, underscoring their potential significance in determining key fruit characteristics. Characterization of SSRs present in fruit-trait-related genes involved examining SSR motifs, SSR numbers, reiterations, average length, and distribution frequency.

Table 1 Distribution characteristics of SSRs motifs

SSR motif	Number	No. of reiterations	Average length (bp)	Percentage (%)
Dinucleotides	328	10	19.46	48.02
Trinucleotides	235	52	18.26	34.41
Tetranucleotides	77	27	17.71	11.27
Pentanucleotides	21	14	21.67	3.07
Hexanucleotides	22	22	26.45	3.22
Total	683	125	20.71	100

The 683 SSR (Simple Sequence Repeat) loci in the fruit-trait-related genes were classified into CDS and intronic-derived SSRs. The results indicated that 157 (23%) of the 683 SSR loci were located in the CDS, whereas 526 (77%) were found in the introns. We could design PCR primers for all the 683 SSR loci. The results obtained in the present study are in congruence with several earlier studies indicating that UTRs and intronic regions harbor more SSRs than the coding regions [14]. Introns have a repeat-unit profile similar to genomic DNA: most intronic SSRs are monomers and/or dimers in different taxonomic groups or species. Analysis of repeat motif distribution in the present study revealed that trinucleotide repeat (87.90%) was the predominant class, followed by hexanucleotide (10.19%) in CDS. In comparison, dinucleotide repeats (61.98 %) were the dominant class in intronic SSRs, followed by tri-nucleotide (18.44%) and tetra-nucleotide (14.45%) (Fig 1). Among the di- and trinucleotide repeats, AT/AT and AAT/ATT were the most abundant motifs, with frequencies of 16.69% and 3.66%, respectively. The number of repetitions of the simple sequences ranged from 4 to 24. The frequency of reiterations ranged from 39.97% (n = 4) to 2.34 (n > 16) (Table 2).

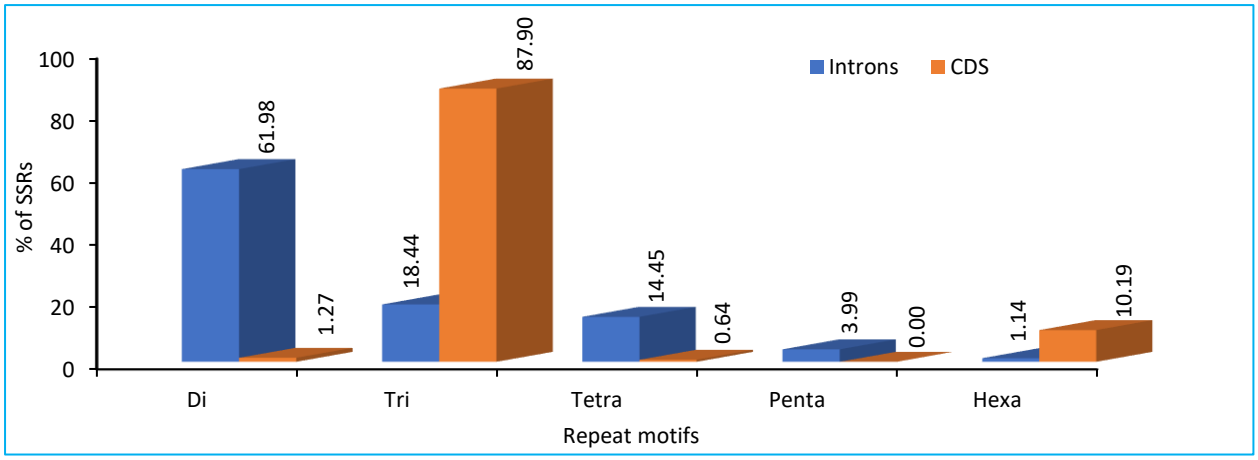

Fig 1 Distribution frequency of repeat motifs in the CDS and introns

We identified a total of 333 fruit-related SSR-containing genes comprising 22 families in jackfruit. The MYB genes were most abundant (77), while the RHF gene was least abundant (02). The number of SSRs per gene varied significantly among the different gene family members. The RNA pol. III and ACS gene families contained the maximum (3.25 SSR/gene), followed by AAA-ATPase (2.92 SSR/gene) and MYB (2.16 SSR/gene), while RHF had the least number of SSR per gene (01 SSR/gene) (Table 3). The study revealed that Myb family genes were significantly enriched in SSR-related genes, indicating its SSR-mediated evolution and divergence. It is well established that SSRs have played critical roles in the evolution and functional diversification of several gene families, particularly stress-related gene families [15]. Such phenomenon may be responsible for a huge diversity in the roles of MYB genes, including a wide range of effects on plant growth, development, and stress resistance, such as anther development, axillary meristem formation, cell-wall thickening, and sperm cell formation [16]. In summary, our study indicated the differential distribution of SSRs in different fruit-related genes and possibly explained the large-scale morphological variation in jackfruits.

Table 2 Frequency distribution of the ten most abundant fruit shape/size trait-related SSRs repeat motifs in *A. heterophyllus*

		No. of Reiteration of the motifs																			
S. No.	**Repeat motifs**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**22**	**24**	**Total**	**Percentage(%)**
1	AT/AT	-	-	-	23	25	17	13	8	8	8	1	3	2		1	2	1	1	1	114	16.69
2	TA/TA	-	-	-	12	16	14	14	6	5	2	-	-	-	1	-	-	-	-	-	70	10.25
3	AG/CT	-	-	-	7	9	7	5	8	3	2	-	-	1	1	-	-	2	-	-	45	6.59
4	TC/GA	-	-	-	9	9	7	4	4	3	3	5	-	-	-	-	1	-	-	-	45	6.59
5	AC/GT	-	-	-	8	7	4	1	4	3	2	1	-	1	-	-	-	-	-	-	31	4.54
6	AAT/ATT	-	7	10	4	2	1	-	1	-	-	-	-	-	-	-	-	-	-	-	25	3.66
7	TG/CA	-	-	-	6	7	4	3	-	2	-	1	-	-	-	-	-	-	-	-	23	3.37
8	AAC/GTT	-	8	8	3	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	21	3.07
9	TTTA/TAAA	12	7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	2.93
10	AAG/CTT	-	6	4	3	1	1	-	-	-	1	-	-	-	-	-	-	-	-	-	16	2.34
	Others	70	110	46	25	12	4	2	3	1	-	-	-	-	-	-	-	-	-	-	273	39.97
	Total	**82**	**138**	**69**	**100**	**89**	**59**	**42**	**35**	**25**	**18**	**8**	**3**	**4**	**2**	**1**	**3**	**3**	**1**	**1**	**683**	

Table 3 Distribution characteristics of SSRs in fruit-trait-related gene families

S. No.	Gene family	No. of SSRs	No. of genes	SSRs/gene
1	MYB	166	77	2.16
2	cyt. P450	123	62	1.98
3	bHLH	67	37	1.81
4	MADS-Box	47	23	2.04
5	Thioredoxin	36	19	1.89
6	DOF	35	17	2.06
7	AAA-ATPase	35	12	2.92
8	OFP	23	12	1.92
9	WUSCHEL	20	11	1.82
10	F-box/WD-40	18	7	2.57
11	LSM	15	7	2.14
12	CLAVATA	13	11	1.18
13	RNA pol. III	13	4	3.25
14	ACS	13	4	3.25
15	UBP	13	5	2.60
16	MTEF	11	5	2.20
17	YABBY	9	4	2.25
18	MSH	7	5	1.40
19	spt	7	4	1.75
20	R-H2 group	6	3	2.00
21	TSO1	4	2	2.00
22	RHF	2	2	1.00
	Total	683	333	2.05

CONCLUSION

The study identified 273,577 perfect SSRs in the jackfruit genome and classified them into 19,934 genic and 253,643 non-genic SSRs. The annotated gene sequences contained 10,445 SSRs out of the 19,934 genic-SSRs. Six hundred and eighty-three SSRs were found in genes associated with fruit traits. The fruit-trait-related genic-SSRs discovered in this study could be used as functional markers for various fruit attributes.

Conflict of interest

The authors of this manuscript have no competing interests.

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LITERATURE CITED

1. Jagadeesh SL, Reddy BS, Swamy GSK, Gorbal K, Hegde L, Raghavan GSV. 2007. Chemical composition of jackfruit (*Artocarpus heterophyllus* Lam.) selections of Western Ghats of India. *Food Chemistry* 102(1): 361-365.
2. Swami SB, Thakor NJ, Haldankar PM, Kalse SB. 2012. Jackfruit and its many functional components as related to human health: A review. *Comprehensive Reviews in Food Science and Food Safety* 11(6): 565-576.
3. Ahmed K, Malek M, Jahan K, Salamatullah K. 1986. *Nutritive Value of Food Stuff*. 3rd Edition, Institute of Nutrition and Food Science. Bangladesh: University of Dhaka.
4. Burkill IH, Birtwistle W. 1966. *A Dictionary of the Economic Products of the Malaya Peninsula*. [2nd Edition]. Kuala Lumpur Malaysia: Published on behalf of the governments of Malaysia and Singapore by the Ministry of Agriculture and cooperatives. pp 2444.
5. Saxena A, Bawa AS, Raju PS. 2009. Optimization of a multitarget preservation technique for jackfruit (*Artocarpus heterophyllus* L.) bulbs. *Journal of Food Engineering* 91(1): 18-28.
6. Ranasinghe RASN, Maduwanthi SDT, Marapana RAUJ. 2019. Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): A review. *Int. Jr. Food Science* 2019: 4327183. doi: 10.1155/2019/4327183. PMID: 30723733; PMCID: PMC6339770.
7. Sarangi PK, Srivastava RK, Singh AK, Sahoo UK, Prus P, Dziekański P. 2023. The utilization of jackfruit (*Artocarpus heterophyllus* L.) waste towards sustainable energy and biochemicals: The attainment of zero-waste technologies. *Sustainability* 15(16): 12520.
8. Tripathi K, Kumar P, Kumar R, Saxena R, Kumar A, Badoni H, Goyal B, Mirza AA. 2023. Efficacy of jackfruit components in prevention and control of human disease: A scoping review. *Jr. Educ. Health Promot*. 12: 361.
9. Shah SS, Gupta A, Karne S, Shinde B. 2017. Immunological evaluation of Artocarpus heterophyllus for determining its antimicrobial and anti-inflammatory activity. *Asian Jr. Pharm. Research* 7(2): 106-110.
10. Collard BC, Mackill DJ. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. B. Biol. Science* 363(1491): 557-572.
11. De Mori G, Cipriani G. 2023. Marker-assisted selection in breeding for fruit trait improvement: A review. *International Journal of Mol. Science* 24(10): 8984.
12. Dirlewanger E, Cosson P, Boudehri K, Renaud C, Capdeville G, Tauzin Y, Laigret F, Moing A. 2006. Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. *Tree Genet. Genomes* 3: 1-13.
13. Du L, Zhang C, Liu Q, Zhang X, Yue B, Hancock J. 2018. Krait: an ultrafast tool for genome-wide survey of microsatellites and primer design. *Bioinformatics* 34(4): 681-683.
14. Li YC, Korol AB, Fahima T, Nevo E. 2004. Microsatellites within genes: structure, function, and evolution. *Mol. Biol. Evolution* 21(6): 991-1007.
15. Yang J, Zhang X, Wang M, Sun Y, Liu C, Li S, Yu Y, Gao Y, Liu F, Zhang X, Kong J, Fan G, Zhang C, Feng L, Xiang J, Li F. 2021. Simple sequence repeats drive genome plasticity and promote adaptive evolution in penaeid shrimp. *Commun. Biol.* 4(1): 186.
16. Ambawat S, Sharma P, Yadav NR, Yadav RC. 2013. MYB transcription factor genes as regulators for plant responses: An overview. *Physiol. Mol. Biol. Plants* 19(3): 307-321.