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ORIGINAL ARTICLE

Nutritional profile and molecular fingerprints of indigenous black jamun (*Syzygium cumini* L.) landraces

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Abstract The indigenous black jamun landraces (*Syzygium Cumini* L.), found in western Gujarat of Gir forest region (India), produced fruits with different size and shape. Fruit morphology like shape, volume, weight, length, girth were examined and black jamun categorized into six landraces viz., BJLR 1 (big fruit, > 11 g); BJLR 2 (medium to big fruit, 8–11 g); BJLR 3 (medium fruit, 6–8 g); BJLR 4 (medium to small fruit, 5–6 g); BJLR 5 (small fruit, 3–5 g) and BJLR 6 (very small fruit, < 3 g fruit weight). The landraces (BJLR 1 and 2) with larger size fruits were accumulated higher amount of moisture, total fat content, sugars, total protein, starch, free amino acid contents. Smaller fruits (BJLR 6) contained higher amount of ascorbic acid—137 and 132 mg%; anthocynin—47.7 and 2.35 mg%; crude fibre 3.05 and 10.5 g%; and total phenol—21.7 and 45.0 mg g^{-1} in their fruit pulp and seed part, respectively with better nutritional profile compared with big and moderate fruited landraces. Nutritional profile of six landraces indicated that fruit pulp accumulated higher amount of soluble sugars (6.51–17.6 mg g^{-1}), anthocyanins (29.7−47.7 mg%) and free amino acids (7.54–18.9 mg%) while that of seeds exhibited higher amount of crude fibre (6017–10.5 g%), ascorbic acid (90–137 mg%), starch (22.8–29.4 g%), total protein (4.72–7.17 mg%), phenols (45–56.7 mg g^{-1}). The black jamun landraces were subjected to ISSR based polymorphic finger prints and genetic diversity analysis. Total 144 bands were amplified across six landraces by 18 UBC primers, of which 94 were polymorphic with 64.2% average polymorphism. Cluster analysis demonstrates the BJLR 6 landraces distinguished from other landraces with 53% similarity.

Keywords *Syzygium cumini* L. · Fruit morphology · Nutritional composition · ISSR polymorphism · DNA finger prints

Introduction

The black jamun (*Syzygium cumini* L.) is an important indigenous plant of the family *Myrtaceae* originally from Indonesia and India. It is a big, evergreen tree widely distributed in different agro-climatic conditions in South Asia but remains underutilized. The black jamun is a tropical tree with oblong opposite leaves that are smooth and glossy, has a terpentine smell. It is a fairly fast growing species that can reach heights of up to 30 m and can live more than 100 years. Its dense foliage provides shade and it is grown just for its ornamental value. The wood is strong and it is water resistant. The plant grows with lance-shaped leaves and greenish yellow flowers. The ripened fruit has a scent and taste of ripe apricots. Fruits with purple flesh are more astringent than the white-fleshed types. The leaves and fruits of black jamun have medicinal properties. The fruit pulp and seeds are sweet, acrid, sour, tonic and cooling, and are used in diabetes, diarrhoea and ringworm.

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The bark is astringent, sweet, sour, diuretic, digestive and anthelmintic (Benherlal and Arumughan 2007).

According to nutritionists, the fruit of black jamun is rich in carbohydrates, minerals and vitamins; and the fruit pulp consisted of glucose and fructose as major sugars contributing sweetness of pulp besides some important minerals such as manganese, zinc, iron, calcium, sodium and potassium (Roy et al. 2013; Kochhar et al. 2006). Jamun is recommended for kapha and pitta doshas. The ripen jamun fruit is well recognized as a liver stimulant, digestive, carminative and coolant. Their hypoglycaemic (lowering blood sugar) property is well recognized in Ayurveda and Siddha system of medicine in India (Paganga et al. 1999). The ripen fruits are purplish black in colour due to the presence of anthocyanins. Fruits have high antioxidant property which contributes to many health benefits. Jamun is highly perishable, therefore, very difficult to store and market at distant places. The presence of oxalic acids, tannic acids, gallic acid and certain alkaloids makes one to feel such an astringency taste.

The molecular markers are used for analyzing genetic diversity because of their abundant polymorphism and the fact that they are independent of environment (Gepts et al. 1993). In black jamun, polymerase chain reaction based random amplified polymorphic DNA (PCR-RAPD) markers were reported for molecular fingerprinting and genetic diversity analysis (Vural 2009; Khan et al. 2010, 2012). Israr et al. (2012) examined genetic diversity analysis in black jamun genotype using inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) primers. The ISSR markers are considered superior to RAPD because it uses repeat anchored or non-anchored primers to amplify DNA sequences between two inverted simple sequence repeats (SSR) (Qian et al. 2001; Zietkiewicz et al. 1994). Based on an ISSR marker linked to a gene of interest, many new markers can be identified in the same region. The ISSR marker does not require a prior knowledge of the SSR targets sequences, high stringency achieved by the annealing temperature and were found to provide highly polymorphic fingerprints (Bornet and Branchard 2001).

The indigenous jamun tree produced different size of fruits with round and oblong shape. The present study aimed to categorize indigenous black jamun landraces of western Gujarat (India), based on fruit size and morphology and to develop nutritional profile of fruit pulp and seed for traditional use (Latitude: 21° 00′ N and Longitude: 71° 00′ E). The study also aimed to ISSR based molecular fingerprints and genetic diversity analysis of indigenous black jamun landraces.

Materials and methods

Plant materials

The indigenous jamun trees, found in gir forest region of western Gujarat (India), were produced fruits of different size, shape and weight. Total six landraces of black jamun were categorized on the basis of fruit weight and size viz., BJLR1 (big fruit); BJLR2 (medium to big fruit); BJLR3 (medium fruit); BJLR4 (medium to small fruit); BJLR5 (small fruit) and BJLR6 (very small fruit). The black jamun fruit ripens in the month of June–July 2015 were harvested from the land races considering three independent replications as individual tree. Fruits were washed with distilled water followed by dry on filter paper. Fruit morphological observations were recorded immediately. The fruits were sealed in polythene bags and stored at − 20 °C for biochemical analysis.

Morphological characterization of fruits

The fruit morphology was examined in three replications as three independent tree of each landraces. Randomly five fruits were selected in each replication and their shape (oblong/round), volume (cm^3), weight (g), length (cm), girth (cm), were recorded. The fruit volume was measured by the water displacement method using graduated cylinder (Teacher's Reference Manual, Grades 4–6 pp. 222–225). The fruit sample was slowly slide into the water and recorded the level of the water rose. Fruit Length and girth were measured by using vernier callipers.

Fresh fruit weight was recorded individual for each replication and were separated manually in pulp and seed. Seeds were further separated to seed coat and kernel for getting individual weight. Pulp to seed ratio was calculated by weight of pulp divided by the weight of seed for five fruits in each replicate. Likewise, kernel to seed coat ratio was calculated by weight of kernel divided by the weight of seed coat.

Biochemical analyses of fruit pulp and seeds

Moisture content of pulp and seed was estimated according to AOAC method (AOAC 1990). Fresh pulp and seeds (10 g) were ground well in a tissue homogenizer with methanol:chloroform:water (2:2:1). The homogenate was centrifuged at 8000 rpm for 15 min at 22 °C and supernatant was collected separately. Dehydrated chloroform fraction was then de-solventized by keeping it at 35 °C in incubator for 2 h and the amount of total fat was quantified gravimetrically (Bligh and Dyer 1959). Total soluble sugars in pulp and seed were estimated by phenol–sulphuric

acid method (Dubois et al. 1956). The methanolic extract of fresh pulp and seeds was refluxed for 6 h at 80 °C and total soluble sugars estimated by phenol–sulphuric acid method (Dubois et al. 1956). Total free amino acid from methanolic extract was estimated using ninhydrin reagent (Lee and Takahashi 1966). Total phenolic compounds was determined using Folins–Ciocalteu reagent (Jayasinghe et al. 2003).

Fruit pulp and seeds were subjected to remove soluble sugars by washing with 80% methanol. The residues were then treated with 10 ml water and 15 ml perchloric acid and extracted for 20 min (Sadasivam and Manickam 1992). Carbohydrate content in the extracts were quantified by phenol sulphuric acid method and starch was quantified as g% by multiplying the result with factor 0.9 (Hodg and Hofreiter 1962). Total crude fibre in pulp and seed was estimated by AOAC method (AOAC 1990). The Ash content was measured by gravimetrically. The differences in weight (g) obtained from crude fibre estimation were utilized to calculate ash content (g%).

For ascorbic acid, the samples were extracted in meta phosphoric acid: acetic acid solution and known aliquot was taken for titration against the indophenol dye until light pink colour persist (Malik and Singh 1980). Anthocyanin content of the fresh pulp and seed were extracted with acidic ethanol the and the absorbance was measured at 535 nm in UV–visible spectrophotometer (Francis 1982). Total protein content was estimated based on nitrogen content by Kjeldahl method, (AOAC, 1990). Total protein content was calculated by multiplying the nitrogen content (calculated from titre value) in the sample with the factor 5.64 and expressed as mg% (Levey et al. 2000).

Fruits were collected from three independent trees in three replicates for each landrace. Analyses were performed in duplicate for each parameter from bulk samples of five fruits per replication. Statistical analysis was performed by subjecting the data to analysis of variance and analyzing them by complete block randomized design (CRD) as statistical tools for interpretation of data (Snedecor and Cochran 1967).

Molecular fingerprints of black jamun landraces

The genomic DNA was isolated from normal leaves of three tree plants per landraces by modified cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987) with some modifications (Oza et al. 2008). In order to perform PCR-ISSR based analysis, the DNA purity and concentration was determined by Picodrop PET01 using software v2.08 (Picodrop Ltd., Cambridge UK). The purity of genomic DNA of landraces was found between 1.76 and 1.83 as ratio of $A_{260/280}$. The concentration of DNA was diluted to 50 ng/µl for PCR-ISSR amplification. Initially, 100 UBC series primers were screened with genomic DNA of two landraces (BJLR1 and BJLR6), as a result 18 primers gave satisfactory polymorphism, which were utilized for polymorphism in all landraces and further analysis.

The amplification was carried out in a 20 µl reaction volume containing 10 mM Tris–HCl (pH 8.0), 1.5 mM $MgCl_2$, 0.1 mM each dNTP, 200 nM primer, 1U Taq DNA polymerase (Merck bioscience, India), and 50 ng of genomic DNA. The polymerase chain reaction (PCR) amplification was performed in an ABI gradient thermal cycler after an initial denaturation at 94 °C for 5 min; followed by 40 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C; with a final 7 min extension at 72 °C (Hakki et al. 2010). After the process, samples of 15 µl of the amplification products were assayed by 1.5% agarose gel containing ethidium bromide, running with Tris borate EDTA (TBE) buffer. The submerge gel electrophoresis (Bio-Rad) was carried out at 80 V for about 100 min. After that, the gel was viewed under UV trans-illuminator (Gel doc) for visualizing separated bands. The 100 bp DNA ladder was loaded in first lane of each gel.

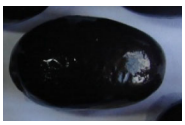
At DNA levels, amplifications were repeated once more for each ISSR primers and only consistent bands were considered for scoring. The NTSYS.PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) system version 2.2 by Exeter Software was used for data analysis (Rohlf 2004). The bands presence or absence were introduced in the form of abinary matrix and a pair wise similarity matrix was constructed using the Jaccard's coefficient. The SIMQUALK programme was used to calculate Jaccard's genetic distance coefficient analysis and a graphical phenogram (dendrogram) of the genetic relatedness among the different landraces was generated by means of the unweighted pair group method with arithmetic average (UPGMA) analysis (Sneath and Sokal 1973). Robustness of the nodes was tested by bootstrap analysis using 1000 resamplings. Size of specific bands of DNA was determined using software Alphaimager 2200, Alpha Ease FC, USA. A polymorphic information index (PIC) for ISSR profile was calculated as $PIC = 1 - p^2 - q^2$, where, p is band frequency and q is no band frequency (Ghislain et al. 1999).

Results and discussion

Fruit morphology of black jamun landraces

Morphological characterization of six landraces of black jamun was recorded as fruit shape, volume, weight, length, girth, pulp to seed ratio and kernel to seed coat ration (Table 1). The fruit shape of six landraces was found to be round and oblong. The fruits of BJLR1, BJLR2, BJLR4

Table 1 Fruit morphology of six landraces of black jamun

Black jamun landraces	Fruit image	Fruit shape	Fruit volume (cm^3)	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Pulp to seed ratio	Kernel to seed coat ratio
BJLR-1		Round	10.70	12.12	3.25	2.32	3.01	5.34
BJLR-2		Round	8.00	9.06	2.53	2.22	2.24	5.36
BJLR-3		Oblong	5.10	6.50	2.58	1.76	1.79	5.16
BJLR-4		Round	4.10	5.32	2.75	1.58	1.73	5.65
BJLR-5		Round	3.50	4.62	2.13	1.56	1.60	5.18
BJLR-6		Oblong	1.80	2.33	1.88	1.22	1.11	3.93
S.Em. ±			0.43	0.41	0.08	0.07	0.07	0.55
C.D. at 5%			1.26	1.20	0.25	0.21	0.20	0.85
C.V. %			7.50	3.78	7.48	8.87	6.60	7.20

Values are mean of three replications; each replication consisted of mean data of five fruits of individual tree

and BJLR5 were round in shape while BJLR3 and BJLR6 were oblong in shape. Roy et al. (2013) also reported that the black jamun fruits are round in shape. The fruit volume of six landraces was ranged from 1.80 to 10.70 cm^3. The significantly highest fruit volume was observed in BJLR1 landraces (10.70 cm^3), while lowest was recorded in BJLR6 (1.80 cm^3). The fruit weight of six landraces was ranged from 2.33 to 12.12 g. The fruit weight was observed highest in BJLR1 landraces (12.12 g), followed by BJLR2 (9.06 g), BJLR3 (6.50 g), BJLR4 (5.32 g), BJLR5 (4.62 g) and BJLR6 (2.33 g). The landraces of black jamun were characterized on the basis of fruit volume and weight viz., BJLR1 (big fruit, > 11 g); BJLR2 (medium to big fruit, 8–11 g); BJLR3 (medium fruit, 6–8 g); BJLR4 (medium to small fruit, 5–6 g); BJLR5 (small fruit, 3–5 g) and BJLR6 (very small fruit, < 3 g fruit weight).

Among the landraces, significant differences were observed in fruit length, girth, pulp to seed ratio and kernel to seed coat ratio. The fruit length of six landraces was ranged from 1.88 (BJLR6) to 3.25 cm (BJLR1). The fruit girth of six landraces was ranged from 1.22 to 2.32 cm. The highest fruit girth was observed in BJLR1 (2.32 cm) followed by BJLR2 (2.22 cm) landraces, while lowest was observed in BJLR6 (1.22 cm). The pulp to seed ratio was found highest in BJLR1 (3.01) and lowest recorded in BJLR 6 (1.11). The kernel to seed coat ratio was elevated in BJLR4 (5.65) followed by BJLR2 and BJLR1 while the lowest was recorded in BJLR 6 (3.93).

Nutritional profile of fruit pulp and seed of black jamun landraces

Different nutritional parameters were measured from fresh pulp and seed of six black jamun landraces and found significant differences as depicted in Table 2. The moisture content was recorded highest in pulp of the BJLR3 (90.6%) followed by BJLR4 (88.8%) while lowest were found in BJLR6 (76.1%), seed moisture was found to be highest in BJLR5 (68.9%) followed by BJLR1 (65.7%) and lowest in BJLR6 (54.9%). Similar to present study, Chowdhury and Ray (2007) reported that the fruit of black jamun content 83.20% moisture. Noomrio and Dahot (1996) studied on biochemical composition of black jamun fruits and reported that fruits are varied in moisture content between 83.70 and 85.80%. However, moisture content from the seeds of black jamun was estimated by Kochhar et al. (2006). They reported seed contained 40.86% moisture. Our study showed variation in seed moisture in the range of 54.9% (BJLR 6) to 68.9% (BJLR 5).

The fat was higher in pulp from BJLR5 (1.81 mg g^{-1}) and lower in that from BJLR3 (0.60 mg g^{-1}). Seed of BJLR 2 contained the highest (0.87 mg g^{-1}) total fat and the lowest found in BJLR 4 (0.23 mg g^{-1}). Noomrio and Dahot (1996) reported that 0.15–0.30 g% fat in black jamun fruit, which agreed with our results. However Kochhar et al. (2006) studied biochemical composition of black jamun seed and reported that seed contained 1.55 mg% fat. Total soluble sugar was significantly found highest in fruit pulp of BJLR 3 (17.6 mg g^{-1}) and lowest in BJLR 1 (6.51 mg g^{-1}), While in seed, the content was recorded highest in BJLR 1 (2.16 mg g^{-1}) followed by BJLR 3 (2.01 mg g^{-1}) and lowest in BJLR 4 (mg g^{-1}).

Crude fibre was found to be higher in pulp of BJLR 3 (3.65 mg%) followed by BJLR 1 (3.52 g%) and lowest in BJLR 2 (2.95 g%), While in seed, the content was highest in BJLR 6 (10.53 g%) and lowest in BJLR 1 (6.17 g%). Ash content was found significantly in the range of 0.93 g% (BJLR2) to 1.02 g% (BJLR3, BJLR5). Differences were not evident for ash content in pulp and seed tissues of black jamun landraces.

Table 2 Nutritional composition of fruit pulp and seed of six black jamun landraces

Sr. No.	Parameters	Fruit parts	BJ LR 1	BJ LR 2	BJ LR 3	BJ LR 4	BJ LR 5	BJ LR 6	S Em±	CD at 5%	CV%
1.	Moisture, g%	Pulp	82.4	79.5	90.6	88.8	82.9	76.1	0.55	1.71	1.15
		Seed	65.7	62.4	63.0	56.1	68.9	54.9	0.92	2.83	2.56
2.	Total Fat, mg g^{-1}	Pulp	0.81	0.84	0.60	1.03	1.81	1.28	0.02	0.07	3.72
		Seed	0.78	0.87	0.53	0.23	0.30	0.40	0.01	0.04	4.15
3.	Total soluble sugars, mg g^{-1}	Pulp	6.51	11.1	17.6	11.8	14.8	7.3	0.53	1.64	7.99
		Seed	2.16	1.97	2.01	1.03	1.48	1.64	0.06	0.17	5.61
4.	Crude fibre, g%	Pulp	3.52	2.95	3.65	3.00	3.06	3.05	0.05	0.14	2.44
		Seed	6.17	6.47	7.27	8.74	10.4	10.5	0.05	0.15	1.03
5.	Ash content, g%	Pulp	1.01	0.93	1.02	1.01	1.02	1.01	0.01	0.02	1.26
		Seed	1.00	0.99	1.01	0.99	0.98	1.00	0.01	0.02	1.10
6.	Ascorbic acid, mg%	Pulp	66	88	80	113	105	137	0.73	4.05	2.72
		Seed	108	105	90	137	96	132	0.86	2.07	3.29
7.	Anthocynin, mg%	Pulp	29.7	41.1	43.0	37.2	39.6	47.7	0.37	1.14	1.62
		Seed	0.86	1.07	1.28	2.35	1.71	2.35	0.20	0.60	1.08
8.	Starch content, g%	Pulp	19.5	15.1	21.4	21.9	22.4	15.4	0.50	1.54	4.48
		Seed	24.9	23.6	29.4	25.8	25.3	22.8	0.55	1.70	3.77
9.	Total protein, mg%	Pulp	3.51	4.46	5.43	5.95	3.53	3.16	0.17	0.52	6.75
		Seed	5.14	4.72	7.07	7.14	5.80	5.78	0.08	0.16	1.50
10.	Total phenols, mg g^{-1}	Pulp	28.9	20.0	23.4	13.0	21.8	21.7	0.05	0.12	1.31
		Seed	48.4	56.7	54.4	56.6	54.3	45.0	0.21	0.64	3.68
11.	Free amino acids, mg%	Pulp	7.54	14.6	15.5	18.5	18.9	12.9	0.17	0.51	1.96
		Seed	7.99	8.78	8.78	4.84	9.90	8.55	0.18	0.57	3.91

Values are mean of three replications; Analysis carried out in duplicate within replication from bulk samples of five fruits per tree for each landraces

S. Em. ± = Standard error of mean; *CD* Critical differences, *CV%* Coefficient of variance

Ascorbic acid content was found to be varies between 66 and 137 mg% in pulp. The ascorbic acid content was recorded in high amount in seed compared with pulp. The content was found significantly highest in the pulp (137 mg%) and seed (132 mg%) of small fruited BJLR 6 landraces. The content was recorded lowest in pulp of BJLR 1 (66 mg%) and seed of BJLR 3 (90 mg%). The anthocynin was found to be highest in the pulp of BJLR 6 (47.7 mg%) and lowest in BJLR 1 (29.7 mg%), In seed, the content was recorded to be highest in BJLR 4 and BJLR 6 (2.35 mg%) while lowest in BJLR 1 (0.86 mg%). Overall, The BJLR1 landraces is poor in anthicynin and ascorbic acid content in their fruit pulp and seed compared with BJLR 6.

Total protein was found to be significantly highest in the pulp of BJLR4 (5.95 mg%) and lowest in BJLR 6 (3.16 mg%). Seed protein was recorded highest in BJLR4 (7.14 mg%) and lowest in BJLR2 (4.72 mg%). In pulp, starch content was significantly found highest in BJLR 4 (21.9 g%) and lowest in BJLR2 (15.1 gm%), In seed, highest content was recorded in BJLR 3 (29.4 gm%) and lowest in BJLR6 (22.8 gm%). Total phenol was found significantly highest in pulp of BJLR6 (28.9 mg g^{-1}) and lowest in BJLR4 (13.0 mg g^{-1}). While, seed phenol was recorded highest in BJLR6 (56.7 mg g^{-1}) and found lowest in BJLR1 (45.0 mg g^{-1}). Overall, phenol content was examined higher in seed tissue of all six landraces compared with fruit pulp. In pulp, free amino acid was recorded highest in BJLR 5 (18.5 mg%) and lowest in BJLR 1 (7.54 mg%). In seed, the content was recorded highest in BJLR 5 (9.90 mg%) and lowest in BJLR 4 (4.84 mg%). Pulp contained higher amino acids compared with seed of six landraces of black jamun.

Roy et al. (2013) described two different types—big and small size fruits of black jamun. The small fruits are round in shape, grow wild and have very little pulp. The small sized fruits are ideal for processing. They contain high amount of acids, tannins and anthocyanins. The big fruits are oblong in shape, have more pulp and are suitable for fresh marketing. They have high Brix ratio and low contents of acid, tannin and anthocyanin which agreed with present results except fruit shape.

Kochhar et al. (2006) studied on biochemical composition of black jamun seed and reported that it contained 40.86% moisture, 4.16 mg crude protein, 1.55 mg extractable fat, 2.16 mg ash, 1.28 mg crude fibre, 90.85 mg total carbohydrate, 29.20 mg starch, 361.40 mg poly phenol and 393.96 kcal energy per 100 g of edible portion, which partially support the present data of biochemical composition of fruit pulp of black jamun. Chowdhury and

Table 3 Polymorphism pattern obtained by ISSR markers across six landraces of black jamun

Sr. no.	Name of primer	Primer sequence (5′…3′)	Marker range (bp)	Polymorphic bands			Monomorphic bands	Total bands	%Poly morphism	PIC value
				S	U	T				
1.	UBC 807	$(AG)_8T$	351–2630	2	4	6	4	10	60.0	0.790
2.	UBC 810	$(GA)_8T$	636–1770	8	0	8	2	10	80.0	0.835
3.	UBC 814	$(CT)_8A$	570–1629	1	4	5	2	7	71.4	0.821
4.	UBC 815	$(CT)_8G$	659–1115	10	1	11	0	11	100.	0.500
5.	UBC 817	$(CA)_8A$	402–1612	4	3	7	3	10	70.0	0.867
6.	UBC 822	$(TC)_8A$	443–1852	3	3	6	2	8	75.0	0.837
7.	UBC 823	$(TC)_8C$	287–1271	1	2	3	7	10	30.0	0.822
8.	UBC 834	$(AG)_8YT$	277–1531	2	5	7	5	12	58.3	0.894
9.	UBC 835	$(AG)_8YC$	319–1292	1	1	2	3	5	40.0	0.849
10.	UBC 836	$(AG)_8YA$	946–1581	4	0	4	0	4	100.0	0.675
11.	UBC 840	$(GA)_8YT$	378–1622	2	3	5	2	7	71.4	0.813
12.	UBC 844	$(CT)_8RC$	293–1987	6	2	8	6	14	57.1	0.901
13.	UBC 848	$(CA)_8RG$	656–1943	3	1	4	0	4	100.0	0.612
14.	UBC 855	$(AC)_8TT$	274–1769	7	1	8	0	8	100.0	0.853
15.	UBC 856	$(AC)_8TA$	924–1877	1	0	1	2	3	33.3	0.595
16.	UBC 862	$(AGCA)_3$ $(GC)_3$	220–1663	5	2	7	2	9	77.7	0.864
17.	UBC 864	$(ATGA)_3$ $(TG)_3$	251–1753	1	0	1	5	6	16.6	0.826
18.	UBC 889	DBD $(AC)_7$	607–1283	1	0	1	5	6	16.6	0.814
Average				3.4	1.8	5.2	2.8	8	64.2	0.79
Total				62	32	94	50	144	–	–

S Shared, *U* Unique, *T* Total Polymorphic Bands, *PIC* Polymorphism information content

Ray (2007) reported that the fruit of black jamun contained 83.2% moisture, 14.0 g reducing sugar, 0.90 g crude fibre, 0.25 g ascorbic acid, 0.1 g anthocyanin 0.33 g ash, 0.13 g nitrogen, 1.90 g tannin and 0.30 g fat per 100 g edible pulp. Noomrio and Dahot (1996) studied biochemical composition of black jamun fruits. Fruit contained 83.70–85.80 g% moisture, 0.70–0.13 g% protein, 0.15–0.30 g% fat, 0.30–0.90 g% crude fiber, 14.00 g% carbohydrate, 0.32–0.40 g% ash, 8.30–15.00 mg% free amino acids. Our results agreed with above reported values of researchers except crude fibre and phenolics content which were examined higher in fruit pulp and seed of all black jamun landraces.

Molecular fingerprints and diversity analysis of black jamun landraces

Polymorphic pattern among black jamun landraces

The DNA fingerprinting of indigenous black jamun landraces was subjected to ISSR polymorphism. Initially, 100 UBC series primers were screened with genomic DNA of two landraces (BJLR1 and BJLR6), as a result 18 primers gave satisfactory polymorphism which were utilized for polymorphism in all landraces and further analysis. Band size, number of amplified bands, per cent polymorphism and PIC obtained by ISSR primers were depicted in Table 3. Total 144 bands were produced by 18 UBC primers with average 8 bands per primer. Out of total 144 bands, 50 were monomorphic and 94 were polymorphic with average 5.2 bands per primer. Polymorphic bands were again categorised into shared and unique. Total 62 polymorphic bands were shared with at least two black jamun landraces with an average of 3.4 bands per primer. However, total 32 polymorphic bands were unique and found in at least one black jamun. This unique polymorphic bands was useful for identification of the landraces. The average per cent polymorphism was found to be 64.2%. The ISSR marker UBC-844 produced maximum number of 14 bands, while UBC-856 produced minimum number of 3 bands. The highest PIC value (0.901) was recorded with UBC 844, considering most informative markers, while lowest PIC (0.500) value was recorded with UBC 815 (Table 3).

Specific markers obtained by ISSR profile for landraces

Total 28 unique markers were produced by 14—UBC primers for identification/discrimination of 6 landraces of black jamun (Table 4). Primer UBC 834 were found with

Table 4 Unique specific markers (bp) generated by ISSR primer for black jamun landraces

Sr. no.	Primer	BJ LR 1	BJ LR 2	BJ LR 3	BJ LR 4	BJ LR 5	BJ LR 6	Total unique bands
1.	UBC 807	1235	2612	–	–	–	1697	4
		692	–	–	–	–	–	
2.	UBC 810	–	–	–	–	–	–	–
3.	UBC 814	–	843	–	–	–	696	2
4.	UBC 815	–	789	–	–	–	–	1
5.	UBC 817	1319	–	–	–	1512		2
6.	UBC 822	–	–	–	–	–	1780	3
		–	–	–	–	–	1319	
		–	–	–	–	–	542	
7.	UBC 823	–	–	724	–	–	1216	2
8.	UBC 834	1451	–	–	–	–	711	5
		–	–	–	–	–	498	
		–	–	–	–	–	275	
		–	–	–	–	–	595	
9.	UBC 835	–	–	–	–	–	796	1
10.	UBC 840	–	1386	–	391	–	–	3
		–	591	–	–	–	–	
11.	UBC 844	–	–	–	535	–	–	2
		–	–	–	295	–	–	
12.	UBC 848	–	–	–	–	–	1261	1
13.	UBC 855	–	341	–	–	–	–	1
14.	UBC 862	–	1142	–	652	–	–	2

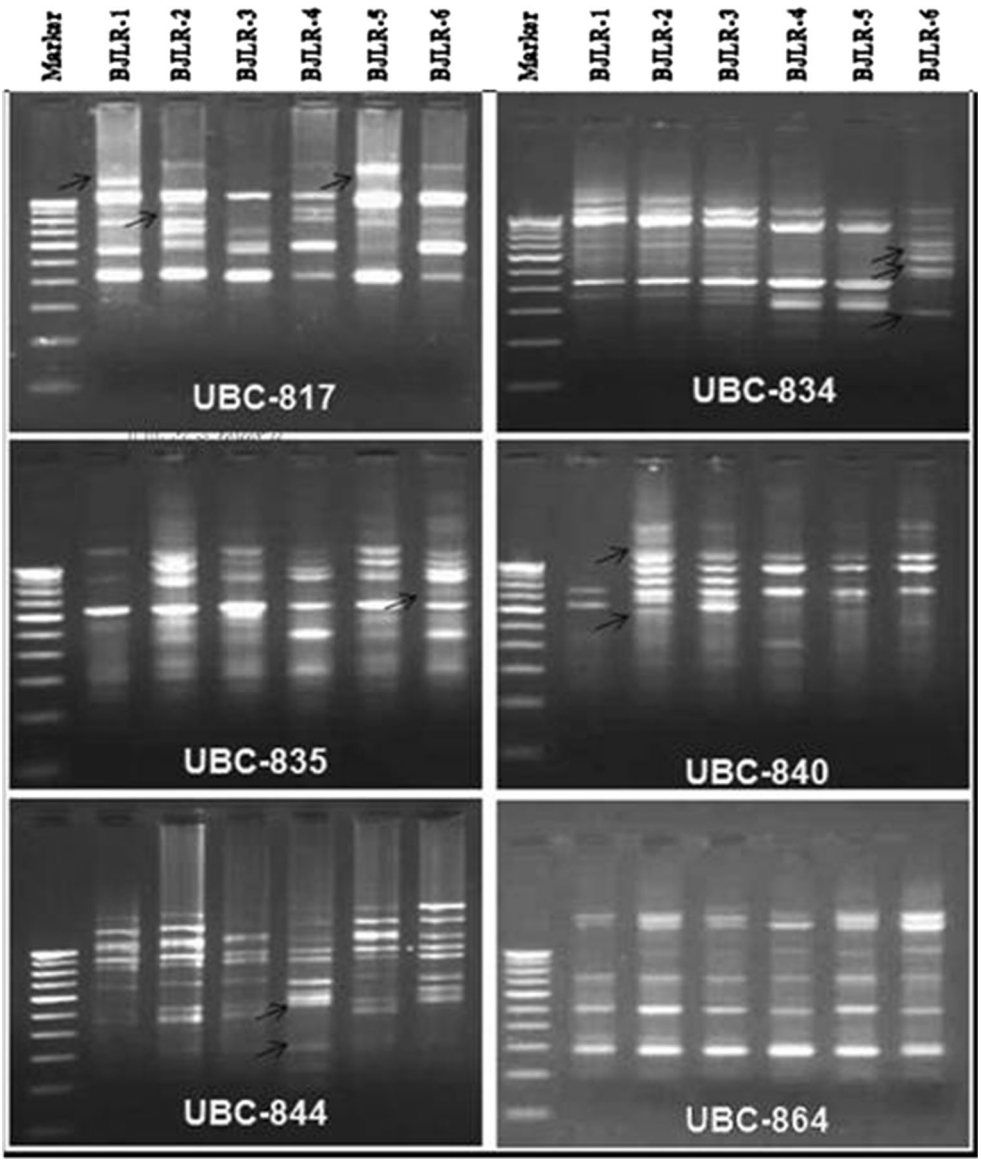


Fig. 1 DNA fingerprints generated by UBC series markers in black jamun (*Syzygium cumini* L.) landraces (Marker—100 bp DNA ladder)

four unique bands (275, 498, 595, 711 bp) for identification of most diverse small fruited BJLR 6 landraces (Fig. 1) with respect to good nutritional profile in their fruit pulp and seed (Table 2) and one unique fragment of 1451 bp for discrimination of big fruit landraces BJLR 1 which was nutritionally poor. Total 4 unique bands were obtained by ISSR primers UBC 807 (1235, 692 bp), UBC 817 (1319 bp) and UBC 834 (1451 bp) for identification of BJLR 1 landraces. Similarly, seven unique bands were examined for BJLR 2, one for BJLR 3, three for BJLR 4 and one for BJLR 5. However, maximum 12 unique bands were found by 6 ISSR primers for identification BJLR 6 landraces, which have been very small fruit size with good nutritional profile with respect to higher ascorbic acid, anthocynin and phenolics in fruit pulp and seed compared with other five indigenous landraces of black jamun.

Phylogenetic relationship among black jamun landraces

Dendrogram constructed using UPGMA based on Jaccard's similarity coefficient for 6 black jamun landraces clearly indicates two main clusters (Fig. 2). Cluster-A consisted of single landraces, BJLR1 (Very big fruit size and nutritionally poor) and it showed 41% similarity with other five landraces. Cluster-B comprised of five landraces, BJLR2, BJLR3, BJLR4, BJLR5 and BJLR6. The highest similarity index value of 0.606 was found between BJLR 2 and BJLR 3 (medium to big fruit size with higher in sugars and protein content) while the lowest similarity index value of 0.435 was between BJLR1 and BJLR4. Jaccard similarity coefficient is between the ranges of 0.41–0.61. In cluster B, the black jamun landraces BJLR 6 were

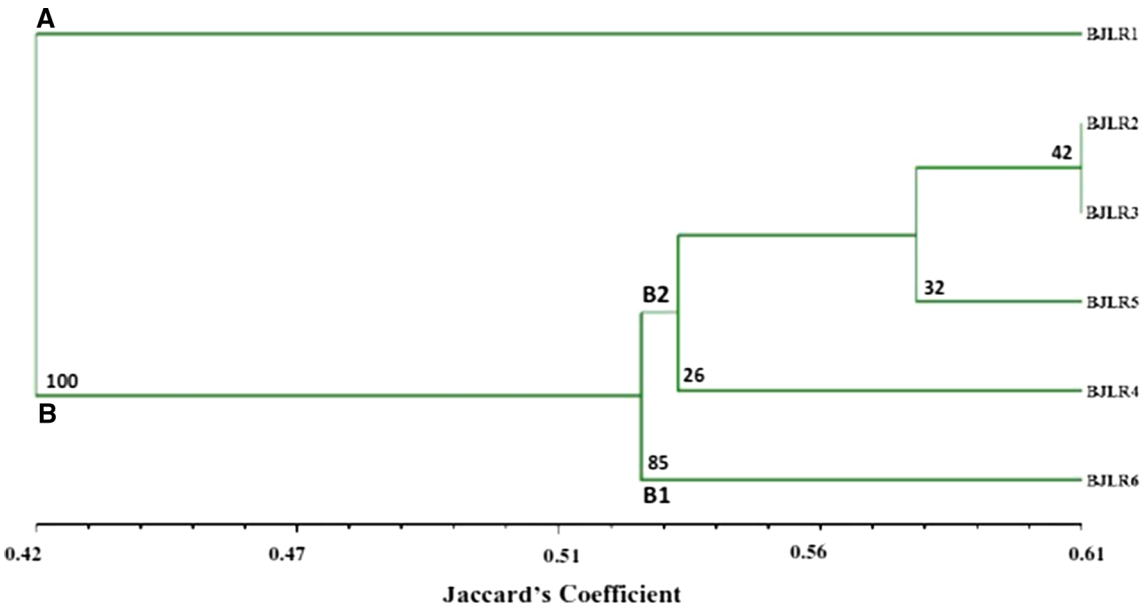


Fig. 2 Dendrogram depicting the phylogenetic relationship among six black jamun landraces based on ISSR data (The numbers represent per cent bootstrap support of each node and NTSYS.PC system version 2.2 by Exeter Software was used for dendrogram construction)

outgrouped from other four landraces with 52% similarity, which have better nutritional profile in their fruit parts (pulp and seed). Thus, six indigenous landraces of black jamun clustered according to their nutritional composition.

Israr et al. (2012) studied genetic diversity in black jamun genotype using DNA marker technology. A set of 30 ISSR primers were taken for DNA fingerprinting, among them 17 ISSR primers produced 70 bands, out of which 43 amplicons were polymorphic with 61.4% polymorphism. The maximum discriminating band was obtained from primer ISSR8. Cluster analysis divided the all genotype into five clusters with ISSR markers. So far, molecular fingerprints of black jamun by ISSR markers were only reported by Israr et al. (2012). However, Khan et al. (2012) studied the genetic variability in two populations of black jamun growing in fluoride rich soils and normal soils located in Rajasthan and Haryana regions of India, respectively using RAPD markers. Khan et al. (2010) used RAPD markers to detect inter and intra levels of genetic variations of black jamun genotypes collected from three major agro-ecological zones of India. A total of 220 amplification products were scored of which 7.50% were polymorphic. The level of polymorphism ranged from 47.69 to 74.87% by RAPD markers. Present study was evident 30–100% polymorphism with ISSR markers. The ISSR markers are considered to be better than RAPD for polymorphism and molecular finger printing (Israr et al. 2012; Qian et al. 2001).

Conclusion

The indigenous black jamun landraces were categorized based on fruit size, shape and weight. Generally, big fruit (BJLR 1) contained higher amount of moisture, total fatty matter, sugars, total protein, starch content, free amino acid. While, smaller fruits (BJLR 6) evident higher amount of ascorbic acid, anthocynin, crude fibre, and total phenol in their fruit pulp and seed with better nutritional profile compared with big and moderate fruited landraces. The small fruits (big seeded) are oblong in shape with little pulp, grow wild and thus ideal for processing. The phylogenetic relationship and diversity analysis of six indigenous black jamun landraces clearly outgrouped landraces based on fruit morphology and nutritional composition. The BJLR 6 (smallest fruit size) landrace having better nutritional profile in their fruit parts (pulp and seed) distinguished from other landraces (big and moderate fruit size) and shared 53% similarity. Total 32 unique bands were amplified by 18 UBC primers to discriminate 6 landraces of black jamun. Primer UBC 834 were found to be most informative as it provided 4 unique bands of DNA finger prints for identification of most diverse BJLR 6 landraces, with respect to nutritional profile, and one unique band for discrimination of big fruited BJLR 1.

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