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Morphogenetic analysis of pineapple cultivars of Tripura

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ABSTRACT

Pineapple is grown in India in diverse agro-ecological zones and Queen, Kew and Mauritius are grown in commercial plantation and farming systems. Selections of superior genotypes of existing varieties are important in identifying potential regions for area expansion in new region as well. Morphological and molecular diversity of four pineapple cultivars of Tripura were undertaken. High phenotypic variances were observed for number of suckers, number of slips, crown weight, fruit weight, percentage of heart rot and percentage of cannery recovery. On the other hand heritability of most of the characters was found very high, except fruit length and number of eyes per fruit. In morphological analysis, clone, belonging to Mauritius showed dissimilarity with Kew but similarity with Queen. Ten ISSR and 20 RAPD primers were tested for their ability to give reaction to pineapple DNA. Among them, 8 primers showed polymorphism. However, RA_1 and IS-12 gave only monomorphic bands. Level of polymorphism was found very low. Among the RAPD primers, RA_2 showed the highest PIC value (0.30) followed by RA_4 (0.18) and among the ISSR primers IS-9 and IS-6 with 0.28 were found informative. Molecular analysis showed close similarity with Queen and Kew. Clone PQM-1, though was more similar to Queen in morphological parameters was distant from Queen in molecular diversity analysis. Mauritius was almost similar to Queen, which was shown from the very low Euclidian distance coefficient (52.0). The highest dissimilarity coefficient was estimated for Kew (452.8).

Key words: Pineapple, genetic diversity, SSR markers.

INTRODUCTION

Pineapple [*Ananas comosus* (L) Merill.] is one of the commercial fruit crops of India and its cultivation is the age-old practice in North Eastern Hill region, particularly in Tripura. The beauty and virtue of this "Golden Queen" have been extolled by many poets all over the Globe. Pineapple has all qualities required for being an ideal fruit crop for North Eastern India and Tripura as well. The name 'Ananas', which later became the generic name, is derived from Tupi Indian name 'Nana'. Pineapple is a unique fruit for its beauty of appearance, delicate fragrance and excellent flavour. Pineapple cultivation is an age old practice and still going on with various cultivars and different methods among the tribes. Commercial orchards are with various types of forest trees and most of them are deciduous in nature which also protects them from high light intensity during summer months (Prakash *et al.*, 11). At present, Kew and Queen varieties are commercially grown in Tripura and Mauritius is grown mostly in government orchard and experimental farm of research institutes. Owing to a wide range of climatic and edaphic conditions in these regions, mutation would have occurred, causing wide variations. Although many research works have been conducted for analyzing plant morphological and biochemical diversity in pineapple in India and abroad (Coppens d'Eeckenbrugge *et al.*, 4; Perez *et al.*, 9; Heenkenda and Sangakkara, 6; Prakash *et al.*, 10), no proper attempt has been made to study the genetic variability of important traits and diversity of Tripura cultivars. On the other hand, molecular tools have been suggested and practised for understanding true genetic diversity and identification of varieties or ecotypes (Noyer *et al.*, 8; Duval *et al.*, 5; Kato *et al.*, 7; Prakash *et al.*, 10). In the present investigation, field studies were conducted to analyse the genetic nature of traits directly or indirectly related to market and industry needs. Analysis of diversity of pineapple cultivars was also done in view of varietal improvement for commercial cultivation in the humid tropical region of Tripura. Moreover, knowledge on its diversity structure is needed to design new breeding programmes. Morphological diversity was also compared with molecular diversity of pineapple cultivars grown in Tripura.

MATERIALS AND METHODS

Four cultivars each one belonging to Queen, Mauritius, Kew and PQM-1 (Pineapple Queen Mutant-1: a clonal selection from Queen) were grown at Experimental Farm of ICAR Research Complex for NEH Region Tripura Centre, Lembucherra for the last three years (2006-2009). Hundred suckers of each clone were planted in 30 cm × 60 cm × 75 cm

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spacing under double row system during March 2006. The clones were planted in randomized block design with five replications and uniform cultural practices and fertilization were adopted as recommended by Prakash *et al.* (11). Data were recorded on pattern of vegetative growth, plant characters, fruit characters, etc. following the standard procedures (Collins, 3). Biochemical parameters like total soluble solids (TSS) by hand refractometer (ERMA), sugar percentage and titrable acidity were measured on ten randomly selected plants in each plot following standard methods (Ranganna, 12).

From one gram tender leaves of pineapple plant, genomic DNA was extracted by procedure described by Chattopadhyay *et al*. (2). Bulked DNA was subjected to PCR. Each bulk DNA was constituted by genomic DNA pooled from 10 plants. The 25 ml PCR mixture contained 4 ml 2.5 mM DNTPs, 20 ng DNA in 2.5 ml 10 × *Taq* polymerase assay buffer having 1.5 mM $MgCl_2$ and 1.5 u *Taq* polymerase enzyme and 100 ng of primer (Banglore Genei, Bangalore). The PCR products were analyzed on a 1.5% agarose gel in 1 × TBE buffer. Bands were visualized by ethidium bromide staining (0.5 mg/ml) and photographed on UV-trans-illuminator. The efficiency of primers was assessed on the basis of percentage of polymorphic bands and polymorphic information content (PIC) values, ranging from 0.0 to 0.5. [PIC = 1/n Σ 2F (1-F), where, F = proportion of bands per assay unit and n = number of loci detected].

Euclidian distance coefficients were calculated using quantitative data of 16 traits and Jaccard's similarity coefficients were calculated using molecular markers. Dendrograms showing morphological and molecular diversity of genotypes were constructed by Unweighted Pair-Group Method of Arithmetic Mean (UPGMA) algorithm, using SHAN sub-program of NTSYS-pc Ver 2.1 software (Rohlf, 13).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) suggested that all the four types of varieties, namely, Queen, Kew, Mauritius and a mutant PQM-1 significantly differed from one another in respect of all the plant morphological, biochemical and fruit chara cters. High phenotypic variances were observed (Table 1)

Table 1. Genetic analysis of some economically important characters of pineapple in Tripura.

Genetic statistic	D-leaf height (cm)	No. of leaves at red heart stage	No. of suckers	No. of slips	Flowering (%)	Maturity period red heart to harvest (days)	Crown weight (g)	Fruit weight (g)
Mean	97.94 ± 0.78	50.56 ± 0.62	1.46 ± 0.04	3.64 ± 0.08	79.53 ± 0.78	116.75 ± 0.6	327.6 ± 4.17	1279 ± 20.1
ECV	2.05	3.15	6.59	5.67	2.5	1.33	3.29	4.05
GCV	7.47	9.8	16.36	30.37	5.99	11.33	18.48	19.25
PCV	7.75	9.98	17.74	30.9	6.49	11.41	18.77	19.66
Heritability (%)	92.96	90.06	86.06	96.63	85.15	98.64	96.94	95.76
Genetic advance (% of mean)	14.83	18.52	31.27	61.49	11.38	23.18	37.49	38.78
	Fruit length (cm)	Fruit dia. (cm)	No. of eyes/fruit	TSS (°Brix)	Sugars (%)	Acidity (%)	Heart rot (%)	Cannery recovery (%)
Mean	15.43 ± 0.24	14.8 ± 0.14	101.17 ± 2.29	17.25 ± 0.15	12.87 ± 0.13	0.62 ± 0.0	0.21 ± 0.01	30.35 ± 0.45
ECV	4.06	2.5	5.86	2.31	2.6	1.77	10.72	3.83
GCV	4.16	13.37	10.77	6.1	6.19	10.89	97.89	15.37
PCV	5.82	13.6	12.26	6.52	6.72	11.03	98.47	15.84
Heritability (%)	51.23	96.61	77.19	87.48	84.97	97.44	98.82	94.15
Genetic advance (% of mean)	6.14	27.07	19.5	11.75	11.76	22.15	200.5	30.73

in characters, like number of suckers, number of slips, crown weight, fruit weight, percentage of heart rot and percentage of cannery recovery. On the other hand heritability of most of the characters was found very high, except fruit length and number of eyes per fruit. High heritability of most of the traits might be due to clonal propagation of those lines. Similarly, high genetic advance of all the characters except the same two traits, suggested their predictable nature. Fruit length and number of eyes per fruit were highly influenced by the environment, restricting their selection for achieving higher fruit weight (Bartholomew *et al.*, 1).

Ten ISSR and 20 RAPD primers were tested for their ability to give reaction to pineapple DNA. Among them, 8 primers showed reaction (Table 2). However, RA_1 and IS-12 gave only monomorphic bands. Pineapple clones showed polymorphism in rest of the primers. 136 alleles were generated by 8 primers in 34 banding positions. Level of po lymorphism was found very low. Among the RAPD primers, RA_2 showed the highest PIC value (0.30) followed by RA_4 (0.18) (Fig. 1b) and among the ISSR primers IS-9 (Fig. 1a) and IS-6 with 0.28 were found most informative.

All the 16 traits were used in the analysis of morphological and biochemical diversity in pineapple clones of Tripura. Dendrogram revealed that Kew was located totally distant to the rest of the clones (Fig. 2a). Euclidian distance coefficient was used to measure the distances among the clones and distance coefficient

Table 2. PCR-based primers and their efficiency to discriminate pineapple clones of Tripura.

Primer	Sequence (5'-3')	Annealing temp (°C)	PIC (range-0.0-0.5)	Polymorphism (%)
RAPD				
RA1	AGCGCCATTG	36	0.0	0.0
RA2	CATCCGTGCT	36	0.3	18.75
RA4	GTGTGCCCCA	36	0.18	10.0
ISSR				
IS6	GAG AGA GAG AGA GAG AC	52	0.28	16.67
IS9	TGT GTG TGT GTG TGT A	46	0.28	16.67
IS61	GAGAGAGAGAGAGAGAT	50	0.26	15.0
IS11	CAC ACA CAC ACA CAC AG	52	0.22	12.5
IS12	GTG TGT GTG TGT GTG TC	52	0.0	0.0

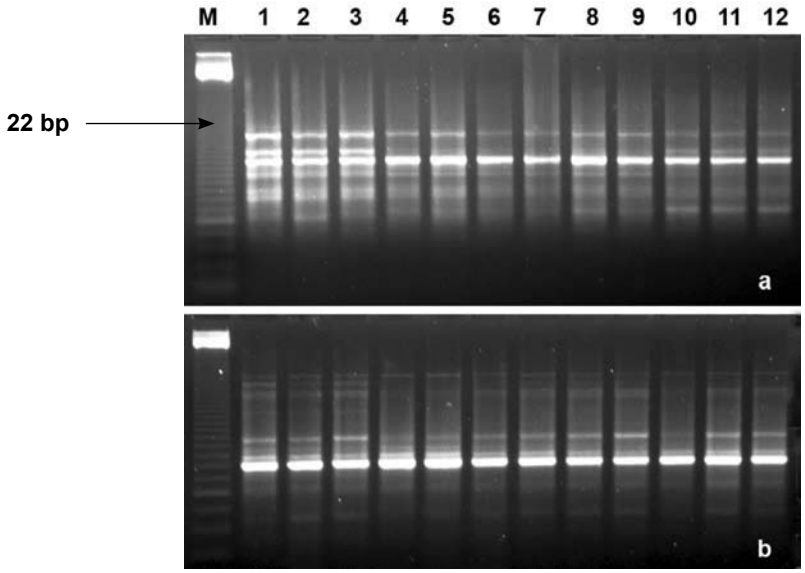

Fig. 1. DNA fingerprinting profile of pineapple cultivars, *viz.*, lane 1-3: PQM-1, lane 4-6: Kew, lane 7-9: Mauritius, lane 10-12: Queen and M: 50 bp DNA ladder generated by a. ISSR primer, IS-9 and b. RAPD primer, RA_4.

matrix was generated (Table 3a). While analyzing the morphological diversity, it was evident that Mauritius was almost morphologically similar to Queen, which was shown from the very low Euclidian distance coefficient (52.0). The highest dissimilarity coefficient was estimated for Kew (452.8).

The RAPD and ISSR primers generated 136 DNA bands which were used in constructing dendrogram showing molecular diversity (Fig. 2b). The clone PQM-1 was distantly located from others. Jaccard's similarity coefficients were used to calculate pair-wise similarity of clones (Table 3b). Unlike the morphological diversity, Mauritius gave almost equal similarity coefficient with Kew and Queen. PQM-1 showed the lowest similarity with Kew as revealed through Jaccard's similarity coefficient matrix (Table 3b). Although PQM-1 showed significant difference with Queen, it was found more similar to Queen

Table 3a. Euclidian distance coefficient matrix of pineapple cultivars using 16 quantitative characters.

Cultivar	PQM-1	Kew	Mauritius	Queen
PQM-1	0			
Kew	291.6	0		
Mauritius	272.8	559.1	0	
Queen	222.3	507.9	52.9	0

Table 3b. Jaccard's similarity coefficient matrix of pineapple cultivars using RAPD and ISSR markers.

Cultivar	PQM-1	Kew	Mauritius	Queen
PQM-1	1			
Kew	0.74	1		
Mauritius	0.79	0.9	1	
Queen	0.79	0.88	0.91	1

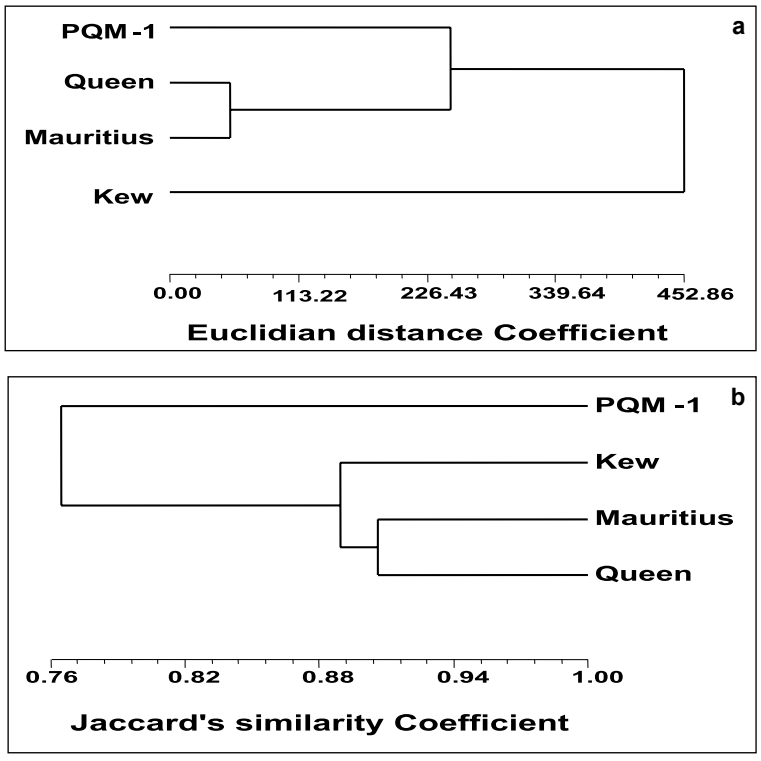

Fig. 2. Dendrogram of pineapple cultivars of Tripura based on (a) quantitative morphological and biochemical characters and (b) RAPD and ISSR markers.

and Mauritius than Kew in both morphological and molecular analyses. In contrary, Kew revealed more dissimilarity with Queen and Mauritius in morphological than molecular analyses (Tapia Campos *et al.*, 14; Prakash *et al.*, 10).

Trend of morphological and molecular diversity of clones of selected varieties of pineapple of Tripura revealed similarity with little diversion. Clone belonging to Mauritius type showed a great similarity with Queen in both the analysis. Similarly, Queen and Kew varieties were found diverse in both the analysis. PQM-1 which was evolved from Queen was found diverse from Queen in molecular analysis. Therefore, getting into the genetic nature of pineapple clones, molecular diversity analysis was found effective. Various clones belonging to all types are being collected from various parts of the state and diversity of these clones will give more comprehensive ideas about pineapple diversity of this state in future.

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