IJCS INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 12, Issue 3, July-September 2021: 1179-1188 www.ijcs.ro

GENETIC VARIABILITY OF SINGLE, DOUBLE AND THREE-WAY CROSS HYBRIDS IN PAPAYA (*Carica papaya*)

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Abstract

The production of high-yielding varieties of papaya is intended to improve both fruit quality and productivity. However, high genetic variability has a significant role in characters selection. In papaya (Carica papaya), genetic variability can be expanded through the hybridization of selected parents. This research aims to determine the genetic variability of 70 papaya hybrids of the Indonesian Tropical Fruit Research Institute's (ITFRI) collection. The research was carried out at the Sumani Experimental Field of ITFRI in Solok (360 m asl), West Sumatera. The study was arranged using randomize block design with 70 papaya hybrids as treatments factors, each with three replications. Hybridization was performed by the back cross, single-cross, double-cross, three-way cross, and selfing. The results show a wide genetic diversity of 70 papaya hybrids, which can be inferred from fruit weight (500-2000g), flesh thickness (1.5-3.5cm), peel hardness (0.70-0.90 kg/cm^2), flesh hardness (0.15-0.4kg/cm^2), and total soluble solids (TSS) (9 - 12.5^{0}brix). The wide genetic diversity can be applied in the selection stage to obtain a superior hybrid with fruits characteristics that include small-medium size, thick flesh, crispy flesh texture, and sweet taste as preferred by the consumer.

Keywords*: Carica breeding; Fruit characteristics; Kybridization; Selection*

Introduction

Papaya (*Carica papaya* L.), which belonged to the genus Carica family Caricaceae, is the most economically important species in a tropical country. The genus is indigenous to tropical America and brought to South East Asia (Philippines) by the Spain merchant during the Spanish exploration [1]. Papaya then spread rapidly to India, Indonesia, Malaysia, and Africa by Portuguese, Dutch, Britain, and French merchants [1]. Today, various varieties are known globally, including Kapoho, Solo Sunrise, Maradol, Tainung, Pusa, Surya, Red Lady, Washington, and Rainbow [2]. In Indonesia, familiar papayas varieties include Calina, Merah Delima, Carisya, and local papaya [3].

Papaya is one of the important fruit crops in Indonesia and cultivated for consumption, pharmaceutical and industry. Furthermore, the fruit is a good source of vitamin C, beta carotene, and antioxidants [4]. Indonesian annual production of the fruit increased year by year from 840.112 tons in 2014 to 986.991 ton in 2019 [5] made Indonesia the third papaya producer in the world after India and Brazil [6].

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Various papaya varieties were well recognized in Indonesia and diverse on term of fruit size, peel and flesh color, taste, flesh thickness, and plant productivity. Indonesian Tropical Fruit Research Institute (ITFRI) have been explored banana cultivars around the Island of Indonesia and found 40 accessions with high genetic diversity [7]. Furthermore, 6 local accessions of papaya in West Sumatra, including Semangko, Tangkai Ungu, Daun Lebar, Gading Merah, Sicincin, Sungai Tarab and 4 in Java such as Dampit, Cibinong, Paris, and MJ9 have been characterized and collected by *Sunyoto et al* [8].

The economic value of papaya has the potential to be exploited since consumer's demand the high quality of papaya is increasing over time [9]. However, the papaya industry in some tropical country, especially in a developing country, is still relatively small and just planted as a side in the home garden and as local consumption [6]. Unsuitable land, pathogen infection, plant quarantine restriction and limited high-yielding varieties were other problems that hinder papaya expansion.

As a country with a high diversity of papaya, Indonesia has a chance to produce a better-yielding variety of papaya to solve a constraint of papaya production. The development of hybrid varieties may be a solution to generate high yielding-varieties, and of course, the diversity of local varieties is defining aspect of this method [10]. The development of high yielding varieties is focused on the production of fruit with a sweet taste, constant productivity (without lag phase), reddish-orange flesh colour, thick flesh and hard texture and thick peel for prolonged self-life (up to seven days after harvest).

One of the significant factors in the conventional papaya breeding program is genetic variability. The hybridisation activity of selected parents from a collection is an alternative method to improve genetic variability papaya. In order to produce hybrids, crossing can either conduct as single, double, and three-way cross hybrids [11] as applied on maize [12, 13], tomato [11], sunflower [14]. Aside from Indonesia, papaya breeding program also have been conducted in several counties, namely Thailand, India, Malaysia, and Brazil [15].

In Indonesia, as represented by the Indonesian Tropical Fruit Research Institute, papaya's hybridisation has also been conducted by applying back cross hybridization, including single-cross, double-cross, three-way cross and selfing of Indonesian papaya parents. However, result characterization and evaluation of their genetic variation have not been explored yet. Therefore, this research aims to evaluate the genetic diversity of the papaya hybrids due to the crossing activity.

Experimental part

Times and experimental design

The research was conducted on Sumani Experimental Fram, Solok Regency, West Sumatera (360 m asl). The study was arranged in a randomized block design with three replications, where each unit contained four plants. The treatments were 70 papaya hybrids resulted from single, double and three-way cross of parent's accessions, namely merah delima (BT1), BT2, BT3, (BT4), dampit (D), solinda (K), local kotobaru (KTBR), sungai tarab (STRB), and lokal daun jarak (DJRK) (Table 1).

Research procedure and data analysis

Before planting, 15kg manure and 1.0kg of dolomite per planting hole were applied to the experimental field for seven days to allow perfect manure decomposition and neutralized soil pH. Forty-five days old papaya seeds were transplanted on the planting hole at a spacing of 2.5 x 2.5m. Plant nursing was conducted on each plant by fertilizer using 250g of Nitrogen

Phosphorus,Potassium (16-16-16) and 50g Potassium Chloride. Plant pests and diseases were managed using pesticide (imidakloprid), acaricide (dicofan), and fungicide (copper and sulfur) with manufacture's dosage recommendation. Weeding was done manually with hoe every two weeks to maintain field sanitation.

Table 1. Seventy papaya hybrids used in this research

Genotypes	Cross hybrid	Genotypes	Cross hybrid
1	(BT2 X BT3) X (BT3 X BT2)	38	BT4 X BT1
2	BT1 X BT4 X BT1 X D	40	BT1 X D X JRK
3	BT3 X BT1	41	BT1 X DX BT3 X D
4	BT2 X BT1 X BT2 X BT1	42	BT2 X BT4 X BT1 X BT4
5	BT3 X D	44	BT2 X BT1 X BT1 X BT3
6	BT4 X BT3 X D	46	D X BT1 X BT1 X BT3
7	BT1 X D X BT2 X BT1	48	BT1 X BT2
8	B4 X BT3 X BT4 X D	49	BT1 X BT2 X BT1 X BT3
9	BT1 X BT2 X BT1 X D	50	BT2 X BT3 X BT4 X BT3
10	D X BT1 X BT1 X D	51	BT2 X BT4 X BT2 X D
11	BT1 X D	52	BT2 X BT1
12	BT2 X BT3 X BT1 X BT4	53	BT1 X D X BT1 X D
13	BT3 X D X BT2 X D	54	BT4 X BT3 X BT1 X BT2
14	D X BT1 X BT2 X BT1	55	BT2 X BT3 X BT3 X BT4
15	BT2 X BT4 X BT2 X BT4	56	BT2 X BT3 X BT3 X BT1
16	BT2 X BT4	58	BT2 X BT1 X BT1 X BT2
17	BT1 X BT4 X BT1 X BT2	59	BT3 X DJRK
18	BT1 X KTBR	61	BT1 X D X K
19	BT1 X BT2 X BT2 X BT4	62	BT4 X D X D X BT1
20	BT2 X BT1 X BT3 X BT4	63	BT2 X BT3 X BT2 X BT3
21	BT4 X BT1 X BT2 X BT1	67	D X BT2
22	BT4 X BT3 X BT3 X BT4	68	BT3 X BT4 X BT2 X BT3
24	BT3 X STRB	70	BT1 X BT2 X BT3 X BT4
25	BT2 X BT1 X BT3 X BT1	76	BT1 X DBT2 X BT4
26	BT1 X BT4 X D X BT1	78	BT1 X BT2 X BT4 X D
27	BT2 X BT4 X D X BT1	79	BT2 X BT3 X D X BT1
29	BT4 X BT1 X BT2 X BT1	82	D X BT4
30	BT4 X BT3	83	BT1 X BT2 X BT1 XB T2
31	D X BT2 X BT4 X BT1	86	D X BT1
32	BT2 X BT3 X BT1 X BT3	87	BT3 X DJRK
33	BT3 X BT1 X BT2 X D	90	BT3 X K
34	BT2 X BT1 X BT2 X BT3	93	BT4 X BT3 X BT4 X BT3
35	BT4 X BT1 X BT4 X D	96	BT2 X BT1 X BT1 X BT4
36	BT2 X BT4 X BT4 X D	97	BT2 X BT1 X BT2 X D
37	BTK X BT3	98	BT4 X K

Note: Merah Delima (BT1), BT2 = Balitbu Tropika 2, BT3 = Balitbu Tropika 3 = Carmina, BT4 = Balitbu Tropika 4 = Carmida, Dampit (D), Solinda (K), Lokal Kotobaru (KTBR), Sungai Tarab (STRB), and Lokal daun jarak (DJRK)

Five fruits per hermaphrodite plants were characterized, including fruit weight, length, diameter, fruit cavity width, flesh thickness, flesh and peel hardness, and total soluble solids.

Analysis of variance was calculated using SAS software to determine the genetic variance. Mean squares (MSt) of treatments and mean squares of error were then used to estimate genetic variance (GV) and phenotypic variance (PV) based on the relations below:

GV = MSt - MSe/replication;

PV = MSe (sum of genetic variance and environment variance)

Genetic variability can be determined by the deviation standard of the genotypic and phenotypic variance observed [16]. A wide GV defined as GV > 2 and a narrow GV if GV ≤ 2. Similarly, a wide PV defined as PV > 2 and a narrow PV if PV ≤ 2 [17].

Further, to construct the intraspecific and subgrouping tree within papaya cultivars, hierarchical clustering was conducted to morphology character data using Unweighted Pair Group Method with Arithmetic Mean/UPGMA method option and Jaccard dissimilarity index on PAST [18].

Results and discussion

A significant difference in all characters of tested hybrids showed that all characters' variability was mainly affected by genetics. All characters have a wide genotypic and phenotypic variance, except for fruit diameter (Table 2). A wide genetic variability regenerates a wide phenotypic variability; however, a vice versa does not always occur due to the environmental effect [19].

Table 2. Analysis of variance, Genotypic, and Phenotypic Variance

Fruit parameters	Middle square	Genotypic variance	2x StdGV	GV criterion	Phenotypic variance	2x StdPV	PV criterion
Stalk length (cm)	1.88**	0.45	0.21	Wide	0.63	0.21	Wide
Weight (g)	340771**	97394	38133	Wide	113590.5	37938	Wide
Length (cm)	32.03**	8.99	3.59	Wide	10.68	3.57	Wide
Fruit circumference (cm)	61.44**	14.38	6.99	Wide	20.48	6.84	Wide
Fruit Diameter (cm)	6.86*	0.84	0.84	Narrow	2.29	0.76	Wide
Peel hardness (kg/cm^2)	0.73**	0.11	0.09	Wide	0.24	0.08	Wide
Cavity width (mm)	2.44**	0.72	0.27	Wide	0.81	0.27	Wide
Flesh thickness (cm)	0.28**	0.08	0.03	Wide	0.09	0.03	Wide
Flesh hardness (kg/cm^2)	0.82**	0.33	0.17	Wide	0.6	0.09	Wide
TSS (^{o}brix)	2.29**	0.59	0.26	Wide	0.76	0.25	Wide

StdVG = standard deviation of genotypic variance, StdVP= standard deviation of phenotypic variance

A wide variability, both genotypic and phenotypic, gives a better chance for selection success. However, the selection of high-yielding papaya was directed based on consumer desires, especially for fruit characters, including small or big, thick flesh, hard texture flesh, sweet taste or high total soluble solids (TSS). This study found that the variability in flesh texture and peel hardness of 70 papaya hybrids did not found on four Punjab (India) papaya

varieties. *Manu et al* [20] said that four papaya variety of Punjab, India varied in term of fruit weight and TSS, but did not vary on flesh hardness. Therefore, selection can be made by choosing hybrids with hard flesh texture and peel. This finding was also crucial as the basis for product improvement and sustainability.

The reason why consumers tend to choose fruit with long stalk is understandable since it can reduce damage risk when fruits on the distribution process, so fruit stalk also correlated with fruit shelf-life [21]. On the contrary, papaya with sort fruit stalk can induce friction among the fruits and creates damage even when the fruits still bear on trees. Most of the tested hybrids are grouped into short stalk (3 – 3.9cm) (Table 3). As much as 20 tested hybrids had the longest fruit stalk (4 – 7.5cm) (Table 3).

Another character that defines the high yielding of papaya is fruit size. The consumer also tended to choose the papaya with a big fruit size for their daily consumption (Fig. 1). Several types of research implied that papaya fruit sizes are classified into small, medium, and big size [8, 22-24). Fruit size is mainly affected by the genetic factor, although environmental also significantly stimulates the phenotype [8].

Fig. 1. Types of papaya that consumers like

In this present research, the fruit size was categorized into three groups, includes 30 smalls (500 – 700g), 34 mediums (800 – 1490g), and 6 bigs (1500 – 2000g) (Table 3).

Table 3. Frequencies distribution of papaya hybrids based on several fruit characteristics

Value Range	Frequencies	Genotypes
		Stalk length (cm)
2.0 - 2.9	13	1, 5, 15, 22, 30, 32, 34, 51, 58, 63, 83, 90, 97
3.0 - 3.9	37	35, 82, 59, 16, 21, 87, 6, 40, 41, 3, 12, 19, 36, 54, 4, 9, 56, 98, 29, 67, 38, 8, 70, 14, 50, 37, 10, 62, 76, 33, 53, 61, 52, 78, 20, 11 and 13
4.0 - 7.5	20	2, 7, 17, 24, 25, 26, 27, 31, 42, 44, 48, 49, 55, 68, 79, 93, 96, 86, 46 and 17
		Weight (g)
500 - 790	30	1, 3, 5, 6, 9, 13, 15, 16, 17, 19, 21, 22, 26, 29, 30, 31, 32, 34, 36, 37, 40, 49, 54, 55, 56, 63, 67, 76, 90, 97
800 - 1490	34	2, 4, 7, 10, 11, 12, 14, 20, 27, 35, 38, 41, 42, 44, 48, 50, 51, 52, 53, 58, 59, 61, 62, 70, 78, 82, 83, 87, 98, 8, 24, 25, 33 and 46
1500 - 2000	6	18, 68, 79, 86, 93 and 96
		Length (cm)
15 - 20	33	1, 3, 5, 7, 9, 12, 13, 15, 17, 19, 21, 22, 26, 29, 30, 31, 32, 34, 36, 37, 42, 48, 49, 54, 55, 5, 63, 67, 70, 76, 83, 90 and 97
21 - 25	28	2, 4, 6, 8, 10, 11, 14, 16, 20, 27, 33, 35, 38, 40, 41, 44, 50, 51, 52, 53, 58, 59, 61, 62, 78, 82, 87, and 98
26 - 35	9	18, 46, 68, 79, 93, 96, 24, 25 and 86
		Fruit circumference (cm)
20 - 25	13	9, 21, 35, 51, 53, 58, 61, 63, 67, 76, 87, 90 and 97
26 - 29	40	1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 15, 16, 17, 19, 22, 24, 25, 26, 29, 30, 31, 32, 34, 36, 37, 40, 42, 46, 48, 49, 50, 54, 55, 56, 78, 79, 82, 83, 98
30 - 40	17	8, 14, 20, 27, 33, 38, 41, 44, 52, 59, 62, 70, 86, 18, 68, 93 and 96
		Fruit diameter (cm)
5 - 8	25	6, 9, 13, 15, 16, 17, 19, 21, 29, 31, 34, 37, 40, 49, 55, 67, 68,76, 82, 86, 90, 96, 97 and 98
9 - 11	43	2, 3, 4, 5, 8, 10, 11, 12, 14, 20, 22, 24, 25, 26, 27, 30, 32, 33, 35, 36, 38, 41, 42, 44, 46, 48, 50, 51, 52, 53, 54, 56, 58, 59, 61, 62, 63, 70, 78, 79, 83, 87 and 93
12 - 15	2	18,75
		Peel hardness (kg/cm^2)
0.70 - 0.79	28	1, 6, 7, 8, 9, 10, 11, 12, 16, 18, 22, 26, 30, 32, 35, 36, 38, 40, 44, 46, 51, 52, 58, 59, 62, 67, 86 and 90
0.80 - 0.90	42	2, 3, 4, 5, 13, 14, 15, 17, 19, 20, 21, 24, 25, 27, 29, 31, 33, 34, 37, 41, 42, 48, 49, 50, 53, 54, 55, 56, 61, 63, 68, 70, 76, 78, 79, 82, 83, 87, 93, 96, 97 and 98
		Flesh thickness (cm)
1.5 - 2.0	2	35, 46
2.1 - 2.6	39	1, 3, 5, 7, 9, 10, 11, 12, 13, 15, 16, 19, 20, 21, 26, 27, 29, 30, 31, 32, 33, 34, 37, 38, 40, 51, 53, 56, 58, 59, 61, 63, 67, 76, 86, 87, 90, 97 and 98
2.7 - 3.5	29	2, 4, 6, 8, 14, 17, 22, 36, 41, 42, 44, 48, 49, 50, 52, 54, 55, 68, 70, 78, 82, 83, 96, 18, 24, 25, 62, 79 and 93
		Flesh hardness (kg/cm^2)
0.15 - 0.20	16	1, 8, 12, 16, 17, 18, 20, 22, 26, 30, 32, 35, 46, 50, 54, 62
0.21 - 0.32	43	11, 44, 52, 55, 59, 5, 6, 24, 25, 37, 49, 86, 90, 7, 9, 14, 34, 41, 3, 10, 36, 40, 78, 13, 51, 58, 2, 4, 15, 19, 31, 33, 42, 48, 53,56, 61, 63, 68, 70, 82, 83, 96
0.33 - 0.4	11	21, 27, 29, 38, 67, 76, 79, 87, 93, 97, 98
		TSS (^{o}brix)
9.0-10.5	8	18, 21, 24, 25, 33, 53, 61, 78
10.6-11.5	14	11, 13, 27, 51, 58, 62, 68, 76, 79, 83, 86, 87, 96, 98
11.6-12.5	34	1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 15,16, 17, 19, 20, 22, 26, 29, 30, 31, 32, 34, 35,36, 37, 38, 40, 41, 42, 44, 46, 48, 49, 50, 52, 54, 55, 56, 59, 63, 67, 70, 82, 90, 93, 97

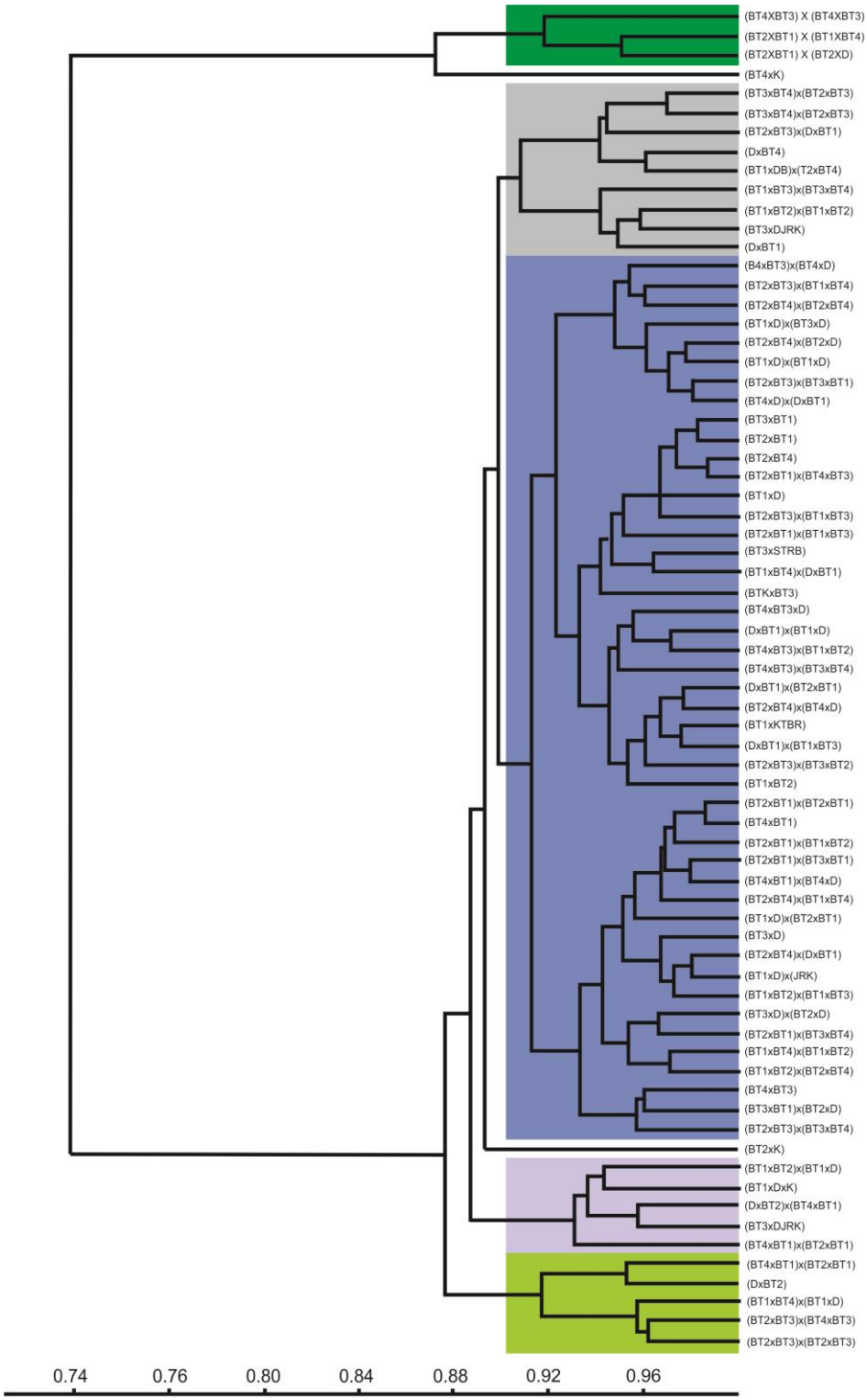

Fig. 2. Clustering analysis of papaya hibrid using jaccard coeficien index

The fruit stalk length of 70 tested papaya hybrids was 15-35cm, whereas the biggest fruit lengths were 26-35cm with a diameter 5-15cm. Most of the hybrids were grouped into small and medium fruits, where are only two other hybrids with big fruit diameter (12-15cm).

Peel and flesh hardness of papaya fruit are correlated with fruit shelf life. The harder peel and flesh, the longer the fruit shelf, so that consumers prefer to choose the hard texture of papaya fruits. In this research, the peel hardness values of 28 hybrids were ranged from 0.7 - 0.9kg/cm^2, and classified into the soft category, whereas 42 hybrids had a level of 0.8 – 0.9kg/cm^2 peel hardness and classified into the hard category. Most of the tested hybrids were a sweet taste for TSS characters and excluded eight hybrids with low TSS values (9 - 10.5^{o}brixs).

The clustering analysis was also performed to explore the genetic variation using the morphological character. A total of 70 hybrids of papaya was clustered into five groups with a coefficient similarity between 0.87 – 0.96 (Fig. 2).

Although papayas are still a single species, they vary morphologically. The variation between papayas showed the effectiveness of hybridization performed by the Indonesian Tropical Fruit Research Institute's (ITFRI).

Most papaya was grouped in the third cluster (purple) with morphological character medium stalk length (3.0 – 3.9cm), medium weight (800 – 1490g) and medium circumference (26 – 29cm). In addition, first cluster (green colour) was characterized by high flesh hardness (0.33 – 0.4kg/cm^2), peel hardness (0.80 – 0.90kg/cm^2) and length (26 – 35). The next production of high-yielding papaya may perform in the first cluster characterized with high flesh hardness and the fourth cluster characterized with length stalk (4.0 – 7.5cm). The hybridization between the two characters is expected to get papaya with characters that are liked by consumers, i.e., length stalk and flesh hard.

Conclusions

The 70 papaya hybrids of ITFRI collection had a wide genotypic and phenotypic variance. The variability can be observed on fruit characteristics, namely weight, flesh thickness, flesh and peel hardness, and TSS. The selected papaya hybrid has a fruit weight of 500-1500 g/fruit, flesh thickness (2.7-3.5 cm), flesh hardness 0.21-0.24kg/cm^2, sweet taste (TSS 11.6-12.5° brix). Further selection should be conducted on these hybrids to produce superior hybrids, which consumers prefer.

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Received: February 20, 2021
Accepted: August 13, 2021