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# Cross-species amplification of selected SSR markers to jackfruit and its related species

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## ABSTRACT

**Out of 28 SSR markers screened, seven (25%) markers were found to stable, reproducible and highly polymorphic amplifications in 16 *Artocarpus heterophyllus* accessions and 5 related species. The number of alleles per marker ranged from 6 to 17 with a PIC per marker ranging from 0.73 to 0.89. The haplotype also showed unique banding patterns in the 21 accessions of *Artocarpus* across the seven primers with 67% similarity. This denotes a potentially successful interfamilial amplification and transferability of the selected SSR markers to jackfruit.**

**Key words**: *Artocarpus heterophyllus*, transferability, microsatellite markers.

## INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is a member of the family Moraceae which comprises over 1,050 species of tropical and subtropical trees and shrubs coming from 37 genera. The genus *Artocarpus*, comprising of 70 species, and two small neotropical genera, *Batocarpus* and *Clarisia,* form the tribe *Artocarpeae* (Williams *et al*., 15). Jackfruit originated in the Western Ghats of India but distributed throughout Bangladesh, Burma, India, Indonesia, Malaysia, the Philippines, Thailand and Sri Lanka (Goswami and Chackrabarti, 5).

In Philippines, jackfruit is considered as a high value crop regarded for its great economic potential. It is an important component of subsistence farming systems as its fruit serves as a secondary staple food contributing to food security and livelihoods of the poor. In addition, the tree has numerous uses including timber, fuel, fodder, medicinal and industrial products. From 2014 to 2016, Philippine domestic production of jackfruit was recorded at 43.4 thousand metric tons (PSA, 10).

Morphological diversity of jackfruit has already been thoroughly studied in India (Jagadeesh *et al*., 6), Bangladesh (Khan *et al*., 7), and Florida in the United States (Schnell *et al*., 13). Using amplified fragment length polymorphism (AFLP) markers, several molecular characterization studies investigated the genetic diversity among jackfruit germplasm collections (Schnell *et al*., 13). Witherup *et al*. (16) developed microsatellite markers for *Artocarpus* species. Tonogbanua and Espino (14) screened for potential microsatellite markers from citrus for use in varietal identification of jackfruit; however, the set of primers used were insufficient to discriminate DNA profiles between registered jackfruit varieties in the Philippines. A high number of polymorphic microsatellites or simple sequence repeats (SSRs) markers have high potential for genetic mapping, marker-assisted selection and genotyping; thus, particularly for species with no SSR markers, cross-species amplification and transferability of developed microsatellite markers to crop families, such as Moraceae (Balachandran *et al*., 1) has been studied. Thus, to increase the number of microsatellite markers in jackfruit, the study aimed to cross-amplify SSR markers from selected crop species to jackfruit and its related species.

## MATERIALS AND METHODS

The study was conducted at the Molecular Plant Breeding Laboratory, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), Laguna, from March to May 2018. Sixteen *A. heterophyllus* accessions, and one accession each of *A. camansi, A. treculianus, A. altilis, A. sericicarpus* and *A. lacucha* from the collection of the Institute of Crop Science, UPLB, were utilized in this study.

Genomic DNA (gDNA) was extracted from fresh young leaf samples of jackfruit through cethyl trimethyl acetyle bromide (CTAB) method (Doyle and Doyle, 3) with modifications from Rasco *et al*., (11). One gram of deveined leaf tissue from each accession was pulverized in liquid nitrogen with a pinch of poly

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vinyl pyrrolidone (PVP) and was added to the 8 mL pre-heated extraction buffer at 65°C. The buffer contained 2% CTAB, 0.1 M Tris-HCl, 1.4 M NaCl, 0.02 M EDTA and 0.2% beta-mercaptoethanol. The solution was incubated further at 65°C for 1 hr. and gently inverted twice every 15 min. Approximately 500 µL from each extract was mixed gently with equal volume of chloroform: isoamyl (24:1 v/v) and centrifuged at 10,000 rpm for 10 min. at room temperature. Then, 200 µl of 2 mMNaCl solution with 4% polyethylene glycol (PEG) was added and refrigerated for 20 minutes in a -4 °C freezer. The mixture was centrifuged at 10,000 rpm for 5 min. at room temperature. The supernatant, about 400-500 µL, was transferred to sterile 1.5 mL tubes and was mixed gently with equal volume of cold isopropanol until the DNA precipitated. The sample was kept overnight at -20 °C and was subsequently centrifuged at 10,000 rpm for 5 min. The pellet was washed twice with 70% ethanol (200 µL), decanted, air dried, resuspended in 40 µL TE buffer (10 mMTris-HCl, pH 8.0, 1 mM EDTA) with 0.5 µl RNAse (20mg/1mL), incubated at 37 °C for an hour, and stored at -20 °C for further analysis.

The quantity of the extracted gDNA was checked through the $A_{260}/A_{280}$ ratio using the BIOTEK EPOCH™ UV-VIS microplate spectrophotometer. The quality was checked using 1.0% agarose gel electrophoresis run in EurogentecMupid One electrophoresis system and stained with Biotium Gel Red™. The gels were viewed under UV light in ClinxGenoSens 1510 Gel documentation and analysis system.

SSR primers from *Ipomoea*, *Manihot* and *Saccharum* were tested for transferability in jackfruit accessions (Table 1). The test was carried out in a 10-µL reaction containing 30 ng/µL of DNA, 1× PCR buffer, 0.3-0.4 µL MgCl2, 0.3-0.4 µL each of dNTPs, 0.3-0.4 µL primers, and 0.1 µL of Taq DNA polymerase. Amplifications were performed in a T100 Biorad thermocycler with the following conditions: 95 °C for 3 min., followed by 35 cycles of 30 sec. at 95 °C, 30 sec. at specific primer annealing temperature and 1 min. at 72 °C with a final extension of 5 min. at 72 °C. Using radioactively labelled 20-bp ladder DNA as a size standard, 20 µL of amplified products were electrophoresed on 6% denaturing polyacrylamide gel electrophoresis (PAGE) with 6 M urea and 1× TBE for about 3 hr. at 100 V and 500 W. The gels were stained with Gel Red dye and visualized using the gel documentation system.

Reproducibility of the screened markers was established by running PCR two to three times with the best runs subjected to molecular marker scoring. Amplifications of each sample were identified through the presence of clear and unambiguous bands which were scored as either 1 for presence or 0 for absence of DNA stretch.

**Table 1.** SSR primers used in polymerase chain reaction (PCR).

| SSR primers | Motif | AT (◦C) | Expected Size (bp) |
|---|---|---|---|
| CAS16* | $(CT)_7$ | 64 | 166-172 |
| CAS17* | $(CT)_{10}$ | 65 | 244-255 |
| SSR19* | $(CT)_8(CA)_{18}$ | 55 | 214 |
| SSR20* | $(GT)_{14}$ | 55 | 143 |
| SSR21* | $(GA)_{26}$ | 55 | 192 |
| SSR28* | - | 55 | - |
| SSR38* | $(CA)_{17}$ | 55 | 122 |
| SSR45* | $(CT)_{27}$ | 55 | 228 |
| SSR51* | $(CT)_{11}CG(CT)_{11}(CA)_{18}$ | 55 | 298 |
| SSR63* | - | 55 | - |
| SSR100* | $(CT)_{17}TT(CT)_7$ | 55 | 210 |
| SSR103* | $(GA)_{22}$ | 55 | 272 |
| SSR106* | - | 55 | - |
| SSR175* | $(GA)_{38}$ | 55 | 136 |
| IBSSR01** | (GA)14 | 58 | 188 |
| IBR13** | $(TTC)_6$ | 58 | 225-298 |
| Sach3*** | $(AAAG)_6$ | 54.7 | 138 |
| Sach8*** | $(GGAA)_3$ | 54.7 | 149 |
| Sach11*** | $(AATA)_3$ | 55.1 | 153 |
| Sach16*** | $(TATT)_3$ | 54.8 | 140 |
| Sach21*** | $(CAAA)_3$ | 54.9 | 150 |
| Sach24*** | $(GTT)_4$ | 54 | 151 |
| Sach27*** | $(AATA)_4$ | 54.8 | 150 |
| Sach30*** | $(CTG)_4$ | 56.8 | 150 |
| Sach33*** | $(CAG)_7$ | 56.5 | 145 |
| Sach34*** | $(CAGG)_3$ | 56.7 | 101 |
| Sach36*** | $(AT)_6$ | 55.1 | 122 |
| Sach48*** | $(CTGCTC)_3$ | 55.1 | 139 |

**Manihot*-based, ***Ipomoea*-based and ****Saccharum*-based SSR primers

Allelic diversity at a given locus for a pool of genome was measured using PIC through the following formula:

$$PIC = 1 - \sum_{i=1}^{h} P_i^2 \quad (1)$$

where $p_i$ is the frequency of the $i^{th}$ allele out of the total number of alleles at an SSR locus and n is the total number of alleles for that locus.

Scored polymorphic markers were used to create haplotypes. Presence and absence of bands are indicated by shaded and unshaded regions,

respectively. Banding patterns in the identified polymorphic markers were used to establish genetic similarity of 16 accessions of *A. heterophyllus* and its related species. Similarity matrix was generated using Dice's coefficient and a dendrogram was created using un-weighted pair group method with arithmetic means (UPGMA) and sequential, agglomerative, hierarchical and nested clustering parameters (SAHN) program of Numerical Taxonomy and Multivariate Analysis System (NTSYS)-pc version 2.1 (Rohlf, 12).

## RESULTS AND DISCUSSION

Genomic DNA quantity of *Artocarpus* DNA showed that 173.6 – 2137.6 ng/µl DNA were obtained from 1 g young leaf tissue. The highest amount of DNA was obtained in *A. camansi* and the lowest from *A. treculianus*. The DNA of the samples had good quality since the $A_{260}/A_{280}$ ratio was recorded to be in between 1.80 and 1.97 (Table 2). This suggests that the preparations were free of proteins and polyphenolic/polysaccharide compounds that reduce the efficiency of the replication process (Lorenz, 9).

Seven out of the twenty-eight (25%) SSR primers screened produced stable amplification and polymorphisms across the 16 *A. heterophyllus* accessions. This comprised one *Ipomoea*-based, two *Manihot*-based and four *Saccharum*-based markers (Table 3). Compared to the congeneric cross-amplification of *A. altilis* markers to *Artocarpus* spp. by Witherup *et al*. (16), a relatively low potential transferability rate of the screened markers was observed in the study. This can be explained by the wide evolutionary distance between the source and target species (Liewlaksaneeyanawin *et al*., 8). On the other hand, the number of alleles per marker ranged from 6 to 17 with a PIC per marker of 0.73 to 0.89 (Table 3). This denotes that the markers screened were highly polymorphic with a PIC value of greater than 0.5 (Botstein *et al*., 2). Furthermore, the haplotype, which is the summary of the banding patterns produced using SSR primers across samples, demonstrated the diversity of the accessions. Unique banding patterns were observed in the 21 accessions of *Artocarpus* across the different primers (Fig. 1).

Besides, the SSR markers produced clear banding patterns, indicating a greater possibility of successful transfer (Fan *et al*., 4). Thus, despite the genetic distance between the crop species of the screened primers and *Artocarpus,* the stable, reproducible and highly polymorphic amplification patterns observed across the accessions denote potential transferability of the cassava, sugarcane and sweet potato microsatellite markers to jackfruit. Similarly, successful interfamilial amplification and transferability of SSR markers were also observed in Euphorbiaceae and Rutaceae to Araceae (Rasco *et al*., 11).

The seven cross-amplified SSR primers successfully differentiated *A. heterophyllus* from five other *Artocarpus* spp. at 0.67 similarity coefficient (Fig. 2). At 75% similarity, cluster I contained all *A. heterophyllus* species while *A. camansi* and *A. altilis* were grouped in cluster II, and *A. sericicarpus* and *A. lacucha* were grouped in cluster IV. These results

**Table 2.** Quantity and quality of DNA based on the absorbance ratio of the genomic DNA.

| Accessions | $A_{260}/A_{280}$ | ng/µL |
|---|---|---|
| *A. heterophyllus* 1 | 1.91 | 1338.7 |
| *A. heterophyllus* 2 | 1.91 | 922.9 |
| *A. heterophyllus* 3 | 1.93 | 1118.8 |
| *A. heterophyllus* 4 | 1.92 | 1036.5 |
| *A. heterophyllus* 5 | 1.92 | 1114.4 |
| *A. heterophyllus* 6 | 1.93 | 609.5 |
| *A. heterophyllus* 7 | 1.98 | 874.3 |
| *A. heterophyllus* 8 | 1.88 | 361.0 |
| *A. heterophyllus* 9 | 1.88 | 606.4 |
| *A. heterophyllus* 10 | 1.94 | 636.1 |
| *A. heterophyllus* 11 | 1.75 | 294.7 |
| *A. heterophyllus* 12 | 1.82 | 398.2 |
| *A. heterophyllus* 13 | 1.85 | 235.8 |
| *A. heterophyllus* 14 | 1.91 | 595.9 |
| *A. heterophyllus* 15 | 1.85 | 453.9 |
| *A. heterophyllus* 16 | 1.81 | 668.9 |
| *A. camansi* | 1.97 | 2137.6 |
| *A. altilis* | 1.93 | 763.4 |
| *A. treculianus* | 1.80 | 173.6 |
| *A. sericicarpus* | 1.94 | 450.9 |
| *A. lacucha* | 1.88 | 232.3 |

**Table 3.** Polymorphism information content (PIC) of 7 cross-amplified SSR markers in *Artocarpus* accessions.

| Primers | Motif | No. of Alleles | PIC |
|---|---|---|---|
| CAS17 | $(CT)_{10}$ | 12 | 0.86 |
| SSR21 | $(GA)_{26}$ | 9 | 0.80 |
| IBR13 | $(TTC)_6$ | 13 | 0.80 |
| Sach3 | $(AAAG)_6$ | 11 | 0.84 |
| Sach8 | $(GGAA)_3$ | 17 | 0.89 |
| Sach34 | $(CAGG)_3$ | 16 | 0.83 |
| Sach48 | $(CTGCTC)_3$ | 6 | 0.73 |

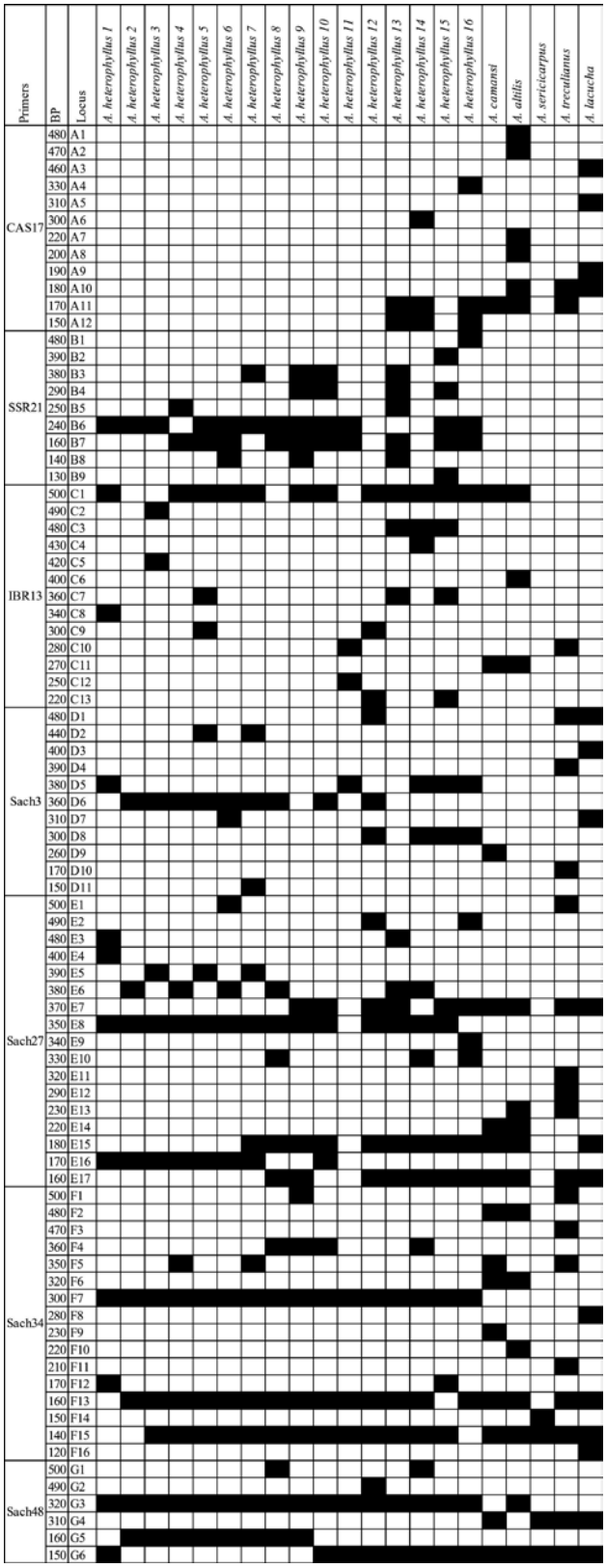


**Fig. 1.** Haplotype analysis for the 21 *Artocarpus* accessions using the 7 cross-amplified SSR markers.

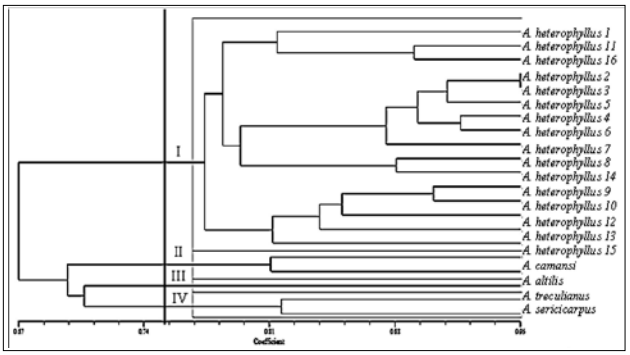


**Fig. 2.** UPGMA dendrogram of *Artocarpus* accessions based on Dice's similarity coefficient using 7 cross-amplified SSR markers.

coincide with the phylogenetic tree of *Artocarpus* proposed using *ITS* and *trnL-F* genes (Zerega *et al*., 17), and *rbcL* and *matK* genes (Williams *et al*., 15). The correct discrimination among the different *Artocarpus* species also validates the potential success in transferring the microsatellite markers to jackfruit.

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