Characterization of Selected Jackfruit Germplasm Accessions for Fruit Shape Through Morphological and Marker Based Assay

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ABSTRACT
The study involved characterization of Jackfruit accessions with obloid (round) versus ellipsoid fruit shape. The morphological characterization was based on tree, fruit and seed descriptors. The Jackfruit accessions exhibited wide range of differences. The dominant tree growth habit was erect type, with medium to low branching density and branching pattern was irregular in most of the trees. With respect to fruit rind weight, it was more than fifty to sixty per cent in accessions with obloid fruit, than ellipsoid fruits. The rind weight ranged from thirty to forty per cent of the total fruit weight. Based on fruit and flake quality characters, the accessions such as Allilugatta 5, Kumaraswamy, Manipur Parmesh and Swarna in obloid fruits and Ashoka Red, Ashoka Yellow, Byrahachandra, Janagere and NSP in ellipsoid fruits have been identified for commercial purpose. Among ten RAPD primers screened, two primers showed higher PIC value (0.24) viz., OPA 4 and OPAH 4. Similarly among ten ISSR markers screened, ISSR - 868 (0.31) showed higher PIC value. These can be used effectively to probe the genetic variations among the Jackfruit accessions.

Key words: Accessions, Genetic variability, ISSR, Jackfruit, RAPD.

INTRODUCTION
Jackfruit (Artocarpus heterophyllus Lam.) belongs to the family Moraceae, the biggest tree borne fruit, spread across Western Ghats, cultivated widely in Southern states of India, as well as in North- Eastern and Eastern parts of India. Though, it is indigenous to rain forests of Western Ghats, it has huge diversity in South Eastern Asian countries viz., Bangladesh, Sri Lanka, Malaysia, Vietnam and Indonesia (Nazrul 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.

India is considered as the second largest producer of jackfruit in the world. In India, it has wide spread distribution in various states viz., Assam, Tripura, Bihar, Uttar Pradesh, the foothills of the Himalayas and South Indian States of Kerala, Tamil Nadu and Karnataka. The jackfruit production in Karnataka is estimated to be around 193.47 Metric tons (NHB, 2016).

Jackfruit is a medium-sized evergreen tree, grows well in varied climactic conditions with minimum care and maintenance (Bose and Mitra, 1985). The tree produces heavier yields than any other tree species and bears the largest known edible fruit (up to 54 kg). The jackfruit tree has extensive range of applications in food industries. The tender fruit is used as vegetable, mature fruit for preparation of value added products such as chips, papad, dried and dehydrated flakes/flake powder and the flakes of ripe fruits are high in nutritive value; every 100 g of ripe flakes contains 191-407 mg potassium, 20-37 mg calcium, 38-41 mg phosphorus, 0.5-1.1 mg iron and 11-19g carbohydrates. Thus, it is aptly called as “poor man's food” (Nazrul et al., 2004) as it is cheap and plentiful during the season. The nutritious seeds are boiled or roasted and eaten like chestnuts, added to flour for baking, or cooked in dishes. The tree is also known for its durable timber and used largely for musical instruments and wooden carvings, because of its color and anti-termite properties. The by-products from the tree like leaf and fruit waste provides valuable fodder for cattle, pigs and goats. Jackfruit wood chips yield a dye, which is used to give the famous orange-red color to the robes of Buddhist priests. In addition, many parts of the plant, including the bark, roots, leaves and fruits have medicinal properties. Despite numerous advantages, the popularity of jackfruit as a commercial crop is poor owing to wide variations in fruit quality, fruit size, shape, pulp color and non-availability of quality planting material etc.

So far, only a limited number of varieties have been developed and released in India, particularly in Southern and Eastern states of India. Most of these are selected from natural populations and released after performance trials. However, local genotypes have different names based on their variability in yield, fruit shape, flake color, total sugars and so on. Cultivar identification and estimation of genetic
diversity using morphological data are limited, as they are environmentally influenced and there are few distinctive traits. Thus, the present study focuses on identification of jackfruit genotypes with obloid fruits popularly called as Rudrakshi Jackfruit, which are distinct with definite fruit shape. The fruits are usually obloid and weigh around two to five kg. The fruit bearing is quite high ranging from 50 to 500 fruits per tree depending on the age of the tree. The pulp colour varies from cream, yellow to orange. These accessions will be evaluated in comparison to accessions with ellipsoid fruits, weighing more than five kg. These are suitable for table purpose as well as for processing into value added products.

The jackfruit accessions with small, round shaped fruits, weighing 2-5kgs with cream, yellow and orange colour pulp are preferred for small family size. Thus, the main focus of the study was to investigate morphological and molecular characterization of jackfruit accessions with distinct fruit shapes.

Hence, the study “Characterization of selected Jackfruit germplasm accessions for fruit morphological traits and RAPD and ISSR - based markers” has been conducted, with the following objectives.

a) Characterization of jackfruit germplasm accessions differing for fruit shape.
b) RAPD and ISSR marker assay based polymorphism among jackfruit germplasm accessions differing for fruit shape.

MATERIALS AND METHODS

Plant materials

Twenty jackfruit accessions with two distinct fruit shape’s viz., obloid (Allilugatta 1, 2, 3, 4, 5, 6, Chidayya, Gundainaru, Kumaraswamy and Manipur Parmesh) and ellipsoid (Arsikere, Ashoka Red, Ashoka Yellow, Ashwathnarayan, Byrachandra, Janagere, KT 7, NKT 1, Siddesh and Suresh) were collected from different jackfruit growing districts in Karnataka. Observations on plant, fruit and seed characters viz., Plant descriptors, Fruit descriptors, Seed descriptors were recorded as per the descriptors provided by IPGRI for Jackfruit by using Swarna and NSP as check varieties for obloid and ellipsoid fruits, respectively (IPGRI, 2000).

Morphological data analysis

The tree, fruit, flake and seed qualitative and quantitative characters were recorded as per the parameters given by Jackfruit descriptors IPGRI, Rome. The one way ANOVA was done where mean, standard error and critical differences were calculated (Table 1; Table 2; Fig 1; Fig 2).

DNA extraction and purification

Fresh and tender leaves, free from pest and disease infection were collected from each accession, rinsed with distilled water and surface sterilized with 70% alcohol. The leaves were stored at -20°C and used for isolation of genomic DNA by CTAB method (Shyamalamma et al., 2008; Kavya et al., 2019).

Plant genomic DNA was isolated by modified CTAB method (Dellaporta et al., 1983; Shyamalamma et al., 2008; Kavya et al., 2019). The isolated DNA was treated with RNaseA 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 (Nano Drop Technologies, USA).

PCR amplification

PCR amplification was performed following Williams et al., (1990) protocol with minor modifications. Fifteen RAPD and ten ISSR primers, showed clear and distinguishable amplification and were selected for further analysis. Three independent replications were carried out to check the reproducibility of the primers. PCR reaction was carried out with a total volume of 20µl and 15µl in for RAPD and ISSR primers, respectively. Amplifications were performed in a MJ Research PTC- 100 Thermocycler (Bio-Rad

Table 1: Quantitative traits observed on fruits in jackfruit accessions with obloid fruit shapes.

| Accessions     | Fruit wt. (kg) | Fruit rind wt. (g) per kg fruit | No. of flakes | Individual flake wt. (g) | Wt. of flakes per kg fruit (g) | TSS (% Brix) | Flake/seed ratio |
|----------------|----------------|---------------------------------|---------------|--------------------------|---------------------------------|--------------|------------------|
| Allilugatta 1  | 2.68a          | 705.00a                         | 15.33a        | 24.33bc                  | 279.66                          | 27.20b       | 6.00             |
| Allilugatta 2  | 0.82a          | 510.00a                         | 16.00ab       | 20.80b                   | 327.00                          | 26.93bc      | 3.40             |
| Allilugatta 3  | 2.34a          | 477.00a                         | 14.33ab       | 30.20ab                  | 459.00                          | 25.76c       | 4.80             |
| Allilugatta 4  | 4.14c          | 460.00c                         | 22.33cde      | 21.46c                   | 478.00                          | 25.90c       | 7.70             |
| Allilugatta 5  | 2.10c          | 480.00c                         | 15.33c        | 34.45d                   | 496.33                          | 28.33c       | 7.80             |
| Allilugatta 6  | 2.83c          | 485.00c                         | 20.66c        | 21.96c                   | 560.00                          | 26.33c       | 7.10             |
| Chidayya      | 1.80a          | 640.00a                         | 29.00c        | 11.60a                   | 336.33                          | 27.33c       | 3.40             |
| Gundainaru    | 0.6a           | 110.00a                         | 13.66c        | 36.56c                   | 497.33                          | 31.00a       | 5.40             |
| Kumaraswamy   | 2.01a          | 420.00a                         | 15.33c        | 37.46d                   | 574.66                          | 25.86c       | 4.70             |
| Manipur Parmesh | 5.18a     | 425.00a                         | 15.66c        | 36.70d                   | 565.00                          | 24.64d       | 5.70             |
| Swarna        | 8.48a          | 250.00a                         | 15.00c        | 46.10c                   | 707.33                          | 22.50a       | 5.40             |
| Mean          | 2.99           | 457.00a                         | 17.51         | 29.27                    | 475.57                          | 26.50        | 5.00             |
| SEs±          | 0.42           | 33.95                           | 1.13          | 2.63                     | 32.13                           | 0.61         | 0.51             |
| CD            | 1.28           | 102.76                          | 3.43          | 7.76                     | 97.26                           | 1.87         | 1.56             |
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Table 2: Quantitative traits observed on fruits in Jackfruit accessions with ellipsoid fruit shapes.

| Accessions  | Fruit wt. (kg) | Fruit rind wt. (g) per kg fruit | Individual flake wt. (g) | No. of flakes per kg fruit | Wt. of flakes per kg fruit (g) | TSS (°Brix) | Flake/seed ratio |
|-------------|----------------|---------------------------------|--------------------------|---------------------------|--------------------------------|-------------|------------------|
| Arsikere    | 3.26           | 420.00                          | 17.93                    | 34.00                     | 595.00                        | 22.66       | 22.60            |
| Ashoka Red  | 6.40           | 435.00                          | 61.50                    | 9.33                      | 556.66                        | 19.16       | 19.10            |
| Ashoka Yellow | 8.70         | 270.00                          | 29.86                    | 24.33                     | 717.66                        | 30.66       | 30.60            |
| Ashwathnarayan | 8.04     | 460.00                          | 22.15                    | 24.00                     | 531.66                        | 27.33       | 27.30            |
| Byrachandra  | 11.13          | 300.00                          | 46.20                    | 15.00                     | 675.00                        | 27.13       | 27.10            |
| Janagere     | 8.47           | 410.00                          | 38.33                    | 14.66                     | 563.33                        | 25.06       | 25.00            |
| KT 7         | 8.48           | 430.00                          | 28.80                    | 21.33                     | 573.66                        | 21.33       | 21.30            |
| NKT 1        | 9.28           | 438.00                          | 29.70                    | 19.66                     | 582.66                        | 25.66       | 25.60            |
| Sridh       | 6.09           | 400.00                          | 29.70                    | 19.66                     | 578.33                        | 25.66       | 25.60            |
| Suresh       | 2.20           | 685.00                          | 20.49                    | 15.66                     | 285.00                        | 26.00       | 26.00            |
| NSP          | 23.73          | 385.00                          | 38.44                    | 16.00                     | 586.66                        | 27.33       | 27.30            |
| Mean         | 10.36          | 406.81                          | 33.04                    | 19.42                     | 570.18                        | 25.27       | 25.20            |
| SE±          | 0.86           | 27.30                           | 4.13                     | 2.50                      | 32.19                         | 3.2         | 3.00             |
| CD           | 2.62           | 82.82                           | 12.51                    | 7.70                      | 97.40                         | 2.16        | 2.15             |

Table 3: RAPD markers with major allele frequency, allele number, gene diversity and PIC values.

| Primer | Major allele frequency | Allele Number | Gene Diversity | PIC value |
|--------|------------------------|---------------|----------------|-----------|
| OPP 6  | 0.85                   | 1.85          | 0.19           | 0.16      |
| OPAH 20| 0.90                   | 2.00          | 0.15           | 0.13      |
| OPAH 5 | 0.90                   | 2.00          | 0.17           | 0.15      |
| OPAH 4 | 0.78                   | 1.82          | 0.28           | 0.22      |
| OPP 1  | 0.92                   | 2.00          | 0.13           | 0.12      |
| OPP 3  | 0.90                   | 1.75          | 0.15           | 0.13      |
| OPA 2  | 0.80                   | 1.50          | 0.22           | 0.17      |
| OPA 5  | 0.84                   | 1.50          | 0.21           | 0.16      |
| OPA 4  | 0.76                   | 2.00          | 0.30           | 0.24      |
| OPA 1  | 0.86                   | 2.00          | 0.21           | 0.18      |
| Mean   | 0.83                   | 1.84          | 0.22           | 0.19      |

Fig 1: Jackfruit accession “Swarna” with obloid fruit shape.

Fig 2: Jackfruit accession “NSP” with ellipsoid fruit shape.
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Labsoratories, 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 for 45 seconds, primer extension at 72°C for 45 seconds and a final extension at 72°C for 10 min. These primers amplified well at annealing temperature varying from 31- 40°C for RAPD and 27-62°C for ISSR primers. PCR products were resolved on 3% (w/v) agarose gels, visualized and documented using an Alpha InnotechFluorochem, San Leandro, CA, USA (Fig 3).

RAPD and ISSR profile Analysis

The amplified fragments obtained from each RAPD and ISSR primers were scored manually for their presence (1) or absence (0) and a genetic dissimilarity matrix was developed using Squared Euclidean Distances, which estimates all pairwise differences in the amplification products. 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. After scoring and computing the allelic data, a dendrogram was constructed 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 (Table 3; Table 4). The genetic similarity matrix was also observed between the genotypes using the Jaccard’s co-efficient.

RESULTS AND DISCUSSION

The fruit characters of obloid and ellipsoid were compared, the fruit weight was less in obloid types, ranging from 0.61kg to 5.18kg, however, in check variety Swarna the fruit weight was 8.48kg. The flake number varied from 13.66 to 29.00 per kg fruit. The individual flake weight varied from 11.60g to 34.46g and in Swarna, it was 46.10g. Flake weight per kg fruit ranged from 279.66g to 574.66g and in Swarna it was higher (707.33g). Pulp TSS content ranged from 22.50 to 31.00 °Brix. The fruit rind weight was less in Swarna (250g) compared to other obloid types, with a range of 110g to 705g. The obloid fruit shapes are popularly called as Rudrakshi types, as they possess unique fruit shape,

Table 4: ISSR markers with major allele frequency, allele number, gene diversity and PIC values.

| Primer | Major allele frequency | Allele Number | Gene Diversity | PIC value |
|--------|------------------------|---------------|----------------|-----------|
| 868    | 0.65                   | 2.00          | 0.39           | 0.31      |
| 823    | 0.97                   | 1.50          | 0.04           | 0.04      |
| 846    | 0.89                   | 2.00          | 0.18           | 0.15      |
| 907    | 0.92                   | 2.00          | 0.13           | 0.12      |
| 818    | 0.92                   | 2.00          | 0.13           | 0.12      |
| 906    | 0.83                   | 1.80          | 0.23           | 0.19      |
| 832    | 0.75                   | 2.00          | 0.32           | 0.25      |
| 839    | 0.90                   | 2.00          | 0.17           | 0.15      |
| 842    | 0.84                   | 1.40          | 0.17           | 0.13      |
| 857    | 0.75                   | 1.80          | 0.31           | 0.24      |
| Mean   | 0.83                   | 1.85          | 0.22           | 0.19      |

Fig 3: RAPD and ISSR gel profile pictures analysed for RAPD and ISSR primers among twenty two Jackfruit genotypes.

Legend:

Lane 1. Allilugatta 1
Lane 2. Allilugatta 2
Lane 3. Allilugatta 3
Lane 4. Allilugatta 4
Lane 5. Allilugatta 5
Lane 6. Allilugatta 6
Lane 7. Chidayya
Lane 8. Gundainaru
Lane 9. Kumaraaswamy
Lane 10. Manipur Parmesh
Lane 11. Swarna
Lane 12. Arasikere
Lane 13. Ashoka Red
Lane 14. Ashoka Yellow
Lane 15. Ashwathanarayan
Lane 16. Byrachandra
Lane 17. Janagere
Lane 18. KT-9
Lane 19. NKT-1
Lane 20. Siddesh
Lane 21. Suressh
Lane 22. NSP
Lane 17. Janagere
Lane 18. KT-9
Lane 19. NKT-1
Lane 20. Siddesh
Lane 21. Suressh
Lane 22. NSP
Lane 17. Janagere
Lane 18. KT-9
Lane 19. NKT-1
Lane 20. Siddesh
Lane 21. Suressh
Lane 22. NSP
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wherein all fruits in a tree will be round and fruit weight also ranges from 0.50 to 5.00 kgs.

In case of jackfruit accessions with ellipsoid fruit shapes, the fruit weight ranged from 2.20kg to 11.13kg. The check variety NSP recorded higher fruit weight (23.73 kg). The flake number varied from 9.33 to 34 per kg fruit. The individual flake weight ranged from 17.93g to 61.50g. The weight of flakes per kg fruit also varied largely from 285.00g to 675g per kg fruit weight. Pulp TSS content also varied from 19.16 °Brix to 30.66 °brix. Fruit rind weight varied from 270.00g to 685g per kg fruit weight.

The jackfruit accessions with distinct fruit shapes (obloid and ellipsoid) were subjected to RAPD and ISSR marker analysis to study the genetic variability among these accessions. The RAPD markers OPP6, OPAH 4, OPAH 5, OPAH 20, OPP 1, OPP 3, OPA 2, OPA 4, OPA 5, OPN 1 and ISSR markers 868, 823, 846, 907, 818, 832, 839, 842 and 857 presented polymorphism with PIC values varying from 0.12 to 0.24 and 0.04 to 0.25, respectively. The gene diversity RAPD markers ranged from 0.13 to 0.30 and in ISSRs it was from 0.04 to 0.30 (Table 3 and 4).

The dendrogram was constructed using RAPD and ISSR marker data together. The analysis grouped the jackfruit accessions into two distinct clusters. Cluster I and Cluster II. The cluster I was again divided into two sub clusters, Cluster IA and IB. The obloid types were grouped in cluster II and IB whereas, all ellipsoid types were grouped in Cluster IA (9 accessions) and IB (2 accessions) (Fig 3 and 4).

The quality of fruits plays an important role in judging utility for table purpose and processing. In the present study twenty two jackfruit accessions were selected based on two distinct fruit shapes and were compared for fruit and flake quality traits for further classification and selection for commercial utilization.

Fruit quality traits such as fruit shape, fruit weight and fruit pulp quality was found to be good in most of accessions with obloid fruit shapes. These accessions were mainly collected to assess their suitability for table purpose, as they exhibit round fruit shape and less weight (<5kg), very ideal to carry for table purpose and also suits well for a small family. Among the accessions studied, Allilugatta 6, Kumaraswamy and Manipur Parmesh exhibited higher flake weight with bigger flakes and average weight of twenty flakes were also on par with the check variety Swarna, thus these accessions have been conserved for future utilization and perpetuation.

Similarly the fruit quality traits were assessed in jackfruit accessions with ellipsoid fruit shapes. These accessions are mainly aimed for higher pulp recovery and their use in processing industry. The traits such as, fruit weight (>10kg), weight of flakes per kg fruit (586g to 717.66g), individual flake weight (30g to 61.50g) and TSS (>25 °Brix). The accessions, Ashoka Yellow, Janagere, Byrachandra and Ashoka Red were selected for the above characteristics as most of these accessions were better for fruit and flake characteristics than the check variety (NSP) used in the present study. Similar reports on fruit quality traits have been made by several scientists in jackfruit Azad and Haq (1998); Singh and Srivastava (2000) and Wangchu et al., (2013).

An interesting observation was made in the present study with respect to fruit rind weight. It was more than fifty to sixty per cent in accessions with obloid fruits when compared to accessions with ellipsoid fruits, where the rind weight ranged from thirty to forty per cent of the total fruit.
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weight. It was observed from the present study that there existed a difference in the fruit rind weight while comparing the obloid and ellipsoid types of jackfruit accessions, where 55-60% of the obloid fruit weight and 30-35% of the ellipsoid fruit weight was of its rind. Thus, there is a need to survey and identify jackfruit accessions with obloid fruit shapes with better fruit and flake characteristics and lower (30 to 40%) rind weight. Similar reports have been made by Jagadeesh et al. (2007).

Rudrakshi fruit size and shape remains same under varied agro-climatic conditions. Thus, it’s an interesting aspect to probe and find out genomic regions controlling the trait, which could be incorporated into improved hybrids during crop improvement programs.

Based on the dendrogram constructed, we can clearly distinguish two major clusters with Jackfruit accessions especially for the fruit shape rather than the region of collection. Cluster IA consisting of accessions with ellipsoid fruit shape which are already in the process of commercialization, as they possess excellent fruit and flake quality traits. Further, cluster IB and cluster II was composed of all obloid types and two ellipsoid type jackfruit accessions. The grouping in these clusters was again based on fruit shape and most of the accessions with obloid fruit shape popularly called as a Rudrakshi types grouped together, indicating the genetic relations among the obloid types. These accessions were also collected from different geographical locations, for assessing their suitability for commercial utilization. Similar studies on variations in fruit size and shape in Jackfruit accessions collected from different locations are reported by Shyamalamma et al. (2008) and Kavya et al. (2019).

Thus, the RAPD primers with higher PIC value (0.24) viz., OPA 4 and OPAH 4 and ISSRs (0.25) viz., 832 and 857 can be used effectively to probe the genetic variations among the jackfruit accessions.

Future line of work
1. The jackfruit accessions with obloid fruit shapes (Rudrakshi types) collected from wide geographical locations, need to be screened in large number to identify suitable types for table fruit purpose.
2. The markers linked to fruit shapes need to be identified for Marker Assisted Selection in Jackfruit Breeding programmes.

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