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GENETIC DIVERSITY AND FRUIT QUALITY OF SEVERAL POMELO "JERUK BALI" (*Citrus grandis* L. Osbeck) CULTIVARS IN BALI

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ABSTRACT

This study aimed to determine the genetic diversity and fruits quality of the "Jeruk Bali" cultivars grown in Bali. This research was conducted in all regencies and city in Bali, during 2016. Furthermore, several cultivars of "Jeruk Bali" were genetically analyzed based on RAPD markers using 10 primers. Analysis of the quality of fruit is based on physical properties and chemical content. Eighteen cultivars of "Jeruk Bali" obtained have a fruit morphological character with round, short round, and piriform fruit shapes, which are red, pink, cream and white flesh color. RAPD analysis results at 53% similarity level are grouped into 5 groups. The first group was only one cultivar, the second group consisted of 13 cultivars, the third and fourth groups were only one cultivar, while the fifth group consisted of two cultivars. The analysis of the diversity between cultivars based on the combination of physical and chemical properties of the fruit with hierarchy method on similarity level about 85% in a group is obtained by 4 (four) groups. Groupings by combination of physical and chemical properties of the fruit are not synchronized in their entirety with dendograms based on their genetic diversity. This illustrates the physico-chemical properties of "Jeruk Bali" fruit in general is not fully genetical expressed, but also influenced by conditions of environmental growth.

Keywords: "Jeruk Bali", genetic diversity, fruit quality

INTRODUCTION

"Jeruk Bali" or pummelo (shaddock) (*Citrus grandis* L. Osbeck) is native to Indonesia, widely cultivated in India, China, Thailand and other East Asian regions (Davies and Albrigo, 1994), needs to be handled seriously in cultivation and utilization in Indonesia (Setiawan and Sunarjono, 2003). "Jeruk Bali" has another name *C. maxima* (Burm.) Merr., *C. decumana* L., *C. aurantium* var. *Grandis* L.

and *C. aurantium* var. *Decumana* L. (Niyomdham, 1992; Setiawan, 1999; Hamilton, *et al*. 2008). "Jeruk Bali" is a good source of vitamin C and antioxidants (Rahman *et al*., 2003; Pichaiyongvongdee and Haruenkit, 2009).

Identification of genetic diversity of crop cultivars needs to be done for use in their development and cultivation. In addition, the evaluation of fruit quality also needs to be done to identify the nutritional compound of fruit as well as to know the response of plant varieties to the changing of the growing environment (Somantri *et al*., 2008). Biochemical analysis with isoenzymes in citrus plants, among others, is also used to distinguish the acidity level of citrus cultivars (Rahman *et al*., 2001). Excellence pomelo cultivars are generally determined based on the delicious fruit flavors (sweet and sour taste balance) as well as the water content of the juice (juicy) (Rahman *et al*., 2003; Paudyal and Haq 2007).

"Jeruk Bali" fruit are generally considered to be ripe when the fruit skin color, the content of the juice and the ratio of total soluble solids with acidity and other internal components have reached the level of acceptance of visual minimum or palatability of consumers (Paudyal and Haq, 2007; Rukmana, 2009; Lado, *et al*., 2014; Riaz, M. *et al*, 2015). "Jeruk Bali" fruits are a source of calories, minerals and excellent for relieving thirst due to high water content (up to 86 g per 100 g of fruit flesh). The leaves can be used for epilepsy, chorea, and cough seizures (Niyomdham, 1992; Direktorat Tanaman Buah, 2012).

RAPD molecular markers are PCR applications used to detect the presence of a DNA polymorphism in a population or interpopulation. Polymorphisms produced by PCR RAPD techniques are due to changes in nucleotide bases, deletions, and

insertions (William *et al*., 1990). The advantage of the RAPD PCR technique is that it takes only a small quantity of DNA samples, is cost effective, easy to learn, and easy to obtain primers (Azrai, 2005). The use of molecular markers with RAPD PCR techniques has been widely used for early plant breeding activities, such as analyzing the genetic diversity of wani Bali (Rai, I N., *et al*., 2008), identifying genetic diversity among barley cultivars (Fernández *et al*., 2002), and detecting polymorphisms in distance plants due to gamma-ray radiation (Dhakshanamoorthy, D. *et al*., 2010). Evaluation of fruit quality including total soluble solids content (TSS), total titrated acids (TA), vitamin C, and flavonoid compounds (Pichaiyongvongdee and Haurenkit, 2009), are required to supplement the description of the "Jeruk Bali" that were grown in Bali.

MATERIALS AND METHODS

This research was conducted in all regencies/cities in Bali province. Molecular analysis was conducted in the laboratory of Genetics and Plant Breeding of Faculty of Agriculture UGM, and fruit quality observation was done at Agricultural Technology Technology Laboratory of Faculty of Agricultural Technology, Udayana University, Denpasar.

The method of genetic diversity testing using Random Amplified Polymorphic DNA (RAPD) method, while observation of fruit quality evaluation is done by sensory analysis (organoleptic) and analysis of chemical compound (nutrient) content according to the method of observation of each specification of the compound. DNA extraction methods (Doyle and Doyle, 1990) use the materials used are fresh "Jeruk Bali" leaves. The purified DNA is then quantified by using

Gene Quant to determine the concentration of DNA obtained.

DNA amplification is done by PCR (Polymerase Chain Reaction) reaction which aims to multiply DNA sequences based on the primary used. The PCR reaction was performed at a total volume of 10 µl for each PCR tube. Each PCR reaction consisted of 5 µl Go Taq® Green (Promega) PCR mixtures, 0.25 µl 100 µM primers (Sigma-Proligo), 2.5 µl sample DNA (template) and 2.25 sterile aquabidest. DNA amplification is done with PCR System BOECO tool. The DNA of PCR was then electrophoresed using 1.0% (w/v) agarose which has been added florosafe DNA stain as dye, in TBE buffer with 100 volt for 45 min. The results were then visualized with UV light. The DNA data obtained was analyzed using binary data by scoring, ie score 1 for the emerging band and score 0 for the band that did not appear (Williams, JGK., *et al*., 1990). The data obtained by scoring the electrophoresis results for each individual on a certain size, and the binary data is then analyzed using the help of software Genalex 6.1 and Ntsys pc 2.2.

Observation of fruit quality evaluation used three pieces of ripe and healthy fruit in accordance with the criteria and characteristics, namely: smooth shiny smooth fruit skin, the colors are brighter and feels heavy, with PTT content around 7 - 10 ^{o}Brix, generally between 24 - 30 weeks after flowering (Setiawan, 1999; Mahardika and Susanto, 2003; Lado, J. *et al*., 2014). Sensory analysis in this study was conducted to identify consumer preferences and match the acceptability level of citrus fruits from various cultivars. The variables observed in fruit quality evaluation are: (a) percentage of fruit parts, including edible portion; (b) the vitamin C content of the fruit was measured using iodometric titration method (Cahyadi, 2006); (c) fruit

juice pH measured with Jenway 3010 digital pH meter; (d) total titrated acid content (TA) was measured by titration using 0.1 N NaOH until the pH of fruit juice reached 7 (AOAC, 2005); (e) total soluble solids (TSS) content, calculated as the degree of Brix (^{o}Brix) measured using a refractometer; (f) flavonoid levels were performed by spectrophotometry using aluminum chloride reagents (Chang and Wen, 2002); (g) sensory analysis performed ie. hedonic test and scalar quality test. The test was conducted by 10 semi-trained panelists, consisting of students and laboratory technicians of the Faculty of Agricultural Technology Udayana University (Wagiyono, 2003).

RESULTS AND DISCUSSIONS

The genetic diversity of "Jeruk Bali" cultivars in Bali is based on RAPD marker test. Based on the varied distribution and morphological features, and the origin of the "Jeruk Bali" plant, 18 cultivars were selected to test their genetic diversity with RAPD markers. The list of samples analyzed using codes in accordance with the district / municipal sites where the plants were grown (Table 1).

Table 1. List of samples "Jeruk Bali" which analyzed

No.	Local name	Sample Code
1.	Juuk Bone Badung	BAD-01
2.	Juuk Saba Badung	BAD-02
3.	Jerungga Bangli	BAN-03
4.	Juuk Saba Badung	BAD-03
5.	Juuk Bali Jembrana	JEM-02
6.	Muntis Buleleng	BUL-01
7.	Juuk Bali Jembrana	JEM-01
8.	Juuk Bali Jembrana	JEM-03
9.	Muntis Buleleng	BUL-02
10.	Muntis Buleleng	BUL-03
11.	Jeruti Karangasem	KAR-01
12.	Juuk Saba Denpasar	DEN-02
13.	Juuk Saba Denpasar	DEN-03

14.	Juuk Saba Denpasar	GIA-02
15.	Jeruti Karangasem	KAR-02
16.	Juuk Bone Gianyar	GIA-01
17.	Jerungga Klungkung	KLU-01
18.	Juuk Bali Tabanan	TAB-01

Stages of RAPD analysis is started from DNA extraction, DNA quantification, DNA dilution, DNA amplification and electrophoresis. DNA amplification is done by PCR reaction. Ten selected primers were applied the DNA obtained from the extraction of the "Jeruk Bali" samples of Bali, in the order of nucleotide bases as shown in Table 2. DNA concentrations in this study were obtained from 400 ng / µl in the 'Juuk Saba' Denpasar (DEN-03) and 'Jerungga' Klungkung (KLU-01) cultivars, up to 3996 ng / µl in the 'Juuk Saba' Badung (BAD- 02), while the purity of DNA obtained from 1.33 to 2.00. DNA purity is determined by the ratio of A260 / A280 (Sambrook *et al*., 1989; Chen, H. *et al*., 2010). The number of amplified DNA fragments obtained in this study ranged from 5 to 15 bands depending on the type of primer and the genotype being analyzed. The smallest amount of DNA fragment (5) was obtained on the primary use of OPA-8, while the highest number of DNA fragments (15) was obtained on primary use of OPD-8 (Table 2). The ribbon pattern on agarose gel which is the result of genomic DNA amplification can be grouped into two categories: polymorphic band and monomorphic band. Polymorphic bands are a picture of DNA bands that appear at certain sizes, but in other samples no DNA bands are found in these sizes. The monomorphic band is a band in some samples that has no variation (Williams *et al*., 1990).

Table 2. Primers and their sequences, and the number of DNA bands umplified from 18 cultivars of jeruk bali pomelo in Bali

Primer	Sequences	Number of bands		Total
	5' – 3'	Monomorphic	Polymorphic	
OPA-1	CAGGCCCTTC	0	13	13
OPA-2	TGCCGAGCTG	0	9	9
OPA-5	AGGGGTCTTG	0	7	7
OPA-7	GAAACGGGTG	0	9	9
OPA-8	GTGACGTAGG	0	5	5
OPD-2	GGACCCAACC	2	10	12
OPD-3	GTCGCCGTCA	0	10	10
OPD-5	TGAGCGGACA	0	9	9
OPD-7	TTGGCACGGG	0	12	12
OPD-8	GTGTGCCCCA	1	14	15
Total		3	98	101
Persentase (%)		2,97	97,03	

The result of visualization of DNA amplification with 10 genetic diversity genotypes 18 (eighteen) Bali citrus cultivars based on RAPD marker test is presented in Fig. 1. Dendogram profile of genetic diversity is presented in Fig. 2. The result of genetic diversity analysis of 18 Balinese citrus cultivars based on RAPD marker test with 10 primers at similarity level about 53 percent yielded 5 (five) groups, that is first group (I) consisted of one cultivar. Second group (II) consists of 13 cultivars, third group (III) and fourth group (IV) each only one cultivar and the fifth group (V) consists of two cultivars. It appears that at a similarity level of 53 percent, some cultivars are specifically separated ie Gianyar 'Juil Bone' cultivars (GIA-01) as well as 'Jerungga' Klungkung which is a group with 'Juuk Bali' Tabanan (TAB-01). The separation of Gianyar 'Juice Bone' cultivars (GIA-01) corresponds to some of the specific properties possessed by these cultivars that are different from those of other cultivars.

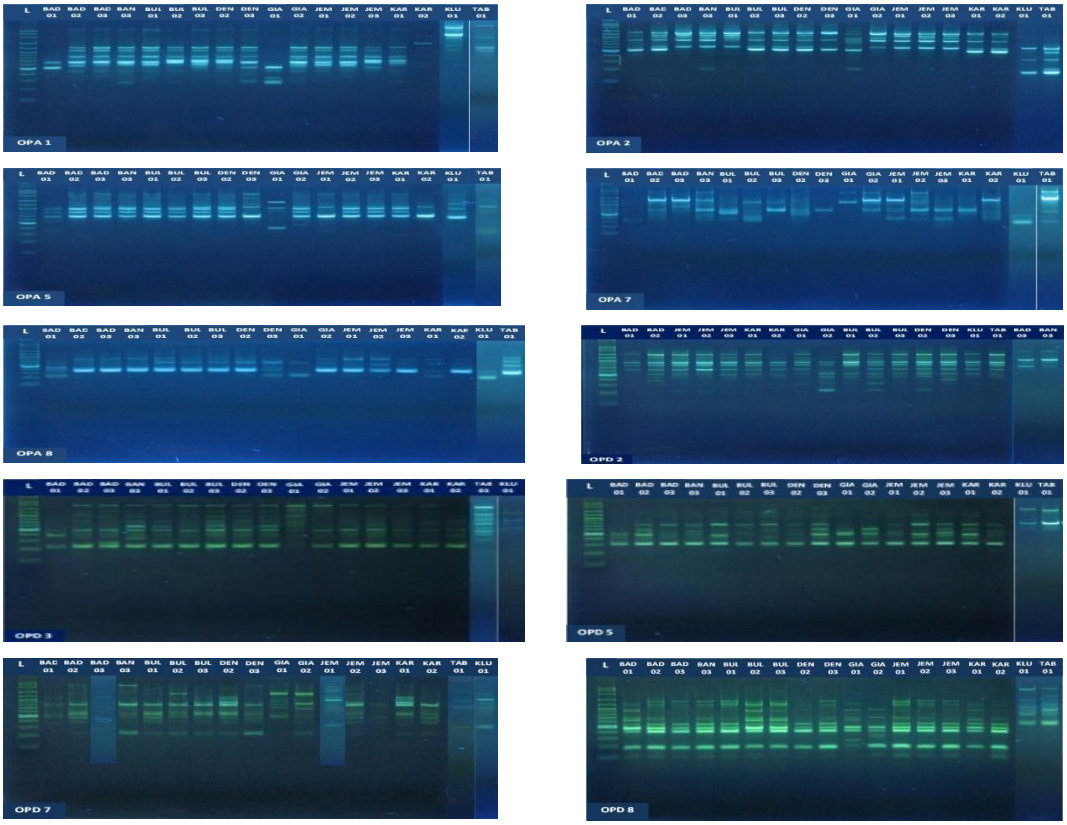

Fig. 1. DNA amplification with 10 primers of 18 (eighteen) pomelo cultivars based on RAPD method

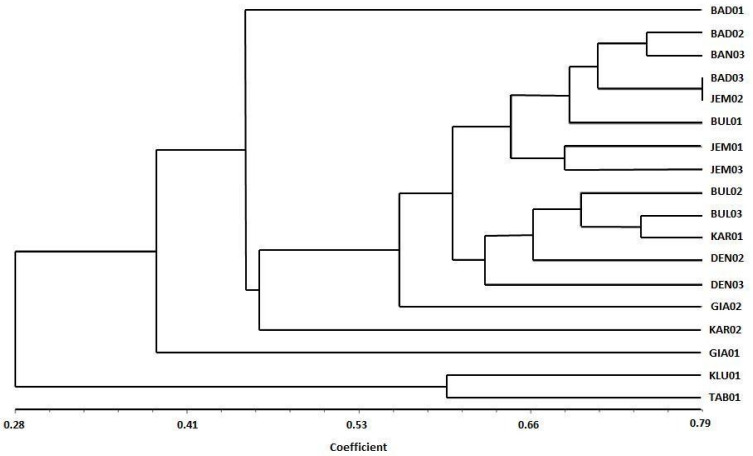

Fig. 2. Dendogram profile of genetic diversity of 18 (eighteen) "Jeruk Bali" cultivars based on RAPD marker test

DNA profile of 18 cultivars of pomelo in Bali which was amplified by using primer OPA-1, OPA-5, OPA-7, 3- OPD, OPD-7 and OPD-8 showed a specific band that characterizes some distinguishing trait for kultivar- Certain cultivars with other cultivars. For example, the primary use of OPA-1 there are specific band OPA-1_ 350 shows as a differentiator pomelo 'Juuk Bone' Gianyar (GIA-01) with other pomelo cultivars (Fig. 3).

Physical characteristics include fruit weight, skin color and fruit flesh (Fig. 4), portions of edible parts per fruit and several other physical characteristics. While the chemical character of the fruit is intended to contain some chemical compounds or nutrients present in the flesh of grapefruit, such as vitamin C content, total acids, total dissolved solids, and flavonoids in fruit juice. The color of "Jeruk Bali" bark on observed cultivars ranges from bright green, yellowish green to yellow. While the color of juice sac varied, ranging from white-pink, reddish to red white (Table 3; Fig. 4.). Red-colored juice generally contains higher antioxidants than brightly colored juices (Pichaiyongvongdee and Haurenkit, 2009). So the possibility of 'Jeruti' Karangasem and 'Juuk Saba' Denpasar has higher antioxidant content than other cultivars. The weight of the average fruit portions are presented in Table 4.

The average pH of the fruit juice fruits ranged from 3.29 on 'Jembran Bali' Jembrana (JEM-03) to 4.89 in 'Bone Badung' (BAD-01) as indicated in Table 5. That were not much different from the pH of grapefruit juice in general and also reported in India that it ranges from 2.94 to 4.44 (Kumar, D. *et al*., 2015).

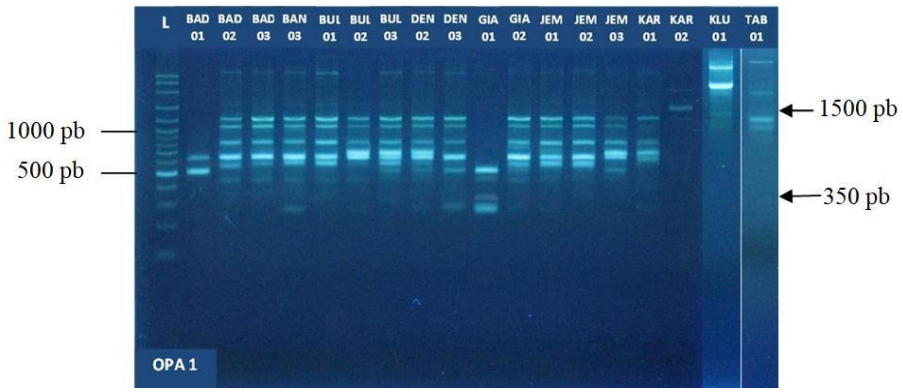

Fig. 3. Profile DNA 18 "Jeruk Bali" cultivars amplification product by using primer OPA-1. The specific band OPA-1_ 350 is the distinction of 'Juuk Bone Gianyar' (GIA-01) (column 10) with other cultivars. And the specific band OPA-1_ 1500 is the differentiator of the 'Jeruti' Karangasem (KAR-02) cultivar (column 16) with other cultivars. L = 1 Kb DNA Ladder

The total titrated acids content (ATT) of Bali oranges in Bali ranges from 0.44% - 0.96 % (Table 5.5). The total titrated acids content is almost the same as that reported for pummelo in India which also ranges from 0.50 - 4.00 percent (Singh, S.K. *et al*., 2015). The total dissolved solids content (PTT) of "Jeruk Bali" juice in Bali is obtained from 7.00 ^{o}Brix in 'Jerungga' Bangli (BAN-03) to the highest of 11.77 ^{o}Brix at 'Juja Saba' Badung (BAD-03). This total dissolved solids content is almost the same as that reported for grapefruit (pummelo) in India which also ranges from 7.40-11.50 oBrix. (Singh, S.K., *et al*., 2015). Sugar in oranges consists mostly of glucose, fructose and sucrose, and has increased during the fruit ripening period (Mahardika and Susanto, 2003).

Total flavonoid levels obtained in grapefruit juice in Bali ranged from 0.61 to 4.99 mg / 100 g (Table 5). Flavonoids are known to be antioxidant compounds that have a very important role in health, for example suspected to have cardioprotective effects and antiproliferative activity (Redha,

A., 2013). The highest total relative flavonoid levels in this study were obtained at Bangli (Jerk) cultivars (BAN-03) which reached 4.99 mg / 100g, much higher than those of other cultivars. This makes the Bang Bang fruit taste "Jerungga" has a harder taste ('pengah') that is harder than the others, because of the nature of flavonoid compounds that can cause bitter taste or bitter on the fruit. The research report Febrianti and Sari (2016) suggested that total flavonoid levels in some tropical fruits such as avocado, guava, citrus fruit, strawberry fruit, papaya fruit, tamarind fruit, apples and mangoes. While in research conducted by Selawa *et al*. (2013), about the flavonoid content of dry and wet leaf binahong leaves, concluded that dry binahong leaf extract is higher in flavonoid levels.

"Jeruk Bali" cultivars observed in this study in general were that have been categorized excellent based on consumer opinions, and the communities around these crops are cultivated. The distinctive characteristic of this sensory judgment besides taste, sweet, and sour also has a distinct bitter bitter taste in Bali called the "pengah" which is a combination of bitter taste and an aromatic gas flavor in the nose. The preferred sensory character of the consumer is owned by almost all observed cultivars, which get a value greater than 4 (four) (Table 6). Three cultivars that have the unpleasant taste were 'Jeruti' Karangasem (KAR-02), 'Juuk Bali' Jembrana (JEM-03), and 'Jerungga' Bangli (BAN-03) have similarities from the height of the growing sites, about 500 meters above sea level.

The analysis of the diversity between cultivars based on a combination of physical and chemical properties of the fruit by hierarchial cluster analysis (Johnson & Wichern., 2007), at 85%

similarity level in a cluster Also formed 4 (four) clusters (Fig. 5.11). As with grouping according to physico-nature and chemical properties, the combination is also not fully synchronized with dendograms based on its genetic characteristics.

Fig. 4. Shape and colour of fruit "Jeruk Bali" cultivars in Bali

This illustrates the physico-chemical properties of grapefruit in Bali in general does not show any major differences, except when viewed based on the content of certain compounds, the color of the flesh, the total flavonoid content, the acid content, and the vitamin C, or the combination of these components and also the growing environmental influences.

The combination of certain properties between physics with clams and morphology is commonly used for the cultivar of the community. For example by mentioning the description of each cultivar found. However, for the purposes of certification of cultivar (fruit) products in order to meet the needs of modern markets, the mention of a cultivar description should also be based on the grouping of its genetic diversity in addition to the mention of morphological identification, the chemical physics of the fruit and the location of its growing cultivation.

Table 5. Content of chemical compound of 18 "Jeruk Bali" fruit cultivars in Bali

Cultivar Code	Vit. C content (mg/100g)	TSS content (^{o}Brix)	Total titrated acids (%)	pH	Total Flav. (mg/100 g)
BAD-01	68,95 ± 1,01	10,00 ± 0,00	0,44 ± 0,02	4,89 ± 0,01	0,61 ± 0,01
BAD-02	61,23 ± 1,75	8,00 ± 0,00	0,58 ± 0,17	4,23 ± 0,03	0,65 ± 0,01
BAN-03	36,70 ± 2,31	7,00 ± 0,00	0,59 ± 0,23	4,45 ± 0,04	4,99 ± 0,02
BAD-03	40,76 ± 0,50	11,77 ± 0,06	0,68 ± 0,04	3,80 ± 0,00	3,52 ± 0,01
JEM-02	76,32 ± 1,33	10,00 ± 0,00	0,57 ± 0,13	3,62 ± 0,02	0,61 ± 0,01
BUL-01	37,12 ± 0,51	9,10 ± 0,10	0,56 ± 0,01	4,33 ± 0,06	2,31 ± 0,02
JEM-01	24,71 ± 0,50	8,00 ± 0,00	0,56 ± 0,12	3,54 ± 0,05	0,61 ± 0,01
JEM-03	44,87 ± 4,08	8,91 ± 0,01	0,78 ± 0,01	3,29 ± 0,02	2,14 ± 0,01
BUL-02	45,36 ± 0,50	11,00 ± 0,00	0,79 ± 0,01	3,87 ± 0,06	3,78 ± 0,02
BUL-03	50,88 ± 2,52	10,00 ± 0,00	0,76 ± 0,01	3,99 ± 0,01	2,14 ± 0,00
KAR-01	36,34 ± 2,76	8,50 ± 0,00	0,36 ± 0,02	4,62 ± 0,01	1,31 ± 0,14
DEN-02	50,88 ± 2,52	10,00 ± 0,00	0,61 ± 0,01	4,39 ± 0,02	1,94 ± 0,02
DEN-03	34,18 ± 0,00	10,00 ± 0,00	0,65 ± 0,10	4,01 ± 0,01	2,26 ± 0,02
GIA-02	34,99 ± 0,24	11,00 ± 0,00	0,96 ± 0,01	3,70 ± 0,00	3,87 ± 0,01
KAR-02	41,96 ± 0,46	10,00 ± 0,00	0,88 ± 0,01	3,98 ± 0,01	3,53 ± 0,01
GIA-01	57,23 ± 1,31	9,07 ± 0,06	0,54 ± 0,13	3,80 ± 0,03	0,61 ± 0,01
KLU-01	44,23 ± 1,01	9,00 ± 0,00	0,71 ± 0,07	4,44 ± 0,01	3,32 ± 2,44
TAB-01	52,34 ± 1,00	9,00 ± 0,00	0,46 ± 0,05	4,32 ± 0,01	1,99 ± 0,03

Table 6. Value hedonic test panelists on juice fruit 18 cultivars of fruit pomelo in Bali

Cultivar Code	Value hedonic test *)				
	aroma	taste	colour	juicines	Average
BUL-03	4,10	4,85	4,90	4,55	4,60
KAR-01	4,20	4,90	4,70	4,40	4,55
DEN-03	4,30	4,80	4,70	4,30	4,53
GIA-01	4,35	4,70	4,35	4,35	4,44
TAB-01	4,45	4,30	4,55	4,00	4,33
JEM-01	4,19	4,38	4,43	4,14	4,29
JEM-02	4,05	4,05	4,25	4,10	4,11
DEN-02	4,10	4,20	3,70	3,90	3,98
BAD-02	3,75	4,10	4,30	3,75	3,98
BUL-01	3,55	4,15	4,15	4,00	3,96
BAD-01	4,10	4,40	3,15	4,15	3,95
KLU-01	3,15	4,30	4,05	4,15	3,91
BUL-02	3,90	4,25	3,60	3,70	3,86
BAD-03	3,45	3,40	3,85	4,15	3,71
GIA-02	3,55	4,20	3,75	3,00	3,63
BAN-03	4,25	2,50	3,00	3,35	3,28
KAR-02	2,90	3,30	3,60	2,00	2,95
JEM-03	3,45	3,15	3,10	2,00	2,93

Description: *) 1 = dislikes; 2 = rather dislike; 3 = neutral; 4 = rather like; 5 = likes

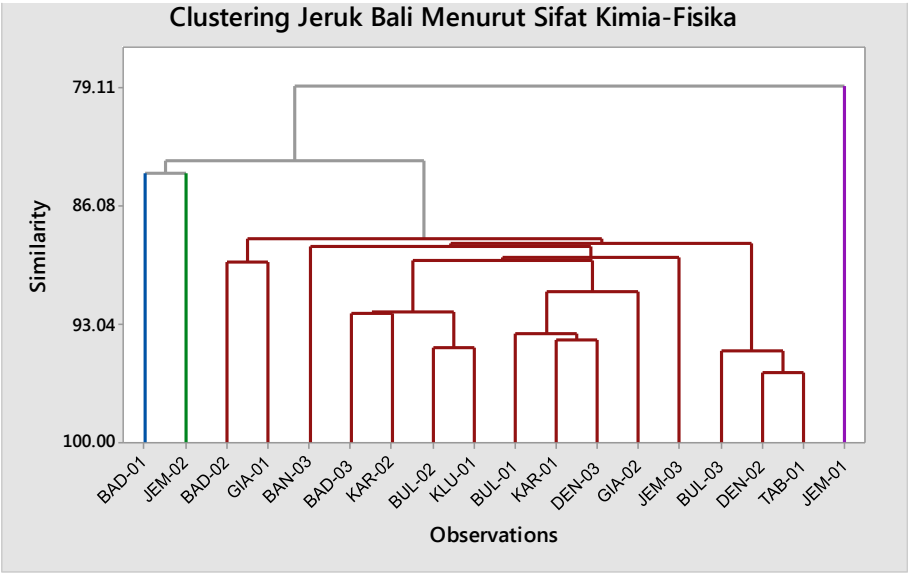

Fig. 6. Dendogram profile of diversity based on physico-chemical properties of fruit of 18 (eighteen) "Jeruk Bali" cultivars in Bali

CONCLUSIONS

RAPD analysis results at 53% similarity level are grouped into 5 groups. The first group was only one cultivar, the second group consisted of 13 cultivars, the third and fourth groups were only one cultivar, while the fifth group consisted of two cultivars. The analysis of the diversity between cultivars based on the combination of physical and chemical properties of the fruit with hierarchy method on similarity level about 85% in a group is obtained by 4 (four) groups. Groupings by combination of physical and chemical properties of the fruit are not synchronized in their entirety with dendograms based on their genetic diversity. This illustrates the physico-chemical properties of "Jeruk Bali"fruit in general is not fully genetical expressed, but also influenced by conditions of environmental growth. Most cultivars were observed highly preferred by consumers, but three less preferred cultivars namely "Jerungga" Bangli (BAN-03), "Juuk Bali" Jembrana (JEM-03) and "Jeruti" Karangasem (KAR-02).

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