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Evaluation of Different Pineapple (*Ananas comosus* Merr L.) Varieties Using Morphological and Genetic Markers in Mauritius

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Abstract: Pineapple is considered as one of the finest fruit grown in tropical and sub-tropical countries. Morphological assessment was done on fruit, leaf, peduncle, growth habit and stem of the plant. Morphological characters, namely those from the fruit and the leaf can be used as reliable criteria to differentiate between the cultivated and the wild varieties. The genetic diversity of the cultivated and wild pineapple varieties was investigated by Random Amplified Polymorphic DNA (RAPD). Genomic DNA was extracted from leaf and leaf base using the CTAB DNA extraction protocol followed by an RNAse treatment. Out of 60 Operon primers, 5 yielded polymorphic bands in all the pineapple varieties. OPP20, OPO03 and OPB18 were the most polymorphic markers. A dendrogram was generated using cluster analysis based on the dissimilarity matrix, performed using un-weighted pair group method arithmetic averages. RAPD markers can be used in identifying genetic diversity of the local pineapple varieties in Mauritius.

Key words: Pineapple, RAPD markers, cultivated variety, morphological characters, molecular characterization

INTRODUCTION

Pineapples (*Ananas comosus* Merr L.) are among the most wanted and popular exotic fruit grown in most tropical and sub-tropical countries (Cobley, 1976). Surmounted by a crown, it is considered as the 'king' of fruits from the Bromeliaceae family. The presence of the golden-yellow colored pineapple is easily noticeable among others in fruit stalls. Reports by Collins (1960) suggest that pineapple originate somewhere in a region including Brazil, Argentina and Paraguay. The modern domesticated pineapple is a result of mutation for seedlessness and continuous breeding for better fruit size, juiciness, sweetness and improved flavor (Samson, 1986). With the development of world trade, the cultivation and use of pineapple has spread worldwide and now it can be found in all the three Mascarene Islands of the Indian Ocean.

Pineapple was introduced in Mauritius in January 1606 by a Dutch governor (Bhugaloo, 1999). In Mauritius, there are different pineapple cultivars within the *Ananas comosus* species: Honey, Bourgault, Queen Victoria, Smooth Cayenne, the dwarf or ornamental cultivar (*Ananas nanus*) and the wild cultivar (*Ananas bracteatus*). The pineapple cultivar Queen Victoria (or denoted simply as Victoria), was introduced in Mauritius by Newman in 1830 (Bhugaloo, 1999) and it has ever since dominated our local and export market. It is very much appreciated for the sweet taste, fragrant smell, small golden-yellow fruit and ability to resist diseases. The pineapple cultivar Smooth Cayenne, also known as 'Ananas maingard' is widely grown across the world both for processing (canning) and fresh fruit consumption. It accounts for 70% of world pineapple production (Botella *et al*., 2000) due to qualities as high yield, good fruit quality and tolerance to stress (Malezieux, 2000). It was introduced in Mauritius from Hawaii in 1931 (Bhugaloo, 1999) but is not as dominant as Queen Victoria. The pineapple cultivar honey was introduced in Mauritius in 1990 by the Food and Agricultural Research council (FARC) from Singapore due to its small fruit size and sweetness. Although, it was targeted to compete with Queen Victoria cultivar for exportation to the European market, it failed to create interest among growers (Bhugaloo, 1999). The pineapple cultivar Bourgault is distinguished by its large fruit size (~2 kg) and conical shape. It is rarely seen on the local market but is produced on a large scale by private companies for the making of fresh juice, jams, jellies and crystallized fruits. The fruit of the wild cultivar (*Ananas bracteatus*) is of a much inferior quality to those of *A. comosus* species. The plant has sharp spines and the fruit has a high acidic taste. It may be used as fences or as an ornamental for decorative purposes. The ornamental cultivar can be easily distinguished by its small fruit size (~15 cm), long and narrow leaves, creamy flesh and small strong spines (Py *et al*., 1987). This dwarf cultivar is another form of wild pineapple and it can also be grown as an in-house

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plant. Although, it is not edible, the small fruit is very useful for decorative purposes and can last for a couple of months. Queen Victoria is the most common variety grown commercially in backyard systems and by few large scale producers while the other varieties namely Bourgault, honey, cayenne and wild are rarely found but do have their place in niche markets.

The juicy and fragrant monocot is very demanding locally and liked by many European countries. Since, the mid 80's, pineapple export to Europe has consistently increased from 10 tons to around 5542 tons. The Mauritian pineapple market has revolutionized towards other value added products (Dehydrated/Crystallized pineapple, juices, pickles etc) due to development in agribusiness. The Government of Mauritius is seeking diversification of the economy of crops to adapt to the new environment (increased tourist number, competition, globalization and climatic change) and reducing dependency on import, promoting export and ensuring self-sufficiency. It is envisaged that the land under sugarcane will be released at a greater pace due to the drastic reduction of the sugar prices and pineapple is regarded as an ideal candidate for replacing sugar-cane if the marketing structure is improved (Central Statistic Office, 2006, 2008).

Most of the trade conducted in the pineapple industry is limited to the Victoria cultivar. This narrow genetic basis makes our industry a fragile and vulnerable one as the outbreak of a major pest or disease can affect the agricultural economy and the export sector. Varietal diversification is an urgent necessity for the fresh fruit market as the other cultivars should be grown on a much larger scale. Mauritius has a flourishing tourist industry whereby pineapple is highly on demand from restaurants and hotels. A diversity of pineapple varieties can reply to different consumer taste. Previous reports on pineapple conducted in Mauritius include those done on cultural practices, post harvest technology and occurrence of pest and diseases (Bhugaloo, 2001; Goburdhun, 1994; Mamet, 1941).

Identification of the local pineapple varieties using morphological characters is prone to produce biased results due to fluctuations in environmental conditions and molecular markers allow efficient determination of genetic differences. No molecular study on the local pineapple varieties has ever been conducted in Mauritius so this molecular study may contribute enormously in the level of detection, characterization and assessment of genetic diversity (Tapia *et al*., 2005). Apart from the fruit, the pineapple leaves are very much used in other countries for the making of cloth (Philippines), ropes and paper (Py *et al*., 1987). This research also creates awareness on the local pineapple varieties such that a multi-faceted industry can be developed to use all the by-products efficiently.

MATERIALS AND METHODS

Plant material: The leaf samples were collected fresh from different locations: Victoria and smooth cayenne variety (Creve Coeur field), Honey variety (Food and Agricultural Research Council, Reduit), ornamental and Bourgault variety (Verger de Labourdonnais) and the wild variety from Nouvelle Decouverte. Healthy and young leaves showing no sign of necrosis or been attacked by pests and or diseases were chosen. The leaf samples were placed in a dark-coloured plastic bag and the spiny margins were peeled off for ease of handling and cutting. All the experiments was carried out with fresh leaf samples from August 2009 to March 2010 in the Molecular Biology Laboratory, Faculty of Agriculture, The University of Mauritius.

Morphological characterization: The morphological characterization was done according to the descriptive terminology by Agarwal (1996) and Collins (1960). It was based on the posture, stem, fruit, peduncle and leaf of the plant. Several parameters of the leaf, fruit and growth characteristics (Agarwal, 1996) were taken into consideration so as to minimize the risk of obtaining biased results.

DNA extraction and RAPD reaction: DNA extraction was carried out according to Ramessur and Ranghoo-Sanmukhiya (2011). The RAPD-PCR reactions were carried out in 30 μL PCR tubes using a Biorad PCR machine. Each 30 μL PCR reaction volume consisted of 1×PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM tris-HCl and 0.1% Tween 20), 3 mM $MgCl_2$, 0.25 mM dNTP, 0.6 mM primer, 1 U *Taq* polymerase, 50 ng DNA template and 17.7 μL sterile water. Negative control lacking DNA template was also included in the PCR reaction. The cycling parameter set comprised of 1 cycle of 90 sec at 95°C, 40 cycles of 30 sec at 92°C, 1 min at 35°C and 3 min at 72°C. This was followed by an extension of 10 min at 72°C and a final delay of 5 min at 15°C. Reaction analysis was evaluated using electrophoresis on a 2% (w/v) agarose gel using 0.5×TBE buffer at 115 V. It was then stained with EtBr for 5 min and destained in running water (5 min). Finally, it was visualized under a UV transilluminator.

RAPD profile analysis: Sixty Operon primers were purchased from Inquaba Biotechnology Industries Ltd., South Africa and used to evaluate genetic diversity. All the screened primers that gave clear and distinct amplification products were used to run with all the six varieties. Each genotype was characterized by its banding pattern using the DNA hyperladder 2 (Bioline) as basepair ladder. The RAPD markers as viewed from the gels after electrophoresis analysis and staining that were converted into a matrix of binary data, where the presence of the band corresponded to value 1 and the absence to value 0. The statistical software NTSYS-PC (Rohlf, 2005) and DARwin 5 software (Perrier and Jacquemoud-Collet, 2006) were used to construct a UPGMA dendrogram using hierarchical clustering. Using NTSYS software, a dissimilarity matrix was calculated utilising Jaccard (1908) coefficient. The matrix was converted to a dissimilarity matrix corresponding to the complement (dissimilarity = 1-similarity). Cluster analysis based on the dissimilarity matrix, was performed using un-weighted pair group method arithmetic averages (UPGMA) (Sneath and Sokal, 1973) of the NTSYS-PC version 2.2 (Rohlf, 2005).

RESULTS AND DISCUSSION

Morphological criteria generally correspond to the qualitative traits that can be scored visually and are used. It is highly influenced by pest and diseases, environment, nutrition, cultural practices, level of maturity and also by human judgement (Chawla, 2002). The major difference observed in the morphological characters between the pineapple varieties were the fruit and the leaf (Fig. 1). Honey and smooth cayenne cultivar had no or very little spines at the leaf tip or along the margin and this is the most evident difference to distinguish them from other varieties. Few sucker slips were observed in the smooth cayenne cultivar as compared to the other varieties and this character has been reported to be highly influenced by environmental conditions (Reinhardt *et al*., 2002).

Significant differences were observed in the fruit shape and size of the different pineapple varieties (Table 1). The biggest fruit was observed in Bourgault and Wild variety making the latter suitable for juice

Fig. 1(a-f): Fruit characteristics of each pineapple variety (a) Queen victoria, (b) Smooth cayenne, (c) Bourgault, (d) Honey variety, (e) Ornamental variety and (f) Wild variety

Table 1: Morphological characteristics of the pineapple fruit

Parameters	Bourgault	Victoria	Cayenne	Honey	Wild	Ornamental
Size						
Small						√
Average		√	√	√		
Large	√				√	
Shape of fruit						
Conical	√					√
Cylindrical	√	√	√	√	√	
Globular	√	√				√
Square shouldered			√		√	
Pointed						
Shape of eye						
Flat			√			√
Narrow	√	√	√	√		
Deep					√	
External fruit colour at maturity						
Golden yellow	√			√	√	√
Orange		√	√			
Red					√	
Colour of flesh						
Cream						√
Yellow	√	√	√	√	√	
Number of crowns						
Single	√	√	√	√		
Multiple					√	√
Thickness of skin						
Thick					√	
Thin				√		√
Normal	√	√	√			
Presence of Spines						
Many	√	√			√	√
Few			√	√		
Colour of crown leaf						
Green		√		√		√
Dark green with red margin	√		√		√	
Length of crown						
Small						√
Long					√	
Average	√	√	√	√		

Table 2: Morphological characteristics of the peduncle

Parameters		Bourgault	Victoria	Cayenne	Honey	Wild	Ornamental
Length of peduncle	Long					√	√
	Average	√	√	√	√		
	Small						
Colour of peduncle	Green	√		√	√		√
	Red					√	
	Yellow		√				
Strength of peduncle	Strong	√	√	√		√	
	Weak						√

making and other value-added products. The ornamental variety had the smallest fruit while an average size was observed in the other varieties. In addition to presence of seeds, a long peduncle and a miniature fruit was observed only in the Ornamental variety which makes it suitable for decorative purposes (Table 2). Another easy way to distinguish between the different varieties is by the fruit colour upon ripening (Fig. 1). A red colour was observed in the wild variety as compared to the orange/yellow colour in the other varieties. A thin skin and a narrow eye-spot were observed in Honey which makes peeling of the fruit easier thus, carving the eyes from the flesh to leave grooves is not required with this variety.

An erect posture, a medium plant height and a less dense canopy were observed in all the *Ananas comosus* species making cultural practices and harvest easier (Reinhardt *et al*., 2002). Research done by Cobley (1976) reveals that *A. bracteatus* (Wild cultivar) and *A. nanus* (Ornamental cultivar) differ from the cultivated types of the *A. comosus* species (cayenne, Bourgault, honey and victoria) by their smaller and less fleshy fruits, numerous seeds, conspicuous floral bracts, thinner peduncles, colour and flavor of flesh and form of leaf margin. Other reports indicate the use of leaf, fruit and flowers for distinguishing the cultivated species (Tapia *et al*., 2005). However, some of the results obtained were contradicting as a stronger peduncle and very fleshy fruits was observed in the Wild cultivar (Fig. 1). Some possible reasons are the lack of optimum conditions required for growth and development and the ability of pineapple to

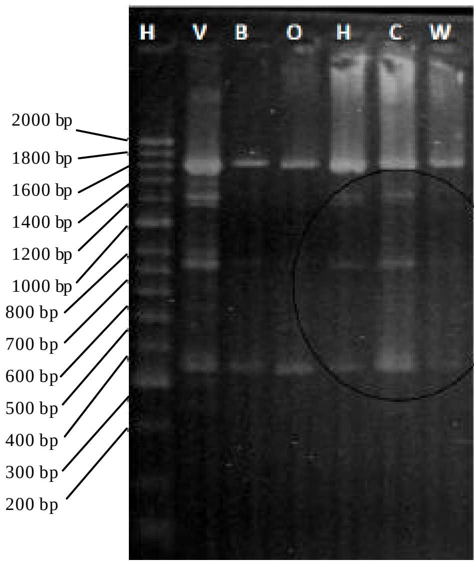

Fig. 2: Amplification products from all six pineapple varieties with primer OPA20. The polymorphic bands are encircled, H: Hyperladder II, O: Ornamental, W: Wild, V: Victoria, B: Bourgault, C: Cayenne, H: Honey, -: Negative control

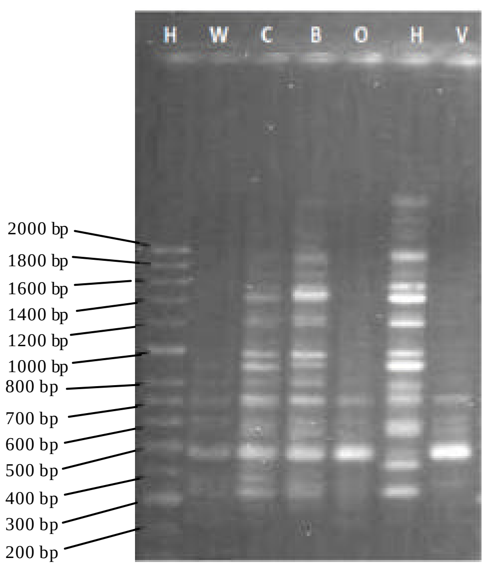

Fig. 3: Amplification products from all six pineapple varieties with primer OPB18, H: Hyperladder II, O: Ornamental, W: Wild, V: Victoria, B: Bourgault, C: Cayenne, H: Honey

adapt to changes in the environment. Overall, morphological characterization has proved to be very efficient to distinguish between the cultivated and wild varieties. Earlier morphological characterization (Reinhardt *et al*., 2002) on the Cayenne cultivar matches

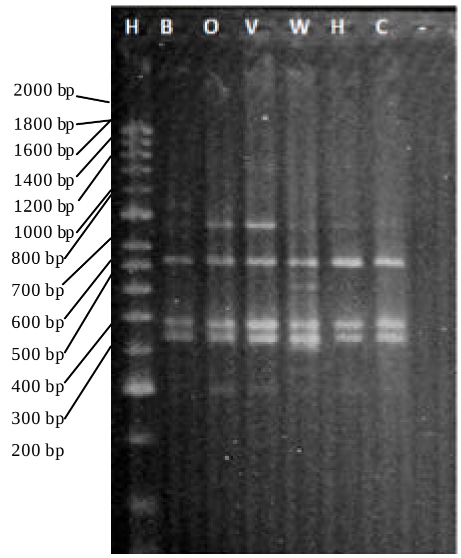

Fig. 4: Amplification products from all six pineapple varieties with primer OPK05, H: Hyperladder II, O: Ornamental, W: Wild, V: Victoria, B: Bourgault, C: Cayenne, H: Honey, -: Negative control

the results of this study. An average length and more resistant peduncle is important for avoiding dropping of fruits which makes the fruit more susceptible to sunburns. Another morphological characterization (Cabral *et al*., 2005) reported presence of a short peduncle, high number of suckers and few slips in the Cayenne cultivar. Production of suckers slips and shoots are highly influenced by stress conditions (Reinhardt *et al*., 2002) which explains the difference in the number of suckers. However, similar result (few slip) was obtained for the Cayenne variety. Features which were not informative to distinguish between the different cultivars as they are present in almost all species of the Bromeliaceae family are namely: Leaf venation, phylotaxy of the leaves and leaf angle.

The Jaccard's similarity analysis depicted a good degree of genotypic diversity existing in the pineapple genotypes studied (Table 4). The minimum and maximum similarity values were 0.607 and 0.803. The dendrogram reflected a good genetic analysis which is based on amplification signals from RAPDS proving that it is a good marker to evaluate the genetic relationships among pineapple varieties (Fig. 7). From the molecular analysis it was surprising to see that those economically important varieties clustered with the wild and ornamental varieties which are cultivated for fence, ornamentals and rarely for the fruit. Separate grouping of the wild pineapple species (*A. bracteatus* and *A. nanus*) from the cultivated one (*A. comosus*) was noted in an earlier study (Tapia *et al*., 2005).

Table 3: RAPD markers and Polymorphism

Primer	No. of markers	No. of Polymorphic markers	Polymorphism (%)
OPB18	19	13	68.4
OPA20	4	2	50.0
OPP20	18	18	100.0
OPO03	19	13	68.4
OPK05	5	2	40.0
Total	65	48	73.8

Table 4: Dissimilarity matrix based on the proportion of shared RAPD fragments among different pineapple varieties

	Victoria	Smooth cayenne	Bourgault	Honey ornamental	Wild
Smooth cayenne	0.2895				
Bourgault	0.3505	0.19710			
Honey	0.2892	0.21470	0.23261		
Ornamental	0.2147	0.32950	0.26980	0.3720	
Wild	0.2326	0.30910	0.32950	0.3943	0.2326

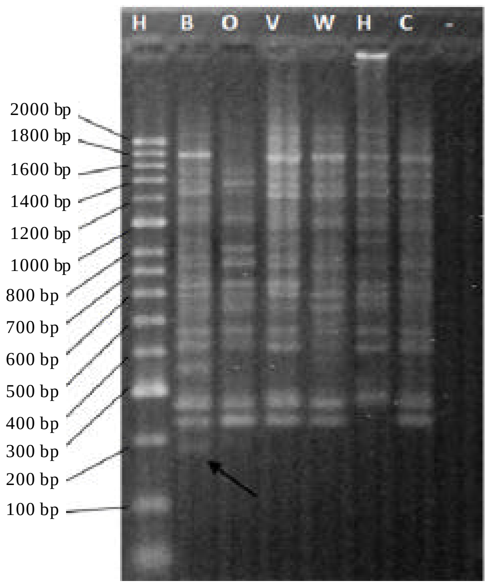

Fig. 5: Amplification products from all six pineapple varieties with primer OPO03, the polymorphic band is highlighted by the arrow, H: Hyperladder II, O: Ornamental, W: Wild, V: Victoria, B: Bourgault, C: Cayenne, H: Honey, -: Negative control

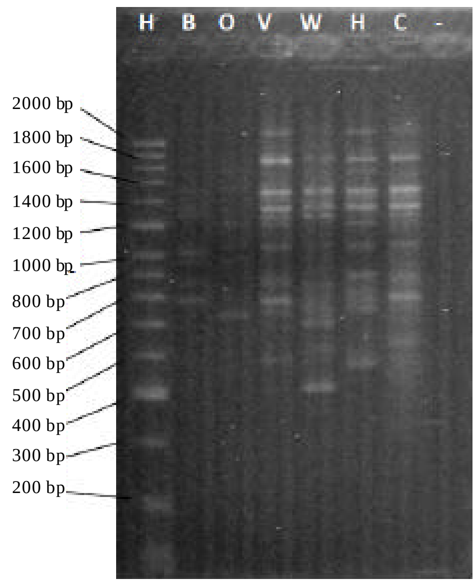

Fig. 6: Amplification products from all six pineapple varieties with primer OPP20, H: Hyperladder II, O: Ornamental, W: Wild, V: Victoria, B: Bourgault, C: Cayenne, H: Honey, -: Negative control

The Bourgault and smooth cayenne variety clustered together in the dendrogram (Fig. 7) based on the analysis of the molecular data (Fig. 2-6). The genetic distance separating these two species is very low (0.19710) due to the sharing of bands in the DNA profile. Primer OPB18 (Fig. 3, Table 3) was very efficient in showing the number of bands shared between the two varieties namely the 1400, 1200, 1000, 800,700, 600, 500 and 300 bp markers. The morphological characters also reveal a high degree of similarity in the length and strength of peduncle, thickness of skin, colour, number and length of crown, colour of flesh, absence of seeds, the number and attitude of leaves. One surprising aspect of this clustering is some of the major phenotypic characters that are different between these two varieties such as the lack of spines, fruit size and shape, leaf shape and colour of stem are not informative. Honey is another cultivar that is closest to smooth cayenne on the basis of morphological traits. The taxonomy of Ananas is based mainly on traits that depend on single genes which may vary with environment. As reflected in the dendrogram, Honey is seen to be the closest relative of Bourgault and smooth cayenne and distantly related to Victoria, Ornamental and wild variety.

Surprisingly, the Ornamental and Victoria variety clustered together despite the large morphological differences like fruit size, colour of flesh, leaves and fruits etc. The Ornamental cultivar is inedible unlike the other cultivars. Very few morphological characters are shared between the two cultivars than compared to the other

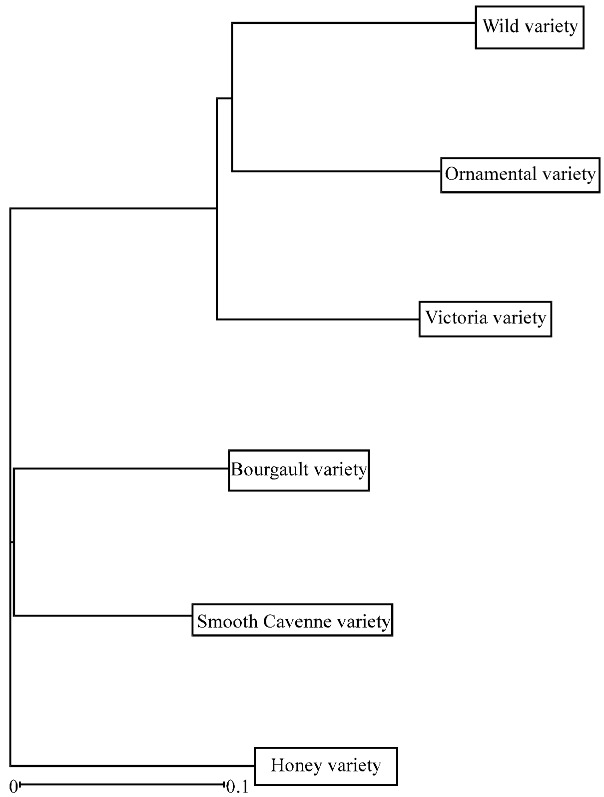

Fig. 7: Dendrogram generated using UPGMA cluster analysis showing the relationships and diversity among the pineapple varieties

varieties. Hence, morphological characters are not the best way to evaluate genetic relationship as the degree of divergence between genotypes at the phenotypic level is not the same (Ramessur, 2008). Again, OPB18 and OPK05 (Fig. 3, 4) were the best to show the similarity due to the number of bands shared namely the 700, 600 and 500 bp. High degree of polymorphism was revealed by primer OPP20 and OPO03 (Fig. 5, 6, Table 3). Similarly high level of polymorphism was found in the cultivated and the wild variety (Carlier *et al*., 2006). Previous studies (Duval *et al*., 2001) showed closer genetic relatedness between *Ananas bracteatus* (wild variety) and *Ananas comosus* (Honey, Smooth Cayenne, Victoria and Bourgault) than with *Ananas nanus* (Ornamental variety).

CONCLUSION

Fruit characteristics were found to be the most reliable markers for differentiating between the local pineapple cultivars. RAPD is a versatile technique to assess the genetic distance which exists between local pineapple cultivars and this study is the first of its kind and paves the way for further molecular studies. Moreover, the local pineapple germplasm represent an untapped and underutilized genetic resource which can be further improved in future breeding programmes. A good understanding of the distribution of genetic variation could help in identifying superior genotypes and for later use in generating new cultivars. These could give a boost to the local pineapple ‘gene pool’ by bringing together the greater adaptability of the landraces to the local agro-climatic conditions and the commercial characters of value from foreign varieties to give birth to appealing varieties and then capture the interest of export markets.

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