Morphological and molecular genetic diversity analysis using SSR markers in Jackfruit (Artocarpus heterophyllus Lam.) genotypes for pulp colour

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Received: 27-06-2018 Accepted: 12-01-2019

ABSTRACT

Jackfruit (Artocarpus heterophyllus Lam.) being an important dryland horticultural tree, grows well with minimum care and maintenance. The study aimed at identifying the genetic diversity present in twenty jackfruit genotypes for different pulp colours. The genotypes were identified for commercial cultivation, based on fruit and flake quality characters viz., fruit weight, fruit rind weight per kg fruit, number of flakes per kg fruit, weight of flakes per kg fruit, individual fresh flake weight and TSS as per the Jackfruit descriptors given by IPGRI, Rome. The best genotypes identified from the study were Swarna, Lalbagh Madhura, Byrachandra, NSP, Ashoka Yellow and NEL and these can be used for commercial purpose. Molecular diversity analysis was also carried out using 22 SSR primers, out of which six primers (SSR 9, SSR 10, SSR 30, SSR 34, SSR 45 and SSR 48) showed polymorphism among twenty genotypes. The genetic similarity coefficient ranged from 0 to 0.96, indicating a vast variation in genetic diversity for pulp colour. A dendrogram was constructed by UPGMA analysis revealed three major clusters. The genotypes with cream and yellow colour pulp grouped in cluster I and II, followed by yellow, orange and red in cluster III. These markers effectively segregated the genotypes based on different pulp colours and hence, can be used for both diversity analysis and in breeding applications.

Key words: Genetic diversity, Jackfruit, Pulp colour, SSR.

INTRODUCTION

Jackfruit (Artocarpus heterophyllus Lam.), a plant native to India and common in Asia, Africa and South America, belongs to the Moraceae family. Grows well in warm, moist regions and also in the rain forests of Western Ghats of India (Rowe-Dutton et al., 1985; Reddy et al., 2004). It is cultivated widely at low elevations throughout India, in many parts of Southeast Asia (Rahman et al., 1999), in the evergreen forest zone of West Africa (Burkill, 1997), and also in the northern Australia (Azad et al., 2007). Due to cross pollination and predominance of seed propagation over a long period of time, there is high degree of variability within the species.

Jackfruit exhibits wide variability in terms of yield, fruit size, shape, pulp colour, taste, aroma and texture. These variations serve as a natural source for divergence studies. This wide range of variation existing in nature aids in the selection of superior desirable types (Jagadeesh et al., 2007). Innumerable variations in sweetness, acidity, flavor and taste are observed in Jackfruit growing areas. Such a wide diversity among clones in Western Ghats of India, the home of jack, offers tremendous scope for studying the variability and for improvement of this crop by clonal selection (Guruprasad, 1981; Samaddr, 1985). The pulp represents about 30% of the jackfruit weight and, due to the carotenoids presence, the pulp colour varies from cream, yellow, orange and red (Jagadeesh et al., 2007). A study carried out by Shyamalamma et al., (2015) on pulp carotenoid composition in different Jackfruit genotypes with varied pulp colours revealed that, the red and orange colour pulp contained significantly higher concentration of ß-cryptoxanthine (45.44 μg/100g and 42.57 μg/100g) followed by ß-carotene (44.20 μg/100g and 43.14 μg/100g), α-carotene (39.40 μg/100g and 37.30 μg/100g) and lycopene (30.20 μg/100g and 27.70 μg/100g) respectively.

A morphological study on custard apple pulp revealed that the orchards developed with improved varieties gave higher pulp yield with better chemical properties, hence justifying for suitable commercial exploitation (Hashmi et al., 2012). Based on the colour of the jackfruit pulp, the genotypes can be classified into four types viz., red, orange, yellow and cream. The genotypes with orange, red and golden yellow pulp colour fetches premium price in the market. The genotypes with orange colour pulp are popularly called as “Chandra Halasu”, meaning the pulp colour resembling the full moon colour. This type of genotypes are in great demand for commercial cultivation, owing to the premium price they fetch in market for table fruit purpose.

SSR markers have been successfully used to study the genetic diversity and relatedness among the genotypes.

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in various perennial crops such as mango (Eiadthong et al., 1999), Prunus (Aranzana et al., 2003), citrus (Barkley et al., 2006), guava (Padmakar et al., 2015). Microsatellites are highly popular genetic markers as they possess co-dominant inheritance, high abundance, enormous extent of allelic diversity, ease of assessing SSR size variation through PCR with pairs of flanking primers and high reproducibility (Singh et al., 2011). The present study aimed at estimating the genetic diversity among the twenty jackfruit genotypes collected from different parts using SSR markers for distinct pulp colour. The morphological diversity provides broader vision on phenotypic traits available in a particular crop. However, the same needs to be probed and confirmed using molecular markers, as they are less prone to environmental influences and also helps to establish trait-marker relations with continued efforts. SSRs and SNPs are the most widely used co-dominant markers since few years. Among the several DNA-based molecular marker technologies available for genetic diversity analysis, SSR markers have been proven as the markers of choice for application in molecular breeding programs (Petchiammal et al., 2015).

**MATERIALS AND METHODS**

**Plant materials:** An extensive survey was conducted in few districts of Karnataka viz., Bangalore rural, Tumkur, Ramnagar, Udipi and Bangalore urban; to find out twenty jackfruit genotypes with superior traits having distinct pulp colour. Five genotypes with four distinct colours, viz., red, orange, yellow and cream were selected for the study (Table 1) (Fig 1A, 1B, 1C, 1D). The morphological characters of jackfruit germplasm for both fruit and seed characters were recorded based on the Jackfruit descriptors given by IPGRI (2000). Approximately, 50g of recently matured leaves (15–20 days old) were collected, washed using distilled water, wiped with 70% (v/v) ethanol, then air dried prior to storage in sealed silver foils at 4°C.

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![Fig 1: Jackfruit genotypes with four distinct pulp colours](image)

A: Red, B: Orange, C: Yellow and D: Cream
Table 1: List of the genotypes used in the study.

| Name of the genotype | Place of collection | Pulp colour      |
|----------------------|---------------------|------------------|
| Nelukunte-1 (NKT-1)  | Nelukunte, Doddaballapur | Coppery Red      |
| Nelukunte-2 (NKT-2)  | Nelukunte, Doddaballapur | Coppery Red      |
| Nelukunte-3 (NKT-3)  | Nelukunte, Doddaballapur | Light Red        |
| Muniyamma Kachahalli (MK) | Kachahalli, Doddaballapur | Coppery Red      |
| KT-12                | Kachahalli, Doddaballapur | Coppery Red      |
| KT-9                 | Kachahalli, Doddaballapur | Dark Orange      |
| Ashoka Red           | Kachahalli, Doddaballapur | Orange          |
| Byrachandra          | Byrapatna, Ramnagara | Orange          |
| KT-17                | Kachahalli, Doddaballapur | Orange          |
| Nelugudige (NEL)     | Nelugudige, Doddaballapur | Orange          |
| Swarna               | UAS, GKVK Bangalore | Yellow          |
| Lalbagh Madhura      | Bangalore Urban      | Yellow          |
| Janagere             | Ramnagara            | Yellow          |
| NSP                  | UAS, GKVK Bangalore | Light Yellow    |
| Ashoka Yellow        | Kachahalli, Doddaballapur | Light Yellow    |
| Horticulture Research Station-2 (HRS-2) | Horticulture Research Station, GKVK, Bangalore | Creamish White |
| TN Big tree          | Hosur, Dharmapuri, Tamilnadu | Cream |
| Virupakshipura (VRT-11) | Virupakshipura, Tumkur | Cream |
| KT-1                 | Kachahalli, Doddaballapur | Cream          |
| Sompadi Gumless (SG) | Udipi, Dakshina Kannada | Creamish White |

**Morphological data analysis:** The fruit, flake and seed qualitative and quantitative characters were taken as per the parameters given by Jackfruit descriptors IPGRI, Rome. The one way ANOVA was done where mean, standard error and critical difference were calculated (Table 2A, 2B).

**DNA extraction and purification:** Plant genomic DNA was isolated by modified CTAB method (Pushpakumara and Harris (2007); Shyamalamma et al., (2008); Singh et al., (2011); Krishnan et al., (2015); Uma et al., (2016). The isolated DNA was treated with RNase A enzyme to remove RNA contamination and the purified DNA was analyzed in an agarose gel (0.8%) stained with Ethidium bromide; later on quantified using a spectrophotometer (NanoDrop Technologies, USA).

**PCR Amplification:** PCR amplification followed the protocol of Williams et al., (1990) with minor modifications. Thirty five SSR primers were used for the study out of which twenty two showed amplification with the extracted jackfruit DNA samples and the bands were obtained (Table 3A, 3B). These were selected for further SSR-PCR analysis. Each PCR reaction was carried out in 15 ul containing 25 ng/ul template DNA (3ul), 2.5 mM each dNTP (1.2ul), 25 mM MgCl2 (0.33ul), 1 Unit Taq DNA polymerase (Sigma- Aldrich Chemicals, Bangalore, India), 10 pmol forward primer, 10 pmol reverse primer in PCR Taq buffer 10X (2ul). Amplifications were performed in a MJ Research PTC-100 Thermocycler (Bio-Rad Laboratories, Bangalore, India), programmed for an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, primer extension at 72°C for 45 seconds, and a final extension at 72°C for 10 min. PCR products were resolved in 3% (w/v) agarose gels, visualized and documented using an Alpha Innotech Fluoroochem, San Leandro, CA, USA.

**SSR Profile analysis:** The genotype profiles produced by SSR markers were scored manually. Each allele was scored as present (1) or absent (0) for each of the SSR loci. A total of twenty two genomic-SSR markers giving consistent expected size products were used for genotyping of twenty genotypes (Fig 2). The primer data was assembled for statistical analysis. The SSR markers were used to visualize the genetic relationships based on pulp colour among the twenty Jackfruit genotypes. Genetic similarity was calculated using the simple matching (SM) coefficient (Sneath and Sokal, 1973). Similarity matrices were obtained using the subprogram SIMQUAL using NTSYS-pc, (version 2.0) (Rohlf, 2000). Clusters were built using the Unweighted Pair Group Method Using Arithmetic Average (UPGMA) procedure. Cluster analysis using UPGMA was also observed using molecular markers in crops like grass pea (Ambade et al., 2015), pigeon pea (Petchiammal et al., 2015). After scoring and computing the allelic data, a dendrogram was constructed by using NTSYS-pc, version 2.02 software. From the NTSYS software, the major allele frequency, gene diversity, allele number and Polymorphic Information Content (PIC) were obtained and the mean was calculated. The genetic similarity matrix was also observed between the genotypes using the Jaccard’s co-efficient.

**RESULTS AND DISCUSSION**

Twenty Jackfruit genotypes with four pulp colours viz, Red, Orange, Yellow and Cream were evaluated for morphological and molecular diversity. Most of the
Table 2A: Fruit and flake quantitative traits based on fruit descriptors data.

| Genotypes   | Stalk Length (cm) | Stalk Diameter (cm) | Fruit Length (cm) | Fruit Width (cm) | Fruit Wt. (kg) | Rind Wt. per kg fruit (g) | Fruit Rind Thickness (cm) | No. of flakes | Wt. of flakes per kg fruit (g) | Wt. of flakes (20 flakes) (g) | Individual Fresh flake Wt. (g) | Wt. of fresh flakes withseed (20 flakes) (g) | Wt. of fresh flakes withoutseed (20 flakes) (g) |
|-------------|------------------|--------------------|-------------------|------------------|----------------|--------------------------|------------------------|--------------|--------------------------------|----------------------------------|----------------------------------|------------------------------------------|------------------------------------------|
| NKT-1       | 4.75             | 2.90               | 37.50             | 24.50            | 6.99           | 427.00                   | 0.97                   | 14.50        | 572.00                          | 39.70                            | 735.50                           | 396.50                                   |                                           |
| NKT-2       | 4.80             | 2.85               | 33.00             | 26.00            | 6.74           | 422.00                   | 0.97                   | 13.90        | 577.00                          | 39.00                            | 290.00                           | 225.00                                   |                                           |
| NKT-3       | 5.00             | 2.10               | 36.00             | 28.50            | 6.87           | 641.00                   | 2.32                   | 9.50         | 411.50                          | 31.73                            | 861.00                           | 649.00                                   |                                           |
| MK          | 4.00             | 1.25               | 45.00             | 21.00            | 8.3            | 335.00                   | 1.40                   | 23.50        | 676.50                          | 27.50                            | 547.50                           | 425.00                                   |                                           |
| KT-12       | 2.50             | 1.85               | 27.00             | 15.50            | 9.26           | 485.00                   | 3.00                   | 19.50        | 446.50                          | 24.02                            | 454.50                           | 328.50                                   |                                           |
| KT-9         | 4.25             | 1.88               | 32.25             | 20.00            | 7.20           | 342.50                   | 1.83                   | 14.75        | 651.00                          | 43.93                            | 845.00                           | 655.00                                   |                                           |
| Ashoka Red  | 5.55             | 2.95               | 40.50             | 16.75            | 6.70           | 458.50                   | 1.10                   | 10.00        | 564.00                          | 40.75                            | 976.50                           | 842.50                                   |                                           |
| Byrachandra | 3.25             | 2.37               | 46.00             | 22.50            | 11.88          | 337.50                   | 0.80                   | 17.00        | 669.50                          | 40.64                            | 878.50                           | 695.50                                   |                                           |
| KT-17       | 3.90             | 2.55               | 36.83             | 21.45            | 6.32           | 291.62                   | 1.70                   | 9.00         | 483.50                          | 52.93                            | 1052.50                          | 843.00                                   |                                           |
| NEL         | 5.15             | 3.30               | 41.00             | 26.83            | 12.50          | 415.00                   | 2.00                   | 15.50        | 610.50                          | 42.15                            | 919.00                           | 838.00                                   |                                           |
| Swama       | 3.75             | 1.63               | 30.75             | 18.75            | 6.94           | 346.00                   | 1.83                   | 14.25        | 600.00                          | 39.00                            | 760.00                           | 624.00                                   |                                           |
| LalbaghMadhura | 7.30         | 3.00               | 42.50             | 19.00            | 8.25           | 525.00                   | 1.75                   | 8.50         | 503.50                          | 67.00                            | 1276.50                          | 1067.50                                  |                                           |
| Janagere    | 6.55             | 1.92               | 37.00             | 22.00            | 8.25           | 420.00                   | 1.50                   | 14.50        | 535.00                          | 35.65                            | 720.00                           | 587.50                                   |                                           |
| NSP         | 5.00             | 4.28               | 54.00             | 28.50            | 23.20          | 425.00                   | 1.60                   | 13.50        | 575.00                          | 43.25                            | 865.00                           | 735.00                                   |                                           |
| Ashoka Yellow | 8.75          | 3.10               | 50.00             | 23.60            | 8.91           | 270.00                   | 2.50                   | 22.50        | 720.50                          | 34.25                            | 680.00                           | 506.00                                   |                                           |
| TN Big Tree | 3.25             | 2.70               | 46.75             | 30.00            | 11.49          | 410.00                   | 1.75                   | 25.50        | 590.00                          | 23.73                            | 471.50                           | 356.50                                   |                                           |
| HRS-2       | 6.25             | 3.03               | 46.75             | 30.00            | 11.49          | 424.00                   | 2.38                   | 19.00        | 551.00                          | 30.31                            | 603.00                           | 414.00                                   |                                           |
| VRT-11      | 4.03             | 1.90               | 60.00             | 15.35            | 4.57           | 329.00                   | 1.00                   | 19.50        | 719.50                          | 36.74                            | 801.00                           | 695.00                                   |                                           |
| KT-1        | 5.13             | 2.15               | 28.25             | 22.50            | 9.87           | 500.00                   | 1.98                   | 20.50        | 446.00                          | 26.00                            | 505.50                           | 333.50                                   |                                           |
| SG          | 5.50             | 2.50               | 31.50             | 22.00            | 6.40           | 350.00                   | 0.87                   | 22.00        | 662.00                          | 29.25                            | 650.50                           | 512.00                                   |                                           |
| Grand Mean  | 4.93             | 2.50               | 38.15             | 22.58            | 9.25           | 407.70                   | 1.66                   | 17.5         | 578.23                          | 36.17                            | 744.65                           | 596.45                                   |                                           |
| CD          | 2.24             | 1.08               | 9.12              | 4.58             | 7.31           | 194.43                   | 1.01                   | 5.78         | 161.54                          | 10.85                            | 114.33                           | 115.50                                   |                                           |
| SEm±        | 0.22             | 0.10               | 1.74              | 1.23             | 0.73           | 19.56                    | 0.10                   | 1.38         | 16.25                          | 1.90                             | 6.17                             | 6.20                                    |                                           |
Table 2 B: Fruit, flake and seed quantitative traits based on fruit descriptors data.

| Genotypes          | Flake length (cm) | Flake width (cm) | TSS (ºBrix) | Rachis/ Fruit core length (cm) | Rachis/ Core diameter (cm) | Seed length (cm) | Seed width (cm) | No. of seeds per kg fruit | Seed weight (100 seeds) (g) | Flake to Seed ratio |
|---------------------|-------------------|------------------|-------------|-------------------------------|---------------------------|------------------|------------------|--------------------------|-------------------------------|-----------------------|
| NKT-1               | 6.70              | 3.50             | 15.00       | 30.50                         | 5.75                      | 3.05             | 1.60             | 14.50                    | 598.50                       | 6.63                  |
| NKT-2               | 5.70              | 3.45             | 21.50       | 28.50                         | 8.25                      | 3.10             | 2.05             | 35.50                    | 473.50                       | 3.17                  |
| NKT-3               | 6.35              | 5.30             | 22.50       | 27.00                         | 7.60                      | 3.25             | 2.15             | 9.50                     | 754.50                       | 4.21                  |
| MK                  | 5.25              | 3.55             | 24.50       | 30.00                         | 5.60                      | 3.45             | 2.35             | 23.50                    | 590.00                       | 4.66                  |
| KT-12               | 4.45              | 3.25             | 26.00       | 21.00                         | 5.90                      | 2.95             | 2.00             | 20.00                    | 575.00                       | 4.18                  |
| KT-9                | 5.75              | 4.50             | 22.80       | 26.00                         | 5.90                      | 4.35             | 2.50             | 14.00                    | 744.00                       | 5.90                  |
| Ashoka Red          | 6.00              | 4.40             | 17.75       | 28.25                         | 6.50                      | 3.55             | 2.25             | 10.00                    | 775.00                       | 5.26                  |
| Byrahanchanda       | 6.50              | 4.25             | 25.50       | 32.40                         | 6.50                      | 3.75             | 2.60             | 17.00                    | 855.50                       | 4.75                  |
| KT-17               | 7.65              | 4.93             | 20.35       | 25.85                         | 5.10                      | 4.20             | 2.70             | 9.00                     | 950.00                       | 5.57                  |
| NEL                 | 5.10              | 5.25             | 22.80       | 33.50                         | 6.00                      | 2.70             | 1.75             | 15.00                    | 345.50                       | 12.20                 |
| Swarna              | 5.50              | 3.55             | 21.75       | 21.00                         | 5.00                      | 3.05             | 1.48             | 14.00                    | 687.50                       | 5.67                  |
| Lalbagh Madhura     | 6.49              | 3.85             | 29.50       | 33.00                         | 5.20                      | 3.75             | 2.55             | 8.00                     | 575.00                       | 11.65                 |
| Janagere            | 4.60              | 3.85             | 25.10       | 27.50                         | 5.50                      | 2.60             | 1.75             | 17.00                    | 535.00                       | 6.66                  |
| NSP                 | 7.85              | 4.45             | 26.50       | 34.00                         | 10.50                     | 3.25             | 1.85             | 13.50                    | 665.00                       | 6.50                  |
| Ashoka Yellow       | 6.15              | 3.25             | 30.50       | 38.50                         | 4.75                      | 3.65             | 2.25             | 22.50                    | 594.50                       | 5.76                  |
| TN Big Tree         | 7.00              | 2.65             | 22.00       | 19.00                         | 8.00                      | 3.65             | 1.70             | 25.50                    | 567.00                       | 4.19                  |
| HRS-2               | 4.33              | 3.17             | 26.77       | 28.00                         | 5.50                      | 2.68             | 2.00             | 19.00                    | 348.00                       | 8.71                  |
| VRT-11              | 5.25              | 4.75             | 24.75       | 26.75                         | 4.65                      | 3.68             | 2.43             | 19.50                    | 667.50                       | 5.50                  |
| KT-1                | 4.73              | 3.38             | 30.75       | 19.25                         | 4.13                      | 3.50             | 2.23             | 20.50                    | 849.00                       | 3.06                  |
| SG                  | 6.10              | 4.25             | 25.40       | 19.50                         | 4.75                      | 3.25             | 2.00             | 22.00                    | 694.50                       | 4.21                  |
| Grand Mean          | 5.87              | 4.03             | 27.40       | 6.05                          | 24.06                     | 3.37             | 2.10             | 17.47                    | 642.23                       | 5.92                  |
| CD                  | 1.19              | 1.44             | 9.75        | 1.41                          | 2.78                      | 0.92             | 2.49             | 6.70                     | 36.05                        | 1.43                  |
| $SE_{a±}$           | 0.63              | 0.145            | 1.80        | 0.68                          | 0.95                      | 0.009            | 0.40             | 1.49                     | 3.60                         | 0.67                  |

Table 3 A: List of SSR primers used for screening Jackfruit genotypes.

| Primer | Sequence |
|--------|----------|
| SSR 2F | GCCGTTCCTTCTACCCCT |
| SSR 2R | CAAACCGAACAAGCCAC |
| SSR 6F | TTGTGAGCTACACCCCG |
| SSR 6R | CAGTTGTCTAGATGGCTCTG |
| SSR 9F | ATGCCTTCCGAGCAAGCACCT |
| SSR 9R | ATGCCTTCCGAGCAAGCACCT |
| SSR 10F | TGCCCGACCTTACACAGAGA |
| SSR 10R | TGCCCGACCTTACACAGAGA |
| SSR 12F | GTCTCAATGTTCAGCGTA |
| SSR 12R | GTCTCAATGTTCAGCGTA |
| SSR 14F | GAAGTGTGTGACCTCTG |
| SSR 14R | CAAGACGTCATCCTGG |
| SSR 17F | GTTGAATGTCTCAGCTTG |
| SSR 17R | CAAATCGGAAGTCCCG |
| SSR 19F | TCTGGTGTCGACAGC |
| SSR 19R | CAAATGGCGAGAAGG |
| SSR 22F | CTCTGTCCTACACAGACT |
| SSR 22R | CACACACCCCTCCT |
| SSR 23F | GCTTTCCTAGAATAAGCCCTG |
| SSR 23R | CAACATCATCAAAAGACATGTCG |
| SSR 30F | CAAACGACCCCCCCTTTC |
| SSR 30R | CACAGGCAACACACTTGG |

Table 3 B: List of SSR primers used for screening Jackfruit genotypes.

| Primer | Sequence |
|--------|----------|
| SSR 33F | GCACAACCATGTATGCA |
| SSR 33R | CACCAACGACCTGAT |
| SSR 34F | CAACGAAAGAAGCCAGAAG |
| SSR 34R | CCACCCCCACATTTGG |
| SSR 35F | CCACATCTCTTCTCGATCCTCCCT |
| SSR 35R | CTGAACTCTCGACCAAAATGACC |
| SSR 36F | CACCATGTCCTTCTCCACCC |
| SSR 36R | CACACACCCCCCT |
| SSR 38F | GCAAAAACCTTTGGG |
| SSR 38R | CACCCCTTCTCGCTC |
| SSR 42F | TCCAAAACACAGCAGC |
| SSR 42R | CACGCAACCTCGTTCCTG |
| SSR 43F | CCAACCGACATCACAAG |
| SSR 43R | CACGGAAACACCGAA |
| SSR 45F | CCACGTGACTTGACCTTACCC |
| SSR 45R | CACGTGCTACCGTACTG |
| SSR 47F | TAGACGGGACCGGAT |
| SSR 47R | CACGTGTCAGCGAC |
| SSR 48F | CCCCACCTCACCACAGC |
| SSR 48R | CAGAATCTCTTGCTTTAGCATCGC |
| SSR 50F | CTGTCCTCTAGTGC |
| SSR 50R | CACAGGAGTAATCTCTGAGG |

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Fig 2: Lane 1-20 represents Jackfruit genotypes in the following order
Red: 1-5 (NKT-1, NKT-2, NKT-3, MK, KT-12);
Orange: 6-10 (KT-9, Ashoka Red, Byrachandra, KT-17, NEL);
Yellow: 11-15 (Swarva, Lalbagh Madhura, Janagere, NSP, Ashoka Yellow) and Cream: 16-20 (HRS-2, TN Bigtree, VRT-11, KT-1, SG).

The various SSR primers amplified for pulp colour were SSR2, SSR6, SSR9, SSR10, SSR12, SSR14, SSR17, SSR19, SSR22, SSR23, SSR30, SSR34, SSR35, SSR36, SSR38, SSR42, SSR43, SSR45, SSR47, SSR48 and SSR 50 from a-v respectively.

Genotypes exhibited ellipsoid fruit shape with green colour rind and spiny surface. The fruit length varied from 26 cm to 54 cm and the fruit width from 15.35 cm to 30 cm. The weight of the fruits ranged between 4.57 kg to 23.20 kg. Based on the number of flakes and weight of flakes per kg fruit, the genotypes in red colour pulp viz., NKT-3 and MK; orange genotypes viz., KT-9, Ashoka Red, Byrachandra and NEL; genotypes with yellow colour pulp viz., Swarna, Lalbagh Madhura, Janagere, NSP and Ashoka Yellow and genotypes with cream colour pulp such as SG can be exploited for commercial purpose, especially for table fruit and value addition purpose in large scale. Most of the genotypes exhibited spheroid shaped flakes and the length of the flake varied from 4.33 cm to 7.85 cm. Rectangular shaped flakes were found in genotypes such as NKT-3, KT-12, KT-9, NEL, Janagere, VRT-11 and KT-1. These genotypes can be exploited further use in preparation of pulp for value added products such as squash, jam and for ice cream. One of the most preferred characteristics while selecting Jackfruit genotypes for commercial cultivation is the seed weight. If the seed weight is low, it is better and preferred. In the present study, low seed weight was recorded in NKT-2, KT-12, NEL, Lalbagh Madhura, Janagere, TN Bigtree, and Horticulture Research Station-2 (HRS-2).

A total of twenty two SSR primers gave good amplification in twenty Jackfruit (Artocarpus heterophyllus Lam.) genotypes. Out of twenty two primers, only nine primers were found to be polymorphic and remaining primers were monomorphic. The SSR markers SSR 2, SSR 9, SSR 10, SSR 17, SSR 22, SSR 30, SSR 34, SSR 45 and SSR 48 amplified and showed polymorphism, but not for a distinct pulp colour in the studied Jackfruit genotypes.

The genetic similarity matrix generated from the SSR marker data showed correlation between pulp colour and similarity of genotypes with in the pulp colour. All the genotypes with red colour pulp were closely related among themselves. The genotypes with red colour pulp were also a bit closely related to the orange colour genotypes. It is also to be noted that the cream colour genotypes had similarity with the yellow colour genotypes rather than orange and red coloured ones (Table 4).

The PIC values for polymorphic markers ranged from 0 to 0.38 and the gene diversity values were found in the range of 0 to 0.50. The mean major allele frequency, allele number, gene diversity and polymorphic information content values were 0.86, 1.50, 0.19 and 0.15 respectively. The primers which showed higher PIC values were SSR 6 (0.38) followed by SSR 19 (0.36), SSR 50 (0.34) and SSR 22 (0.33).

The dendrogram or UPGMA tree constructed from SSR marker data classified Jackfruit genotypes in three major clusters (Figure 3). The major cluster 1 grouped three genotypes viz., SG, KT-1 and VRT-11 with cream colour pulp.
Table 4: Genetic similarity matrix based on Jaccard’s co-efficient observed in Jackfruit genotypes with different pulp colours.

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2  | 0.92| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3  | 0.82| 0.89| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4  | 0.89| 0.89| 0.93| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | 0.92| 0.92| 0.89| 0.96| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6  | 0.79| 0.72| 0.76| 0.76| 0.79| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7  | 0.89| 0.89| 0.93| 0.93| 0.96| 0.82| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |
| 8  | 0.89| 0.89| 0.93| 1.00| 0.96| 0.76| 0.93| 1.00|     |     |     |     |     |     |     |     |     |     |     |
| 9  | 0.79| 0.86| 0.96| 0.89| 0.86| 0.79| 0.89| 0.89| 1.00|     |     |     |     |     |     |     |     |     |     |
| 10 | 0.79| 0.79| 0.82| 0.76| 0.79| 0.79| 0.82| 0.76| 0.79| 1.00|     |     |     |     |     |     |     |     |     |
| 11 | 0.82| 0.82| 0.79| 0.86| 0.89| 0.76| 0.86| 0.86| 0.77| 0.76| 1.00|     |     |     |     |     |     |     |     |
| 12 | 0.88| 0.81| 0.79| 0.86| 0.88| 0.75| 0.85| 0.85| 0.76| 0.75| 0.92| 1.00|     |     |     |     |     |     |     |
| 13 | 0.76| 0.70| 0.73| 0.73| 0.76| 0.76| 0.79| 0.73| 0.71| 0.76| 0.86| 0.85| 1.00|     |     |     |     |     |     |
| 14 | 0.63| 0.63| 0.71| 0.71| 0.67| 0.68| 0.71| 0.71| 0.74| 0.58| 0.71| 0.70| 0.77| 1.00|     |     |     |     |     |
| 15 | 0.63| 0.63| 0.56| 0.71| 0.67| 0.68| 0.71| 0.71| 0.74| 0.58| 0.71| 0.70| 0.77| 1.00| 1.00|     |     |     |     |
| 16 | 0.63| 0.58| 0.50| 0.61| 0.63| 0.63| 0.61| 0.61| 0.59| 0.63| 0.67| 0.71| 0.72| 0.82| 0.82| 1.00|     |     |     |
| 17 | 0.52| 0.52| 0.50| 0.55| 0.56| 0.52| 0.55| 0.55| 0.53| 0.52| 0.60| 0.58| 0.59| 0.73| 0.73| 0.81| 1.00|     |     |
| 18 | 0.43| 0.47| 0.56| 0.46| 0.43| 0.43| 0.46| 0.46| 0.53| 0.47| 0.46| 0.44| 0.50| 0.68| 0.68| 0.63| 0.79| 1.00|     |
| 19 | 0.53| 0.58| 0.56| 0.56| 0.53| 0.40| 0.52| 0.56| 0.55| 0.53| 0.56| 0.47| 0.55| 0.55| 0.55| 0.69| 0.81| 1.00|     |
| 20 | 0.53| 0.58| 0.60| 0.56| 0.53| 0.40| 0.52| 0.56| 0.55| 0.53| 0.56| 0.47| 0.55| 0.55| 0.55| 0.69| 0.81| 1.00| 1.00 |

LEGENDS:
1. NKT-1 6. KT-9 11. Swarna 16. HRS-2
2. NKT-2 7. Ashoka Red 12. Lalbagh Madhura 17. TN Bigtree
3. NKT-3 8. Byrachandra 13. Janegere 18. VRT-11
4. MK 9. KT-17 14. NSP 19. KT-1
5. KT-12 10. NEL 15. Ashoka Yellow 20. SG

Fig 3: Dendrogram of Jackfruit genotypes with different pulp colour generated from SSR primer data.
Cluster II was further classified into two sub-clusters IIA and IIB, which comprised two cream viz., TN Bigtree, HRS-2 and two yellow pulp coloured genotypes viz., AY and NSP. Cluster III comprised large number (thirteen) of the genotypes with yellow, orange and red pulp colour. Interestingly, the Jackfruit genotypes with cream colour pulp were genetically closer with a similarity co-efficient of 0.82, compared to Jackfruit genotypes with red and orange colour pulp in cluster IIIc, which were related very closely with a very similar co-efficient of 0.88. The genetic similarity matrix based on Jaccard’s co-efficient in twenty Jackfruit genotypes with distinct pulp colour showed similarity values ranging from 0.40 to 0.96. The higher (0.96) similarity was observed between genotypes MK and KT-12, both of which exhibited red colour pulp; KT-17 and NKT-3; Ashoka Red with KT-12 and Byrachandra with KT-12. Again the similarity was close (0.93) between NKT-3 with Ashoka Red; Byrachandra and MK; Ashoka Red and MK and finally Ashoka Red and Byrachandra. Again the genotypes with red colour pulp were closely linked to orange colour pulp irrespective of the place of collection indicating the genetic relations for the pulp colour rather than the place of cultivation. The similarity index table provides valuable information on relationships between Jackfruit genotypes with different pulp colour.

Therefore, further studies are required to screen and find out the sequences linked to pulp colours for development of molecular tools which could be used in crop improvement programme in Jackfruit.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Biotechnology-Human resource Development, New Delhi and the Department of Plant Biotechnology, GKVK, University of Agricultural Sciences, Bangalore for providing facilities to carry out the research work and for their constant encouragement.

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