Research Paper

Evaluation of Pineapple Hybrids for Quantitative and Qualitative Characters and Inheritance of Leaf Spine Distribution

I. Kalubowila*, S.M.G.D. David, R.M.M.N.K. Rathnayaka, H.G.B. Darshana

Fruit Research and Development Institute, Kananwila, Horana, Sri Lanka

**Corresponding author: indrakalu@yahoo.com*

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Abstract

Mauritius (Queen type) and Kew (Cayenne type) are the economically important two pineapple cultivars in Sri Lanka. The desirable characters for both fresh market and canning are detached between two cultivars. The aim of the pineapple breeding programme at Fruit Research and Development Institute Horana Sri Lanka was to develop pineapple hybrids with improved yield and quality by crossing Kew and Mauritius. 435 hybrids derived from the cross between Kew and Mauritius and its reciprocal were evaluated for fruit weight, fruit shape, crown weight, eye shape, Brix% and spine distribution of leaf margin. Six promising hybrids having combined characters of ≥ 1700 g fruit weight, cylindrical shape, ≤ 350g crown weight, ≥17 Brix%, spiny at the leaf tip and broad eyes were selected for further evaluation. Spiny tip leaf margin showed by the parent Kew is controlled by the "*ppSs*" genotype while the spiny leaf margin of the parent Mauritius is controlled by the "*ppss*" genotype. The phenotypic segregation of the hybrid progeny was 1:1 (spiny tip: spiny) corroborates the theory of two genes with epistatic interaction of the locus "*P*" (piping) over the locus "*S*" (spiny) in control of leaf margin spine distribution.

Keywords: Hybrids, Kew, Mauritius, Spine distribution

Introduction

Pineapple (*Ananas comosus* L. Merr.) is the most economically important species in the family Bromeliaceae (Bartholomew *et al*., 2003). It is native to Brazil in South America. There are approximately 30 cultivars of *A. comosus* that are grown commercially in tropical and sub-tropical countries around the world. However, for convenience in global trade, the numerous pineapple cultivars are grouped in four main classes; Smooth Cayenne, Red Spanish, Queen, and Pernambuco (Abacaxi), despite much variation in the types within each class (Morton, 1987; Coppens and Leal, 2003). Out of these four types Mauritius variety in queen type is the most popular and widely cultivated in most of the districts in Sri Lanka while Kew variety in Cayenne type is mostly restricted in Badulla and Moneragala districts. The annual extent and production were 4,665 ha and 44,793 t respectively in 2019 (Agstat, 2020). Nearly 90% of the pineapple is consumed as fresh fruit in Sri Lanka.

Mauritius, the most important commercial cultivar in Sri Lanka, has spiny leaves and relatively small fruit,with an average weight of 1.5 kg. This is a well-adapted variety with good qualities for fresh market. The fruit has a pleasant aroma, crispy and golden yellow colour flesh and sweet juice with 14-16% brix and 0.50 - 0.70% acidity. Mauritius is suitable for distant marketing due to good keeping quality and transportability. The fruit cannot be canned well due to its conical shape and deep 'eyes'. Though the Kew is cultivated on a small scale in Sri Lanka it is the most famous variety in the world. It is very good for canning due to its cylindrical shape of fruit and flat eyes on fruit peel. Fruits are larger than Mauritius. When ripe its flesh is pale yellow, translucent, non-fibrous, sweet and sour in taste. It has spineless leaves except at the tips. The keeping quality and transportability are poor in the fruit.

Both of these cultivated varieties are not perfect to fulfill the standard of fresh and canned consumption. Therefore, the pineapple improvement programme in Fruit Research and Development Institute (FRDI) Sri Lanka aims to develop hybrids of pineapple by crossing Mauritius and Kew. The hybrids are expected to have high yield, spineless leaf, cylindrical-shaped fruit, yellow flesh, sweet flavor and good keeping quality by blending characteristics of both parents. In general, plant breeders use the Cayenne type as a parent in crossing because it has the best quality amongst the other cultivars (Py *et al*., 1987; Leal and Coppens, 1996). Hybridization between Queen and Cayenne is expected to be able to generate the new superior varieties having higher quality than both Queen and Cayenne types (Hadiati *et al*., 2011).

The pineapple plant is self-incompatible that is no seed will be formed if self-pollination (selfing) takes place (Py *et al*., 1987; Coppens *et al*., 1993). Therefore, hybridization among pineapple varieties should be conducted to obtain seeds. Hybridization and selection of progenies in the segregating F_1 have been the method of choice for pineapple breeding at the Malaysian Agricultural Research and Development Institute (MARDI). MARDI successfully developed a commercial hybrid 'Josapine' using this methodology

and more promising hybrids particularly those with the 'piping-leaf' character (Chan and Lee, 1996).

This study was carried out to evaluate the quantitative and qualitative characters of 435 hybrids of pineapple generated from crossing between Kew × Mauritius and their reciprocal in the pineapple breeding programme at the FRDI. The study was extended to determine the genotypic combinations associated with control of pineapple leaf margin spinescence in Kew and Mauritius based on the phenotypic evaluation of segregating progenies.

Materials and Methods

Collection and characterization of parental materials, hybridization, seedling management, evaluation and selection of hybrids were carried out at the FRDI from January 2017 to December 2020.

Collection and characterization of parental materials

Kew and Mauritius cultivars were used as parental materials. Kew plants were collected from the Monaragala district and Mauritius was collected from the Gampaha district. Hundred plants of each were established in a crossing block. Phenotypic characterization of parents including fruit weight, crown weight, fruit shape, eye shape, Brix % and spine distribution was done using randomly selected five plants of each parent.

Hybridization

Pollen was collected from flowers at anthesis early in the morning. The anthers were removed from the inflorescence by making three deep triangular incisions into the base of the flower and then gouged them out (Chan *et al*., 2003). The petals of flowers were removed to expose the freshly dehisced anthers with an abundance of pollen. Pollination was carried out from 8:00 am to 12:00 noon.

The anther was picked up with a fine pair of forceps and gently brushed on the stigma of the flower. There is no necessity to emasculate the flower before pollination because the cultivars are self-incompatible. The number of flowers crossed varied from one plant to another depending on the availability of opening flowers at that time.

Seedling management

Seeds produced were then germinated in glass bottles lined with moist filter paper. The sprouts were then transplanted to small coir dust pots for 3 months. Seedlings were subsequently transplanted to the 15 cm × 20 cm poly bags filled with a medium containing a mixture of soil, organic manure, and sand (1:1:1) for six months. After that, the seedlings were moved again to the bigger poly bags (40 cm × 50 cm) containing the same medium mixture until plants producing fruit. The plants were kept in a shed covered with a 50% shade net.

Evaluation of quantitative and qualitative characters of hybrids

Observations were made on the quantitative and qualitative characters of each plant. Parameters observed in quantitative characters were fruit weight (g) weighed without crown and peduncle and crown weight. Qualitative characters covering fruit shape, spine distribution and eye shape were observed following the method of descriptor list for pineapple (IBPGRI 1991)

and the brix value was assessed using juice from the base, middle and top parts of the fruit using a hand refractometer.

Inheritance of leaf spine distribution

Leaf spine distribution in F1 pineapple plants was evaluated in a total of 233 of the progeny of the Kew × Mauritius and 202 plants of the progeny of the Mauritius × Kew. According to the inheritance model proposed by Collins and Kerns 1946, the genotypic combination of *ppSs* (spiny tip) for Kew and *ppss* (spiny) for Mauritius was analyzed. The chi-square statistical test (χ^2) was performed on deviations between observed and expected numbers, based on the genetic hypotheses among hybrid progenies of 233 of Kew × Mauritius and 202 of Mauritius × Kew. The value of χ^2 was obtained by the following expression.

$$\chi^2 = \Sigma [(o_i - e_i)^2 / e_i] \quad \text{..... (1)}$$

where o_i = observed number of plants for the class evaluated

e_i = expected number of plants for the class evaluated.

Hypotheses with the calculated $\chi^2 \geq$ the tabulated χ^2 were rejected; and hypotheses with the calculated $\chi^2 <$ the tabulated χ^2 were accepted.

Results and Discussion

Phenotypic Characterization of parent

The Kew had spiny tip margins and was heavy in size. Fruits are larger than Mauritius. Fruits were in a cylindrical shape and contain cream-white pulp with 17% Brix (Table 1). The parent Mauritius has spiny leaf margins and vigorous growth with long leaves and dark green color. Its fruit has a conical shape and its average fruit weight is 1.09 kg. The pulp has a yellowish pulp color with an intense aroma and total soluble solids are 16 % Brix (Table 1).

Fertility and compatibility

Fertility and compatibility are important considerations in deciding the parents to be used for hybridization. The average number of seeds per fruitlet produced by Kew × Mauritius is lower than the reciprocal (Table 2). Smooth cayenne for instance had low fertility and was a poor female parent because only a few seeds were formed. However reciprocal crosses using smooth cayenne as the pollen donor provided satisfactory seed set in all crosses (Chan, 1986). Smooth cayenne was also shown to be a poor female parent bearing only about 200 seeds per fruit compared with its reciprocal carried 2000 seeds per fruit (Cabral *et al*., 1993).

Table 1. Phenotypic Characterization of parent

Parent	Spine Distribution	Average Fruit weight (g)	Average Crown weight (g)	Fruit shape	Eye shape	TSS (% Brix)
Kew	at the tip	1700	290	Cylindrical	Flat	17
Mauritius	spiny	1093	170	Conical	Projected	16

Table 2. Mean seed set per flower in Kew x Mauritius cross and its reciprocal

Cross	Number of flowers crossed	Number of seeds obtained	Mean seed set/ fruitlet
Kew x Mauritius	2156	6402	2.96
Mauritius x Kew	2374	11804	4.97

Quantitative and qualitative characters of hybrids

Fruit weight closely related to fruit size is an important consideration in selection because of standards set by the industry. The range of fruit size preferred by canary is from 1.23 -2.18 kg that process into fruit cylinder weight of 0.4-0.75 kg (Hapton and Hodgson, 2003). The high heritability of fruit size is shown in hybrid populations generated from large-fruited parents like "Sarawak", which invariably has a high percentage of large-fruited progenies (Chan, 1991).

In the present study, eighteen percent of hybrid plants from Kew x Mauritius cross produced heavier fruits than their female parent (1700g) while 48% of them are heavier than their male parent (1093 g). In Mauritius x Kew progeny 42% of plants produced fruits heavier than their female parent and 14% of them produced fruits heavier than their male parent (Table 3). These results suggested that crossing between Kew x Mauritius and their reciprocal resulted higher fruit weight on more than 14% of the Kew while more than 44% compared with Mauritius.

Hadiati *et al*. (2011) reported that 13.3% of hybrid plants from Cayenne x Queen produced heavier fruits than their female parent while 46.7% of them were heavier than their male parent. In Queen x Cayenne hybrid plants, 40.0% of them produced fruits heavier than their female parent and 27.5% of them produced fruits heavier than their male parent.

The shape of fruit produced from the population of hybrid plants varied from cylindrical to reniform. Hybrids from Kew x Mauritius tended to generate hybrids with shape of fruit of cylindrical (23.7%) while 17.2 % hybrids from its reciprocal (Table 4).

Table 3. The percentage of hybrid plants producing fruit associating parent's fruit weight

Cross	Percentage of hybrid plants producing fruit		
	< 1093 g	1093 - ≤ 1700 g	>1700 g
Kew x Mauritius	34	48	18
Mauritius x Kew	42	44	14

Table 4. The percentage of hybrids based on fruit weight, fruit shape, eye shape and brix value in both progenies

	Fruit shape			Eye shape		Crown weight (g)		Brix%	
	Conical	Cylindrical	Other	Broad	Projected	≤350	>350	≥17	<17
Kew x Mauritius (233 hybrids)	40.7	23.7	35.6	59.6	40.4	46	54	50.6	49.3
Mauritius x Kew (202 hybrids)	41.9	17.2	40.9	43.1	56.9	52	48	57.4	42.5

Fruits with broad eyes are also a desirable character of pineapple. Table 4 shows that hybrids from Kew x Mauritius that have fruits with the broad eye shape and projected-eye shape were 59.6% and 40.3%, respectively. Hybrids from Mauritius x Kew that have fruits with the broad-eye shape and projected-eye shape were 43.1% and 56.9%, respectively.

Crown size is affected the packing and transportation in which the lighter the crown weight is easier to pack and transport. In the current study, hybrids with larger fruits over 1700 g were selected, resulting in an increase in crown weight. Therefore, ≤ 350 g of crown size was concerned to select larger fruited hybrids. The hybrid plants producing ≤ 350 g of crown weight were as much as 46% and 52%, respectively in Kew x Mauritius hybrids and in Mauritius x Kew hybrids (Table 4). The hybrid plants producing small crown (<100g) were as much as 48.0% and 37.5%, respectively in Cayenne x Queen Hybrid plants and in Queen x Cayenne hybrid plants (Hadiati *et al*., 2011).

Smith (1988) stated that TSS is one of the parameters generally used to predict the eating quality of pineapple. Table 4 shows that more than 50% of hybrid plants produced fruits with ≥17°Brix in both progenies. This is probably influenced by Queen either as a male or a female parent since Queen has sweeter fruit flesh than Cayenne (Py *et al*., 1987; Hadiati *et al*., 2003). Smooth cayenne as the female parent generated progenies with higher TSS content in a cross between smooth cayenne and Manzana (Loison-Cobot and Lacoeuilhe, 1990). Hybrid plants bearing fruits with sugar content ≥16° Brix were 23.8% and it was higher on the Cayenne x Queen Hybrids than on the Queen x Cayenne hybrids (Hadiati *et al*., 2011).

Concerning the most desirable characters, six hybrids (KQ-21, KQ -22, KQ- 33, KQ-65, KQ-69, KQ- 127) having combined characters of ≥ 1700 g fruit weight, cylindrical shape, ≤ 350 g crown weight, ≥17 Brix%, spiny at the leaf tip and broad eyes were selected for further evaluation (Table 5).

Table 5. Quantitative and qualitative characteristics of selected hybrids and parents

Accession number	Spine Distribution	Fruit weight (g)	Crown weight (g)	Fruit shape	Eye shape	TSS (% Brix)
KQ -21	at the Tip	1890.5	305	Cylindrical	Flat	18
KQ -22	at the Tip	2300.3	307	Cylindrical	Flat	17
KQ -33	at the Tip	1732.1	308	Cylindrical	Flat	17
KQ -65	at the Tip	2030.1	342	Cylindrical	Flat	19
KQ -69	at the Tip	1762.7	350	Cylindrical	Flat	19
KQ - 127	at the Tip	1793.8	202	Cylindrical	Flat	19
Kew	at the Tip	1700	290	Cylindrical	Flat	17
Mauritius	Spiny at both leaf margins	1093	170	Conical	projected	16

All these hybrids appeared to have similarities to the Kew type with superior fruit weight and sugar content (TSS).

Spine distribution and its inheritance

Based on the spines distribution pattern, the leaf margins of pineapple can be classified into three phenotypes. The completely spineless leaf type and entirely spiny leaf type are designated as 'piping-leaf type' and 'spiny-leaf type', respectively. The third, 'spiny-tip-leaf type', shows spines at the tip of the leaf and sometimes has spines in the region below the tip, separated by a non-spiny leaf margin. The term 'piping' originates from the peculiar structure of the leaf margin, which shows a folded narrow portion of the edge of the leaf. On the other hand, spiny-tip leaf and spiny leaf have a sharp leaf edge (Collins and Kerns, 1946).

Leaf margin phenotype is controlled by two genes, *P* (piping) and *S* (spiny tip), which correspond to piping leaf and spiny-tip leaf, respectively. *P* and *S* loci are independent of each other, and *P* is epistatic to *S* (Collins and Kerns, 1946). Therefore, plants with a *P* locus show piping leaf regardless of the genotype of the *S* locus. Genotypes of 'spiny-tip-leaf type' and 'spiny-leaf type' are *ppSS*/*ppSs* and *ppss*, respectively (Collins and Kerns, 1946).

Evaluations of the "spineless" and "spiny" leaf margins converged to the same hypothesis of segregation when data were analyzed using the chi-square test. Both progenies Kew x Mauritius and Mauritius x Kew were segregated into 1:1 (spiny: spiny tip), expressing the phenotypes of their respective parents (Table 6).

These results confirm the genetic theory proposed by Collins and Kerns (1946) and indicate that the parent "Kew" has genotype "*ppSs*" for spiny tip leaf. It indicates the presence of the locus "*P*" (piping) in recessive homozygosity and the locus "*S*" (spiny) in heterozygosity. The parent Mauritius has the "*ppss*" genotype for spiny leaf margin and it indicates the presence of double recessive homozygosity.

Table 6. Chi-square test (χ^2) for the genetic hypothesis with two loci (P - piping and S - spiny) and epistatic interaction of "*P*" over "*S*" (Collins and Kerns, 1946), controlling leaf margin spinescence

	Progenies Female parent x male parent	
	Kew x Mauritius	**Mauritius x Kew**
Genotypic Hypothesis Phenotype	**"*ppSs*" × "*ppss*" Spiny tip x Spiny**	**"*ppss*" × "*ppSs*" Spiny x Spiny tip**
Phenotypic relation	(spineless: spiny tip: spiny)	
Expected phenotypic ratio	0:1: 1	0:1: 1
Observed number (O)	0:130:103	0:91:111
Expected number (E)	0:116.5:116.5	0:101:101
$(O- E)^2 /E$	0:1.56:1.56	0:0.99:0.99
Calculated χ^2 df=1 *P*=5%	3.12^{ns}	1.98^{ns}

Table 6 presents the results of the chi-square test (χ^2), associated with the genotypic combinations of the 1:1 (spiny: spiny tip) phenotypic ratio related to the cross between the "*ppSs*" genotype and the "*ppss*" genotype. The results indicate that there are no significant deviations between observed and expected results at 5% level of significance.

In the study of Júnior *et al*. (2011) the heterogeneity test was properly applied, since the spineless leaf (*Ppss*) BRS Imperial cv. was used in hybridization as both a female parent and a male parent with "Pérola" and "Pico de Rosa", respectively, both with spiny leaves (*ppss*). The phenotypic segregation of 1:0:1 (spineless: spiny tip: spiny) corroborates the theory of two genes with epistatic interaction of the locus "*P*" (piping) over the locus "*S*" (spiny) in control of leaf margin spinescence.

Conclusions

435 hybrids derived from the cross between Kew and Mauritius and its reciprocal were evaluated for fruit weight, fruit shape, crown weight, eye shape, Brix% and spine distribution of leaf margin. Six promising hybrids(KQ-21, KQ -22, KQ- 33, KQ-65, KQ-69, KQ- 127) combined with most desirable characters i.e. ≥ 1700 g fruit weight, cylindrical shape, ≤ 350g crown weight, ≥17 Brix%, broad eyes and spiny at the leaf tip were selected for further evaluation. The Spiny tip leaf margin showed by the parent Kew is controlled by the "*ppSs*" genotype while the spiny leaf margin of the parent Mauritius is controlled by the "*ppss*" genotype. The phenotypic segregation of the hybrid progeny was 1:1 (spiny tip: spiny) corroborates the theory of two genes with epistatic interaction of the locus "*P*" (piping) over the locus "*S*" (spiny) in control of leaf margin spine distribution.

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