JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2022, VOL. 5(2), 141-154

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Morphological and physico-chemical characteristics of three local pineapple [*Ananas comosus* (L.) Merr.] cultivars grown under subtropical region of Bhutan

Ratu Kinley[1]*, Chinta Mani Dhimal[1] and Ganja Singh Rai[1]

1, *Agriculture Research and Development Centre, Samtenling, Department of Agriculture, Ministry of Agriculture & Forests, Bhutan*

ARTICLE INFO

Original Article

Article history:
Received 10 September 2021
Revised 20 December 2021
Accepted 10 January 2022
Available online 21 April 2022

Keywords:
Landraces
Pineapple
Qualitative
Quantitative
Varietal evaluation

DOI: 10.22077/JHPR.2022.4490.1233
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Agriculture Research and Development Centre, Samtenling, Department of Agriculture, Ministry of Agriculture & Forests, Bhutan.
Email: rkinley@moaf.gov.bt

ABSTRACT

Purpose: Although the area under pineapple [*Ananas comosus* (L.) Merr.] cultivation has been increasing over the years in Bhutan, information on the morphological characters and yield performances of the cultivars grown in the country are literally absent. In this study, three landrace cultivars of pineapple (Accession ID: PV1, PV2 and PV3) collected from Sarpang District in Bhutan were evaluated for their morphological and physico-chemical characteristics. **Research method:** The field experiment was carried out in Randomised Complete Block Design with five replications at Agriculture Research and Development Centre, Samtenling, Bhutan in 2018. Seventeen morphological characteristics and five physico-chemical parameters were evaluated to record the variation amongst three accessions. **Findings:** The mean fruit weight of PV1 (1.4 kg), PV2 (0.9 kg) and PV3 (0.5 kg) were statistically significant. The fruit yield of pineapple accession PV1 (47.9 t/ha) was significantly higher than that of PV2 (30.0 t/ha) and PV3 (16.7 t/ha). Accession PV1 (14.3 °Brix) and PV3 (14.4 °Brix) had significantly higher total soluble solid (TSS) than that of accession PV2 (10.4 °Brix). Sugar to acid ratio of PV2 (46.81) was significantly higher than that of PV3 (34.95) but not with that of PV1 (41.54). **Research limitations:** Absence of molecular technique in characterisation of pineapples accessions. **Originality/Value:** This is the first report of characterisation of pineapple cultivars in Bhutan. Based on the result of this study, National Seed Board of Bhutan released Accession PV1 and PV3 as pineapple variety 'Samtenling Kongtsey 1' and 'Samtenling Kongtsey 2' respectively in 2020 for cultivation in Bhutan.

INTRODUCTION

Pineapple [*Ananas comosus* (L.) Merr.] is a tropical fruit plant that belongs to the family *Bromeliaceae*. It is the third most important tropical fruit in the world after banana and mango (d'Eeckenbrugge et al., 2011; Perez et al., 2011). Its mature fruit has exceptional juice and vibrant flavor, and is packed with many types of nutrients, antioxidants and enzymes that have several health benefits. The mature fruit contains sugar, a digesting enzyme bromelin, citric acid, malic acid, vitamin A, vitamin B complex, calcium and potassium (Bartholomew & Malezieux, 1994; Ramallo & Mascheroni, 2012). Its origin has been traced to Brazil and Paraguay in the Amazon basin where the fruit was first domesticated (Adje et al., 2019). Costa Rica, Philippines, Brazil, Thailand and Indonesia are the main pineapple producers in the world supplying nearly 45.5% of the total production and other important pineapple producing countries include India, Nigeria, China, Mexico and Colombia which together contributes about 24% of the total (FAO, 2019).

The taxonomical classification of pineapple went through several modifications in the past and the classification proposed by Coppens in 2003 was internationally accepted (d'Eeckenbrugge et al., 2011). The two genera and seven species of the previous classification were downgraded to one genus (*Ananas* Miller) with two species: *A. comosus* (L.) Merr. and *A. macrodontes* Morren with five botanical varieties (d'Eeckenbrugge et al., 2011; Li et al., 2018). Those five botanical varieties under *A. comosus* include *comosus, ananassoides, parguazensis, erectifolius*, and *bracteatus* (d'Eeckenbrugge et al., 2011; Li et al., 2018; Py et al., 1987). Among these five varieties, *Ananas comosus* var. *comosus* is the most widely cultivated variety throughout the tropical regions both for fresh fruit consumption and processing. Other varieties represent the wild forms with robust growth habits and are mostly grown as fiber crop, ornamentals and live fencing (Bartholomew & Malezieux, 1994).

The five pineapple cultivars for commercial production based on isozyme variation include Cayenne, Queen, Abacaxi, Red Spanish and Perola (Bartholomew & Malezieux, 1994; Li et al., 2018). Among these, Smooth Cayenne cultivar is the most planted cultivar worldwide and dominates commercial production for canning as well as for fresh fruit consumption (Sanewski et al., 2018). The Queen group is very much appreciated for the sweet taste, fragrant smell, small golden yellow fruit and ability to resist diseases (Burhooa & Ranghoo-Sanmukhiya, 2012). The Spanish group is characterised by small to medium vigorous plants with spiny leaves and resistant to mealy bug wilt. It is acceptable for the fresh fruit market but not favoured for canning due to deep eyes and poor flesh colour (Sanewski et al., 2018).

Culturally, pineapple is an important fruit for Bhutan. It is mostly used as table offerings in religious functions (*Tshog*) and other festivals (*Thokey*) besides fresh fruit consumption and processing. Although Bhutan is not a tropical country, the hot-humid environment in the southern part of the country having subtropical climate is suitable for pineapple production. Currently, the pineapple in Bhutan is grown primarily for fresh fruit markets and very little is used in the processing industry. Pineapple is mainly grown in 10 districts in Bhutan with the total production of 72 metric tons (MoAF, 2017). Sarpang district is the largest producer and contributes about 30% to the total pineapple production in Bhutan (MoAF, 2017). It is mostly grown in small scale in scattered manner and usually intercropped with other subtropical fruit plants like areca nut, litchi and mango. Recently, pineapple cultivation in Bhutan is increasing and is becoming an important cash crop for the farmers of the eastern part of country (Dorji, 2019). Pineapple production in the country has increased by four folds within a year in 2018 with total production of 279 metric tons (MoAF, 2018). The low input requirement, assured market and the remunerative price have motivated the farmers to take up the cultivation of the

pineapple. With this increase of production, Bhutan Agro Industry Limited established a pineapple processing plant in Lingmithang under Mongar district in November 2019 which provides an additional market to the farmers in eastern Bhutan (Namgyal, 2019).

Although many landrace cultivars of pineapples are grown in different parts of Bhutan, the data on the pineapple varieties and their yield performances in the country were not available prior to this study. In fact, there was not a single commercial variety available in the country. No studies were conducted on the morphological and physico-chemical characteristics of local pineapple cultivars and their potential utilization in Bhutan. Morphological characterisation of local cultivars can assess the morphological diversity and identify desirable traits in cultivars for selection and crop improvement. The composition of fruit juice also varies according to variety of fruit, maturity, and environmental conditions during the growing season (Brown & Cohen, 1983). For industrial processes, it is important to have the information of the physical and chemical properties of pineapple cultivars grown. Therefore, this study aimed to: (1) characterise three local pineapple landraces grown under subtropical region of Bhutan in terms of their plant morphology, (2) examine the physico-chemical properties of their fruits and (3) assess consumers' preferences among them.

MATERIALS AND METHODS

Planting materials and field trial

A field survey was carried in 2017 in Sarpang district located in the subtropical region of Bhutan to find out the number of landrace cultivars of pineapple grown by the farmers and to collect the germplasms of pineapple for establishment of a field trial. The pineapple germplasm block cum field trial was established at Agriculture Research and Development Centre (ARDC) at Samtenling located at an altitude of 375 meter above sea level (masl) with three distinctly different landrace cultivars of pineapple (accession ID: PV1, PV2 and PV3) grown by the farmers in Sarpang district. The field experiment was conducted in randomised complete block design (RCBD) with five replications. Each plot was 2.4 m long and 1 m wide, and two adjacent plots were separated by a drain of 50 cm wide. The aerial suckers were transplanted at a spacing of 60 × 30 cm in two rows following the standard package of practices. In each block, there were 16 plants out of which 10 plants per accession were randomly sampled out for data collection. Fully ripened fruits that were free from defects such as sun scorch and pest or disease damage, which might have affected the normal ripening process, were sampled for morphological characterisation and physico-chemical analysis.

Quantitative morphological characterisation

Seventeen quantitative morphological characteristics including plant characteristics (plant height, stem diameter, peduncle length and diameter, number of propagules produced) and fruit characteristics (fruit weight, size, number of fruitlets, skin thickness, eye depth, flesh firmness, days to flowering and maturity, yield) were evaluated to record the variation amongst three pineapple accessions. Morphological characterisation was carried out following the "Guidelines for the conduct of tests for distinctness, uniform and stability" for pineapple developed by International Union for the Protection of New Varieties of Plants (UPOV, 2013) and "Pineapple descriptor" developed by International Board for Plant Genetic Resources (IPGR, 1991). Morphological characteristics like fruit weight, size, shape, numbers of slips and suckers produced, fruit colour, plant height, leaf and stem characteristics were examined in this study.

Physico-chemical analysis of pineapple fruit

Physico-chemical analysis was carried out according to the guidelines on "Objective Tests to Determine Quality of Fruits and Vegetables, Dry and Dried Produce" developed by the Organisation for Economic Co-operation and Development (OECD, 2005). Physico-chemical characteristics like total soluble solid (TSS), pH, titratable acidity, juice content and dry matter content were analysed. Fruits were peeled off and cut into pieces and squeezed manually to extract juice. The squeezed pulp was then filtered out through muslin cloth to extract juice. Flesh firmness of the fruit was determined with a hand-held penetrometer using a detachable plunger of 11 mm diameter (1 cm^2).

Determination of total soluble solid (TSS)

Brix degree is generally used as indicator for % soluble solid content. The total soluble solids of pineapple juice samples were evaluated using Vee Gee Scientific BTX-1 Handheld Refractometer having Brix scale of 0-32% with +/-0.2% accuracy.

Determination of pH

The pH of pineapple juice samples was evaluated with pH-2016 Automatic Temperature Compensation (ATC) pen type pH meter.

Determination of titratable acidity

Pineapple juice contains non-volatile organic acids such as malic acid and citric acid, which are readily neutralized by strong bases and can be titrated against standard bases such as sodium hydroxide (Nadirah et al., 2012). After determining the pH, the solution was titrated with 0.1N NaOH up to the end point of pH 8.1. The total acidity was calculated using equation (1) and the results were expressed as percent citric acid content. The measurement was repeated three times per sample.

$$\text{Percentage citric acid} = \frac{\text{Titre value x acid factor} \times 100}{10\ (\text{ml juice})} \quad (1)$$

Where, the acid factor for citric acid is 0.0064.

Determination of moisture content

Moisture content was determined following the Official Methods of Analysis developed by the Association of Official Analytical Chemists (AOAC, 2005). The percent moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \left(\frac{W1 - W2}{W1 - W3}\right) \times 100 \quad (2)$$

Where, W1= Initial pineapple sample weight with crucible, W2= Final pineapple sample weight with crucible, W3= Initial weight of empty crucible

Sensory evaluation of fruit quality

Sensory attributes like fruit appearance, size preferences, fruit colour, aroma, sweetness, tartness, flesh fibrousness were assessed by 20 consumers selected randomly. Hedonic Scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely) was used to assess the consumers preferences of local pineapple accessions (Jones et al., 1955). Percentage of acceptability for each of the sensory characteristic was calculated as follows:

$$\text{Acceptibility (\%)} = \left(\frac{\text{Number of panelists rated>5}}{\text{Total number of panelists}}\right) \times 100 \quad (3)$$

Data analysis

Results were presented as the mean ± standard error of five replicates. The data were subjected to analysis of variance (ANOVA) using the statistical analysis software STAR (Statistical Tool for Agricultural Research) version 2.0.1 (International Rice Research Institute, Los Banos, Philippines). The means were separated using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

Table 1. Plant characteristics of three accessions of pineapple evaluated in 2018 at ARDC Samtenling, Bhutan

Accession ID:	Plant height (cm)	Stem diameter (cm)	Peduncle length (cm)	Peduncle diameter (cm)	Number of peduncle slips	Number of aerial suckers	Number of underground suckers
PV1	64.3 (2.00±) b	4.4 (0.06±) a	17.7 (0.25±)b	2.2 (0.04±)a	2 (0.37±) b	1 (0.34±) b	0 (0.00±) b
PV2	81.9 (10.88±) a	4.7 (0.23±) a	24.5 (0.70±)a	1.9 (0.08±)b	5 (0.70±) a	2 (0.37±) b	0 (0.10±) b
PV3	50.8 (2.52±) b	3.7 (0.15±) b	18.2 (0.44±)b	1.5 (0.10±)c	7 (0.79±) a	8 (0.68±) a	14 (1.15±) a
P-Value=0.05	0.010	0.006	<0.001	<0.001	0.006	<0.001	<0.001
CV (%)	18.12	7.93	6.01	7.76	40.31	29.42	32.19

Means in the same column, followed by the same letter(s) are not significantly different at p ≥ 0.05. The figures given in the parenthesis are standard error of mean.

Table 2. Quantitative fruit characteristics of three pineapple accessions evaluated in 2018 at ARDC Samtenling, Bhutan

Accession ID:	Fruit weight (g)	fruit height (cm)	Fruit diameter (cm)	Number of fruitlets	Skin thickness (mm)	Eye depth (cm)	Days to flowering	Days to fruit maturity	Flesh firmness (kg/cm^2)	Yield (t/ha)
PV1	1436.7 (51.96±) a	16.7 (0.45±) a	11.7 (0.41±) a	106 (3.82±)a	4.8 (0.40±)	1.08 (0.04±) b	290 (1.41±) a	413 (2.47±) a	1.3 (0.02±) c	47.9 (1.73±)a
PV2	899.5 (44.99±) b	13.6 (0.33±) b	9.7 (0.18±) b	72 (0.87±)c	5.8 (0.33±)	1.04 (0.04±) b	285 (1.22±) b	402 (2.20±) b	3.3 (0.31±) a	30.0 (1.50±)b
PV3	502.2 (23.78±) c	10.4 (0.43±) c	9.2 (0.14±) b	83 (2.80±)b	5.9 (0.57±)	1.28 (0.07±) a	280 (2.19±) c	390 (2.62±) c	2.5 (0.35±) b	16.7 (0.79±)c
P-Value= 0.05	<0.001	<0.001	0.001	<0.001	0.335	0.003	0.003	<0.001	<0.001	<0.001
CV (%)	11.16	7.48	7.09	7.77	21.51	6.83	1.03	0.6593	19.38	11.16

Means in the same column, followed by the same letter are not significantly different at p ≥ 0.05. The figures given in the parenthesis are standard error of mean.

RESULTS

Quantitative morphological plant characteristics

The results of the quantitative morphological plant characteristics of three pineapple accessions showed statistically significant differences (P≤0.05) in all the quantitative morphological characters among three accessions (Table 1). Plant height (P=0.010) and peduncle length (P<0.001) was found significantly higher in accession PV2 as compared to PV1 and PV3. The largest stem diameter was recorded in PV2 (4.7 cm) and PV1 (4.4 cm) which were found significantly larger (P=0.006) than stem diameter of PV3 (3.7 cm). However, the largest peduncle diameter (P<0.001) was observed in PV1 (2.18 cm) followed by PV2 (1.90 cm) and smallest in PV3 (1.48 cm). Pineapple accession PV3 produced significantly (P<0.001) more number of underground suckers and aerial suckers, while PV1

and PV2 produced lesser number of aerial suckers and no underground suckers. The peduncle slips produced by PV3 (7) and PV2 (5) were statistically different (P=0.006) from that of PV1 (2).

Quantitative morphological fruit characteristics

The results of morphological fruit characteristics of three pineapple accessions are presented in Table 2. Statistically significant differences were found in all the fruit characteristics (fruit weight, fruit height and diameter, number of fruitlets, eye depth, days to flowering and fruit maturity, and skin firmness) except for skin thickness amongst three accessions evaluated. The largest fruit size was observed in PV1. Accession PV2 produced medium sized fruits while fruits of PV3 were found to be relatively smaller in size. The mean fruit weights of PV1, PV2 and PV3 were 1.4 kg, 0.9 kg, and 0.5 kg respectively. The mean fruit yield of pineapple PV1 (47.9 t/ha) was significantly higher (P<0.001) than that of PV2 (30.0 t/ha) and PV3 (16.7 t/ha) when grown at the plant population of 33,342 plants per hectare. The fruits of PV1 and PV2 were found to have broad flat and sunken fruitlets apex respectively and both had shallow eye depth. In contrast, the fruits of PV3 had prominent fruitlets and deeper eye depth. The results also showed that the days to flowering and maturity varied significantly (P=0.003) among three accessions. Among three accessions evaluated, PV1 was found to be late maturing cultivar taking about 14.0 months from date of planting to full fruit maturity, PV2 about 13.4 months, while PV3 matured early with the maturity period of about 13.0 months (Table 2).

Table 3. Morphological plant characteristics of three pineapple accessions evaluated in 2018 at ARDC Samtenling, Bhutan

Morphological characteristics	Accession ID:			
	Parameters	PV1	PV2	PV3
Plant growth habit	Upright			
	Semi upright	√	√	
	Spreading			√
Size of aerial suckers on stem	Small			√
	Medium	√		
	Large		√	
Size of slips	Small			√
	Medium	√		
	Large		√	
Green colour of upper side	Light			
	medium		√	√
	Dark	√		
Leaf anthocyanin colouration	Absent or very weak			√
	Weak			
	Medium	√	√	
	strong			
	Very strong			
Leaf spines	Absent			
	Present	√	√	√
Density of spines	Sparse	√		

	Medium			√
	Dense		√	
Position of spines on margin	At base only			
	At apex only			
	At base and apex	√		
	Along all Margin		√	√
Size of spines	Small	√		
	Medium			
	Large		√	√
Size of bracts	Small			√
	medium	√	√	
	Large			
Petal: colour of apex	Blue Purple	√	√	√
	Purple red			
Fruit shape	Narrow ovate			
	Medium ovate			
	Oblong	√	√	
	Elliptic			
	Circular			√
Immature Fruit colour	Grey			
	Medium green			√
	Dark green			
	Pink			
	Red			
	Purple	√		
	Brownish purple			
	Dark brown		√	
Fruit predominant colour	Cream			
	Yellow green			
	Green			
	Grey green			
	Light yellow	√		
	Medium yellow			√
	Orange		√	
	Orange red			
	Red			
	Brown			
Fruit size	Very small			
	Small			√
	Medium		√	
	Large	√		
	Very large			
Size of eyes	Small			√

	Medium		√	
	Large	√		
Fruitlets apex	Sunken		√	
	Flat	√		
	Prominent			√
Fruit external aroma	Weak		√	
	Medium	√		
	Strong			√
Flesh colour	Whitish yellow		√	
	Light yellow	√		
	Medium yellow			√
	Yellowish orange			
Flesh fibrousness	Low	√		√
	Medium			
	High		√	
Flesh aroma	Weak		√	
	Medium	√		
	Strong			√
Crown attitude	Upright			
	Semi upright	√	√	
	Spreading			√
Size of crown	Small			√
	Medium	√		
	Large		√	

Qualitative morphological characteristics

The major difference in qualitative morphological characteristics among the accessions were observed in plant growth habits, spine distribution on the leaves, fruit shape, fruit colour, fruit size and the fruitlets apex (Table 3, Fig. 1). Plants of PV1 and PV2 were found to have relatively bigger canopy with semi upright growth habits, while PV3 had smaller plant canopy and spreading growth habits. Accession PV1 had larger and darker green coloured leaves with a superficial brownish-red mottling with silvery-grey or ashy-grey coloured lower surface (Fig. 1e). Its leaves were almost spineless with small spines distributed sparsely at the base and apex of leaves. PV2 plants had light-green coloured leaves with purplish anthocyanin along margins and spines (Fig. 1f). PV3 plants had short, stiff green coloured leaves with greenish spines along the margins (Fig. 1g). Oblong shaped, medium to large sized fruit of PV1 was held on a relatively short and strong peduncle. It was bluish purple in colour during immature stage and turned to attractive yellowish orange colour on maturity (Fig. 1a). Similarly, medium-sized, oblong-shaped immature fruits of PV2 were brownish in colour and turned into dull orange colour upon maturity (Fig. 1b). PV3 fruits were much smaller in size, circular to ovoid shaped and turned golden yellow from green immature fruits on maturity (Fig. 1c, g). Yellowish coloured flesh of PV1 and PV3 fruits had pleasant fruit aroma and less fibre as compared to fibrous and whitish yellow flesh of PV2 fruits.

Fig. 1. Fruit and plant characteristics of three accessions of pineapple evaluated at Agriculture Research and Development Centre, Samtenling, Bhutan in 2018: (a) fruits of PV1 at maturity (left) and at green stage (right), (b) fruits of PV2 at green stage (left) and at maturity stage (right), (c) fruits of PV3 at green stage (left) and at maturity stage (right), (d) spine distribution and its density on leaf of PV1, PV2 and PV3 from top to bottom respectively, (e) plants of PV1, (f) plants of PV2, and (g) plants of PV3.

Physico-chemical characteristics

Statistical significant differences were found in all physico-chemical characteristics evaluated (TSS, juice pH, citric acid percentage and acid sugar ratio) amongst three accessions (Table 4). TSS of juice was found significantly higher ($P < 0.001$) in accession PV1 (14.3°Brix) and PV3 (14.4 °Brix) as compared to TSS of accession PV2 (10.4°Brix). The citric acid percentage was also found significantly higher ($P < 0.001$) in accession PV1 (0.35%) and PV3 (0.42%) compared to that in PV2 (0.23%). The highest sugar to acid ratio of the juice was recorded in PV2 (46.81), which was found significantly higher ($P= 0.018$) than that of PV3 (34.95) but not significantly higher than that of PV1 (41.54). Mean fruit juice pH of three

pineapple accession ranged from 3.9 to 4.6. No statistical significance was found in the juice content and water content of fruits amongst three accessions evaluated.

Table 4. Physico-chemical characteristics of fruits of three pineapple accessions at maturity analysed in 2018 at ARDC Samtenling, Bhutan

Treatments	TSS (°Brix)	Acid percentage (%)	Sugar/acid ratio	pH	Water content (%)	Juice content (%)
PV1	14.30 (0.17±)a	0.35 (0.03±) a	41.54 (3.10±)ab	3.86 (0.07±)b	88.3 (3.03±)	37.4 (2.26±)
PV2	10.38 (0.51±)b	0.23 (0.02±)b	46.81 (3.82±)a	4.61 (0.16±)a	86.8 (0.61±)	33.2 (0.89±)
PV3	14.40 (0.52±)a	0.42 (0.02±)a	34.95 (2.04±)b	3.88 (0.14±)b	83.6 (1.10±)	32.8 (2.13±)
P-Value =0.05	<0.001	<0.001	0.018	0.005	0.277	0.226
CV (%)	5.31	14.45	12.34	7.08	5.02	12.38

Means in the same column, followed by the same letter(s) are not significantly different at $p \geq 0.05$. The figures given in the parenthesis are standard error of mean.

Table 5. Pearson's correlation matrix for six physico-chemical characteristics of three pineapple accession evaluated in 2018 at ARDC Samtenling, Bhutan

Physico-chemical characteristics	AP	TSS	SAR	pH	WC	J C
Acid percentage (AP)	1	0.76**	-0.85**	-0.76**	-0.41	-0.06
TSS (°Brix)	0.76**	1	-0.35	-0.81**	-0.05	0.11
Sugar/acid ratio (SAR)	-0.85**	-0.35	1	0.50	0.50	0.14
pH	-0.76**	-0.81**	0.50	1	0.16	-0.15
Water content (WC)	-0.41	-0.05	0.5	0.16	1	-0.12
Juice content (JC)	-0.06	0.11	0.14	-0.15	-0.12	1

** = Significant at 5% level of probability.

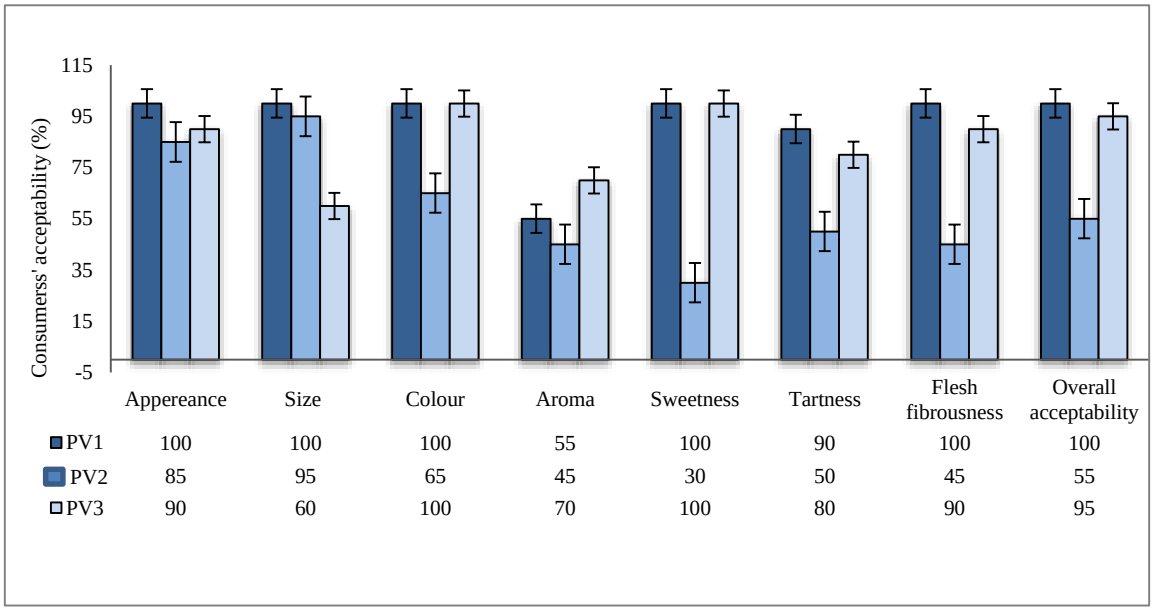

Fig. 2. Sensory evaluation of fruits of three pineapple accessions evaluated at maturity by twenty randomly selected penalists (n = 20) at ARDC Samtenling, Bhutan in 2018. Error bars represent standard error of mean.

Pearson's correlation coefficient analysis between physico-chemical characteristics

Multivariate Pearson's correlation coefficient was conducted amongst six physico-chemical characteristics of three pineapple accessions and represented by correlation matrix (Table 5). Citric acid percentage was found to be associated with TSS of fruit juice ($r=0.76$, $p\leq0.01$) and negatively correlated with sugar acid ratio ($r= -0.85$, $p\leq0.01$) and juice pH ($r= -0.76$, $p\leq0.01$). This indicates that increase in citric acid will result in decrease of sugar to acid ratio and pH, while increase in TSS will lead to increase of acid percentage. A strong negative correlation was found between pH of fruit juice and TSS ($r=-0.81$, $p\leq0.01$).

Sensory evaluation

The result of the sensory evaluation showed that PV1 was the most preferred accession with the overall acceptability percentage of 100% which was slightly higher than the acceptability percentage of PV3 which was 95% (Fig. 2). Pineapple accession PV1 was preferred over other accessions for all the sensory characteristics except for fruit aroma, sweetness and colour. Both PV1 and PV3 were equally preferred for their excellent fruit taste and attractive yellowish orange fruit colour. Consumers preferred PV3 over other accessions for its excellent fruit aroma with the acceptability percentage of 70% which was slightly higher than that of PV1 (55%). However, larger fruit size of PV1 and PV2 were preferred over the smaller sized fruits of PV3. The medium sized fruit of PV2 was least preferred by the consumers because of its dull orange coloured fruit with fibrous flesh, lower TSS and weak fruit aroma at fruit maturity.

DISCUSSION

This is the first study to characterise the morphological and physico-chemical attributes of three local landraces of pineapples grown in Sarpang district located in the wet subtropical region of Bhutan. Based on the results of this study, the National Seed Board of Bhutan under the Ministry of Agriculture and Forests have released accession PV1 and PV3 as variety 'Samtenling Kongtsey 1' and 'Samtenling Kongtsey 2' respectively in 2020 for large scale cultivation by farmers in Bhutan in the absence of known cultivars of pineapple prior to this study (DoA, 2020).

Although morphological and physiological characteristics are often influenced by the environment, morphological characterization of local landraces is important to better understand existing diversity and identify desirable traits for cultivation for specific market purposes and their potential utilization in the future. The results from this morphological and physiological characterisation of three pineapple accessions showed that accession PV1 has similar characteristics of Smooth Cayenne cultivar. According to Sanewski et al. (2018), Smooth Cayenne fruits are ovoid, medium to large fruits (1.5-2.5 kg) held on a relatively short and strong peduncle. The plants of Smooth Cayenne cultivar are a poor producer of shoots and slips (Bartholomew & Malezieux, 1994; Burhooa & Ranghoo-Sanmukhiya, 2012). The production cycle of Smooth Cayenne cultivar is usually longer than most of the other cultivars (TFNet, 2016). Furthermore, Burhooa and Ranghoo-Sanmukhiya (2012) stated that the Honey and Smooth Cayenne cultivars have no or very little spine at the leaf tips or margin, which is the most evident difference to distinguish them from other varieties. On the other hand, the morphological and physico-chemical characteristics of cultivar PV2 are similar to the description of Spanish group with typical square-shouldered (cylindrical) fruits of 1-2 kg with broad, fairly flat and deep eyes, resulting in lower flesh recovery (TFNet, 2016). Plants of pineapple accession PV3 resembles the characteristics of Queen cultivar characterised with compact short spiny green leaves with excessive suckers. Similar trait of

producing excessive ground suckers were recorded as characteristic trait of Natal Queen, a variant of Queen cultivar. The fruits of Natal Queen are attractive golden-yellow colour with deep eyes, less fibrous with crispy flesh and emits pleasant aroma. The average fruit weight varies from 600-800 g (TFNet, 2016). The accession PV3 possesses all the good organoleptic qualities of pineapple.

The selected parameters to predict eating quality in pineapples are: total soluble solids (TSS), titratable acidity, TSS/acid ratio, pH, colour and translucency (Sanewski et al., 2018). According to Lobo and Paull (2017), TSS is the most correlated parameter with eating quality and is used always as quality criteria for selecting for fresh market. Minimum TSS of 12 ^{o}Brix is required in the international trade for consumers acceptance (Bartholomew & Malezieux, 1994). The average TSS of PV1 (14.3°Brix) was within the TSS range of Smooth Cayenne cultivar (12-16 °Brix) as specified in varietal description by TFNet (2016). Similarly, average TSS of PV2 (10.3 °Brix) was also within the range (10-12 °Brix) of Spanish group. TSS of PV3 (14.4°Brix) was slightly higher than the TSS range of Queen cultivar which varies from 10-14°Brix (Sherman & Brye, 2019; TFNet, 2016).

The acid percentage content in PV1 and PV3 were within the range of 0.28 to 1.6% as specified by Lobo and Paull (2017). A sweetness ratio or sugar to acid ratio expressed as % TSS and % citric acid is used as a measure of consumers taste preference (Paull, 1993). According to Py et al. (1987), sugar acid ratio of 20-40 is recommended for consumers taste preferences and international trade market. Therefore, sugar to acid ratio of PV3 is within the range as specified, while that of PV1 is also close to the range mentioned by Lobo and Paull (2017). The pH values of PV1 (3.86) and PV3 (3.88) were found significantly lower than that of PV2 (4.61) but were close to the pH values of Smooth Cayenne (3.54) and Red Spanish (3.49) cultivars reported by Bartolome et al. (1995).

The first plant crop usually takes 16-20 months for harvest and the next ratoon crop takes another 15 months (Rodriguez-Alfonso et al., 2020). However, all three pineapple cultivars were found to mature much earlier, taking about 13 to14 months under wet subtropical climatic condition of Bhutan. According to Carlier et al. (2007), the time taken from planting to harvest depends on the weight or size of the propagule, cultivar and the temperature in the field. In addition, pineapple propagated through crown produces fruits in 18-24 months, slips in 15-20 months and suckers in 14-17 months (Lu et al., 2014; TFNet, 2016). Hence, the difference in the maturity period recorded in this study could be due to the type and the bigger size of the plant propagules used and the temperature in the field.

CONCLUSION

This is the first comprehensive study on the morphological and physico-chemical characterisations of landrace cultivars of pineapple in Bhutan. The data from our study revealed that there were many differences in morphological and physico-chemical characteristics among three accessions of pineapple evaluated. Major morphological differences were observed on both plant characteristics (plant growth habit, spine distribution on leaves, number of slips and suckers) as well as on fruit characteristics (fruit size, fruit shape and colour, fruit aroma, eye profile and days to fruit maturity). Significant differences were also observed on physico-chemical characteristics like juice TSS, acid percentage, acid to sugar ratio and pH of juice amongst three accessions evaluated.

Although only three landrace cultivars of pineapple were characterized in this study, the data presented here can provide the basic information to pineapple growers in Bhutan in choosing the cultivars for growing pineapple for different uses. It also presents the comprehensive data on the diversity of pineapple cultivars currently grown in Sarpang district

situated in the wet subtropical region of the country. PV1 is most suitable for canning and processing purposes owing to its fruit size, shape, broad shallow eyes and good physico-chemical characteristics. Similarly, PV3 has very good organoleptic characteristics and thus, is a good cultivar to grow for table purpose and fresh fruit consumption. Fruits of PV2 are neither attractive nor have any good physico-chemical properties and were least preferred by consumers. Future research should focus on evaluating more pineapple landrace germplasms by including landrace cultivars from other districts in Bhutan located in the subtropical agro-ecological zone. Further, the use of molecular techniques in identifying genetic diversity and characterisation of pineapple cultivars in Bhutan would generate more precise results over this conventional morphological characterisation.

Acknowledgment

The fund for this research was provided by the Department of Agriculture, Ministry of Agriculture and Forests, Royal Government of Bhutan. The authors are grateful to Agriculture Research and Development Centre at Samtenling for human resource and logistic supports rendered in conducting this research.

Conflict of interest

The authors hereby declare that there is no conflict of interest.

REFERENCES

Adje, C., Achigan-Dako, E. G., d'Eeckenbrugge, G. C., Yedomonhan, H., & Agbangla, C. (2019). Morphological characterization of pineapple (*Ananas comosus*) genetic resources from Benin. *Fruits, The International Journal of Tropical and Subtropical Horticulture, 74*(4), 167-179. https://doi.org/10.17660/th2019/74.4.3.

AOAC- Association of Official Analytical Chemists. (2005). *Official methods of analysis* (W. Horwitz & G. W. J. Latimer Eds. 18 ed.). Washington D.C.: Association of Official Analytical Chemists.

Bartholomew, D. P., & Malezieux, E. (1994). *Handbook of Environmental Physiology of Fruit Crops* (Vol. 2). London: CPC Press.

Bartolome, A. P., Ruperez, P., & Fuster, C. (1995). Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars. *Food Chemistry, 53*(1), 75-79. https://doi.org/10.1016/0308-8146(95)95790-D

Brown, M. B., & Cohen, E. (1983). Discussion of statistical methods for determining purity of citrus juice *Journal of the Association of Official Analytical Chemists, 6*, 781-788.

Burhooa, K., & Ranghoo-Sanmukhiya, V. (2012). Evaluation of different pineapple (*Ananas comosus* Merr L.) varieties using morphological and genetic markers in Mauritius. *Biotechnology, 11*(5), 272-279. doi:https://doi.org/10.3923/biotech.2012.272.279.

Carlier, J. D., d'Eeckenbrugge, G. C., & Leitao, J. M. (2007). Pineapple. In C. Kole (Ed.), *Fruits and Nuts* (pp. 331-342). Berlin: Springer.

d'Eeckenbrugge, G. C., Sanewski, G. M., Smith, M. K., Duval, M.-F., & Leal, F. (2011). Ananas. In C. Kole (Ed.), *Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits* (pp. 21-41). Berlin: Springer.

DoA (Department of Agriculture). (2020). *Inventory of Released and De-notified Crops in Bhutan (1988-2020).*Thimphu,Bhutan: Department of Agriculture,Royal Government of Bhutan.

Dorji, T. (2019). *Pineapple cultivation picking up in Khangma, Pema Gatshel. 31 October 2019* Thimphu, Bhutan: Bhutan Broadcasting Services Corporation.

FAO- Foood and Agriculture Organisation. (2019). *Major tropical fruits - Statistical compendium 2018*. Rome, Italy: Food and Agriculture Organisation.

IPGR- International Board for Plant Genetic Resources. (1991). Descriptors for pineapple. Retrieved 15 November 2017, from International Board for Plant Genetic Resources https://www.bioversityinternational.org/e-library/publications/detail/descriptors-for-pineapple/.

Jones, L. V., Peryam, D. R., & Thurstone, L. L. (1955). Development of a scale for measuring soldiers' food preferences. *Food Research, 20*, 512-520. https://doi.org/10.1111/j.1365-2621.1955.tb16862.x

Li, W., Yan, M., Hu, B., Priyadarshani, S., Hou, Z., Ojolo, S. P., & Qin, Y. (2018). Characterization and the expression analysis of nitrate transporter (NRT) gene family in pineapple. *Tropical Plant Biology, 11*(3), 177-191. https://doi.org/10.1007/s12042-018-9209-z.

Lobo, M. G., & Paull, R. E. (Eds.). (2017). *Handbook of Pineapple Technology: Production, Postharvest Science, Processing and Nutrition.* London: John Wiley & Sons.

Lu, X.-H., Sun, D.-Q., Wu, Q.-S., Liu, S.-H., & Sun, G.-M. (2014). Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China. *Molecules, 19*(6), 8518-8532. https://doi.org/10.3390/molecules19068518.

MoAF-Ministry of Agriculture and Forests. (2017). *Bhutan RNR statistics 2017*. Thimphu: Ministry of Agriculture and Forests, Bhutan.

MoAF. (2018). *Bhutan RNR statistics*. Thimphu, Bhutan: Ministry of Agriculture and Forests, Bhutan.

Nadirah, W. W., Jawaid, M., Al Masri, A. A., Khalil, H. A., Suhaily, S., & Mohamed, A. (2012). Cell wall morphology, chemical and thermal analysis of cultivated pineapple leaf fibres for industrial applications. *Journal of Polymers and the Environment, 20*(2), 404-411. https://doi.org/10.1007/s10924-011-0380-7

Namgyal, T. (2019). *Bhutan Agro to help eastern farmers, 13 November 2019*. Thimphu, Bhutan: Kuensel Corporation Limited.

OECD- Organisation for Economic Co-operation and Development. (2005). *Guidance on Objective Tests to Determine Quality of Fruits and Vegetables and Dry and Dried Produce*. Paris: Organisation for Economic Co-operation and Development.

Paull, R. (1993). Pineapple and papaya. In G. B. Seymour, T. J.E., & T. G.A. (Eds.), *Biochemistry of Fruit Ripening* (pp. 291-323). Dordrecht: Springer.

Perez, G., Mbogholi, A., Sagarra, F., Aragón, C., González, J., Isidrón, M., & Lorenzo, J. C. (2011). Morphological and physiological characterization of two new pineapple somaclones derived from in vitro culture. *In Vitro Cellular & Developmental Biology-Plant, 47*(3), 428-433. https://doi.org/10.1007/s11627-011-9342-y.

Py, C., Jean-Jospeh, L., & Teisson, C. (1987). *The Pineapple: Cultivation and Uses*. Paris: Editions Maisonneuve & Larose.

Ramallo, L. A., & Mascheroni, R. H. (2012). Quality evaluation of pineapple fruit during drying process. *Food and Bioproducts Processing, 90*(2), 275-283. https://doi.org/10.1016/j.fbp.2011.06.001.

Rodriguez-Alfonso, D., Isidron-Prez, M., Barrios, O., Fundora, Z., Hormaza, J. I., Grajal-Martin, M. J., & Herrera-Isidron, L. (2020). Minimal morphoagronomic descriptors for Cuban pineapple germplasm characterisation. *Horticultural Science, 47*(1), 28-35. https://doi.org/10.17221/27/2019-HORTSCI.

Sanewski, G. M., Bartholomew, D. P., & Paull, R. E. (Eds.). (2018). *The Pineapple: Botany, Production and Uses* (2nd ed.). London: CABI Publishing.

Sherman, L. A., & Brye, K. R. (2019). Soil chemical property changes in response to long-term pineapple cultivation in Costa Rica. *Agrosystems, Geosciences & Environment, 2*(1), 1-9. https://doi.org/doi:10.2134/age2019.07.0052.

TFNet- International Tropical Fruits Network. (2016). Major fruit: Pineapple. Retrieved 7 November 2017 from International Tropical Fruits Network (TFNet). https://www.itfnet.org/v1/2016/05/pineapple-common-varieties/.

UPOV-International Union for the Protection of New Varieties of Plants. (2013). Guidelines for the conduct of tests for distinctness, uniform and stability for pineapple. Retrieved 17 November 2017 from International Union for the Protection of New Varieties of Plants https://www.upov.int/edocs/tgdocs/en/tg295.pdf.