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Quality Improvement of Pummelo (*Citrus maxima* (Burm.) Merr.) Using Leaf-to-Fruit Ratio Arrangement and Fruit Bagging

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ABSTRACT
Pummelo fruit has a large size, a lot of assimilate will be required for fruit growth and development. The problem of pummelo cultivation is not only assimilate requirement for fruit growth but also the fruit quality, i.e. external and internal quality. The aims of this research were to evaluate the effect of leaf-to-fruit ratio, fruit bagging and their relationship on fruit development and quality. This research has been conducted at University Farm of Bogor Agricultural University from November 2013 until September 2014. The experiment was arranged in completely randomized factorial design with two factors. The first factor was leaf-to-fruit ratio with three levels (50:1, 75:1 and 100:1). The second factor was fruit bagging with four plastic colors (transparent, blue, red and yellow) and control (without bagging). The result showed that fruit of the control treatment dropped because of pest attack. Leaf-to-fruit ratio and fruit bagging affected fruit growth and quality. The highest leaf-to-fruit ratio (100:1) significantly increased fruit weight as compared with lower leaf-to-fruit ratio (75:1 and 50:1), i.e: 746.3, 641.4 and 603.3 g, respectively. There was no significant effect of leaf-to-fruit ratio on edible portion, juice content and vitamin C in all treatments, whereas bagging bag color has significantly affected on fruit quality. Red plastic resulted the largest fruit but poorest quality in total soluble solids and maturity index. Transparent and yellow plastic could be recommended for pummelo bagging, which showed better taste indicated by high total soluble solids, maturity index and vitamin C.

Key words: Pummelo, fruit growth, quality, bagging, leaf-to-fruit ratio

INTRODUCTION
Citrus is one of the main fruits in Indonesia. Citrus production in the country during 2008-2012 was able to fulfilled 86-94% of the citrus domestic consumption (SSR = Self Sufficiency Ratio), therefore, Indonesia needs to import for remain necessity. The import of citrus during 2008-2012 has increased, which was 143,661, 216,917, 204,148, 232,049, 258,446 t respectively. In 2012, the import value of citrus resided in the largest proportion of fruit imports in Indonesia, reaching US $ 256,098,000 (Secretariat General of the Ministry of Agriculture, 2013).

Pummelo (*Citrus maxima* (Burm.) Merr.) is considered to be one of three true basic *Citrus* species together with *Citrus medica* and *Citrus reticulata* based on karyotype analysis (Hynniewta *et al*., 2011). Pummelo has been widely cultivated in Indonesia and defined as one of the leading commodity types of fruit crops according Directorate of Horticulture Agriculture Decree

No. 511/Kpts/PD.310/9/2006. The most widely cultivated pummelo in Indonesia is cv. Nambangan because it has a relatively long shelf life and belong to the potential seedless pummelo (Pangestuti *et al*., 2004; Rahayu, 2012).

Pummelo fruit has a large size, a lot of assimilate will be required for fruit growth and development. There is still lack of information of Indonesian's pummelo related with source sink relationship therefore, it is necessary to investigate. Famiani *et al*. (2000) explained that the source-sink could be manipulated by arranging different level of leaf-to-fruit ratio in branches. Leaf-to-fruit ratio could increase fruit quality of 'Valencia' orange (Yuan *et al*., 2005) and pummelo cv. Hom Hat Yai (Rattanapong, 2006).

The problem of pummelo fruit is not only assimilate requirement for fruit growth but also the fruit external and internal quality. The most important of external quality improvement is fruit appearance. One of the technique to improve the fruit appearance is bagging. Bagging could reduced pests attack and improved fruit quality on guava (Harach and Wanichkul, 2006). Grapefruit bagged by black paper could change the peel color and had influence on TSS/TTA ratio (Hwang *et al*., 2004). The aims of this research were to evaluate effect of leaf-to-fruit ratio, fruit bagging and their relationship on fruit development and quality of pummelo fruit.

MATERIALS AND METHODS

The 5 years old of pummelo cv. Nambangan tree grown at Cikabayan Experimental Research Station, Bogor Agricultural University Dramaga Campus was used for the experiment. Laboratory analysis has been conducted at the Center for Tropical Horticulture Studies and Department of Agronomy and Horticulture, Bogor Agricultural University. Carbohydrate analysis has been carried out at Center for Agricultural Postharvest Research and Development. The experiment has been conducted in Nopember 2013 until September 2014.

Experimental design: This study used a completely randomized factorial design with 2 factors. The first factor was leaf-to-fruit ratio with level of 50:1, 75:1 and 100:1. This treatment carried on selected tertiary branches. The second factor was the color of bagging plastic, i.e. transparent, red, yellow, blue and no bagging as controls. There were 15 combinations of experimental treatments with three replications for each treatment. Microclimate measurements on bagging plastic done in the morning (07.00-9.00), noon (12.00-13.30) and afternoon (15.30 to 17.00). Bagging plastic perforated as much 11 holes on the bottom with diameter ±0.5 cm for air circulation. Pummelo fruit harvested at 25 Weeks After Anthesis (WAA). The experimental data were analyzed using analysis of variance at level $\alpha = 5\%$, followed by Duncan Multiple Range Test (DMRT) at level $\alpha = 5\%$.

Leaf carbohydrate analysis was done in 5, 13 and 21 WWA using the method of Luff Schoorl (Sudarmadji *et al*., 1989). Determination of carbohydrates conducted by titration using a Na-thiosulfate ($Na_2S_2O_3$). Measurement of Total Titrable Acidity (TTA) and vitamin C juice conducted by titration method. TTA titrated using NaOH solution (Nielsen, 1998), while vitamin C using iodine (Sudarmadji *et al*., 1989). Peel pigment was analyzed based on the method of Sims and Gamon (2002) with modification. Flavedo of fruit peel as much as 0.12 g was added with 2 mL acetris (85% acetone+15% tris) then dissolved the extraction solution and rinsed the mortar with 1 mL acetris. The extract solution was placed in microtube and centrifuged at 14 000 rpm for 10 sec, then removed 1 mL supernatant and 3 mL acetris on cuvette, then measured the absorbance spectra on wavelengths 470, 537, 647 and 663 nm.

RESULTS AND DISCUSSION

Microclimate condition within fruit bagging: Measurement of microclimate conditions on internal bagging treatment include temperature, humidity and light intensity (Table 1). Bagging plastic with different colors resulted in difference of internal microclimate conditions. Based on the color spectrum of visible light, red, yellow and blue have each wavelength 620-680, 550-580 and 440-470 nm (Nielsen, 1998) respectively. The larger color wavelength causing the greater light intensity received.

Temperature and relative humidity in fruit bagging bag showed inversely relation. The highest temperature was showed by red plastic, which reached 34.5° C meanwhile the relative humidity was the lowest at 42.7%. Yang *et al*. (2009) explained that the type of plastic bagging with high temperature accompanied by high light absorption affected the rate of fruit development, fruit size and weight. Lechaudel and Joas (2007) reported that temperature affected sink strength. The increasing of temperature increased sink strength to have assimilates translocation for fruit development. Whereas, high humidity inside the bag may reduced sink strength and fruit transpiration and further decreased assimilates flow rate (Zhou *et al*., 2012). Lin and Jolliffe (1996) reported that high light intensity caused high chlorophyll content in fruit peel and had a longer fruit shelf life. The highest light intensity was shown in transparent color bag, which about 62.3% of light transmission to fruits.

Leaf carbohydrate content: There was no significantly difference on leaves carbohydrates content at 5 and 13 Weeks After Anthesis (WAA) for all treatment, but showed significantly difference at 21 WAA (fruit ripening period) (Table 2). Similar result has been reported by Nebauer *et al*. (2011) on the 'Salustiana' orange that the amount of leaves carbohydrates on the highest leaf-to-fruit ratio was significantly higher than the lower leaf-to-fruit ratio in fruit ripening stage. Leaf-to-fruit ratio affected the availability of assimilates for the fruit growth and development. Carbohydrate supply acted as a major limiting factor for fruit enlargement (Goldschmidt, 1999). Nebauer *et al*. (2011) explained that the limited condition of amount carbohydrates, almost of all carbohydrates used for fruit development.

The leaves carbohydrate content of L3 (100:1) was significantly higher at 21 WAA considerably caused by large amounts of carbohydrates production are exceed than the fruit requirement. Iglesias *et al*. (2007) explained that the high carbohydrate translocation to fruit occurred at the

Table 1: Microclimate condition in fruit bagging

Treatments	Mean temperature (°C)	Mean relative humidity (%)	Mean light intensity (lux)
No bagging	32.0±1.1	50.0±3.9	12,800±621
Transparent	33.7±1.0	44.1±3.4	7,969±493
Red	34.5±1.4	42.7±3.5	6,303±363
Yellow	33.0±1.1	46.7±3.1	5,035±402
Blue	33.5±0.9	45.3±3.2	3,870±538

±: Standard deviation

Table 2: Leaf carbohydrate content

	Leaf carbohydrate (%)		
Treatments	5 (WAA)	13 (WAA)	21 (WAA)
Leaf-to-fruit ratio			
50:1 (L1)	11.3	10.9	11.5[b]
75:1 (L2)	11.1	11.2	11.5[b]
100:1 (L3)	11.1	11.0	11.8[a]

In each column, values followed by the same letter are not significantly different at DMRT α = 5%. WAA: Week After Anthesis

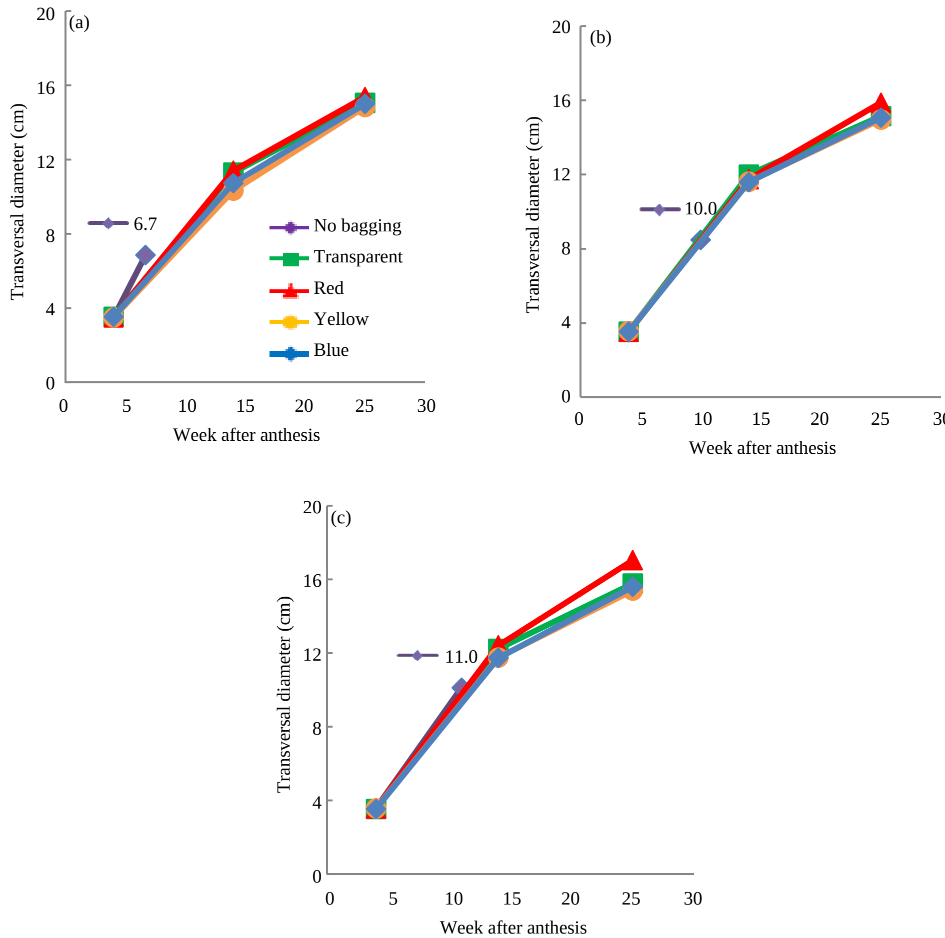

Fig. 1(a-c): Development of fruit transverse diameter, (a) L1 (50:1), (b) (L75:1) and (c) L3 (100:1)

fruit development stage and decreased at the fruit ripening stage. In the leaf-to-fruit ratio 100:1 (L3) treatment has the highest number of leaves per fruit thus potentially providing assimilates higher than the lower leaf-to-fruit ratio. The leaf carbohydrate accumulation in L3 (100:1) treatments at 5, 13 and 21 WAA were 9.1, 9.0 and 9.7 g (dry matter of pummelo leaf = 0.015 g cm^{-2}) respectively.

The development of pummelo fruit: Observations on pummelo fruit in the field showed that not all of samples could survive until harvest (Fig. 1). All of control fruits (without bagging treatment) dropped since fruitlet stage on all of leaf-to-fruit ratio due to the fruit fly attack. Fruit flies insert their eggs into the fruit and the larvae damaged pummelo fruit until dropped. The fruit bagged could survive until harvest.

The development of fruit transverse diameter was rapid until 14 WAA, followed by the slower development until harvest. Similar result was reported by Mahardika and Susanto (2003) that the fruits growth was rapid in early growth stage until two Months After Anthesis (MAA) caused by rapid cell division and enlargement in the period. The highest development of fruit transverse diameter was shown on leaf-to-fruit ratio 100:1 (L3) treatment in all of bagging bag color treatments. This is presumably due to the large number of leaves which causes greater assimilates availability for fruit growth.

Table 3: Chlorophyll of pummelo's fruit peel

Treatments	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophyll total (mg g^{-1})
Leaf-to-fruit ratio			
50:1	0.038	0.019	0.056
75:1	0.041	0.017	0.058
100:1	0.045	0.016	0.060
Bag color			
Transparent	0.060[a]	0.023[a]	0.083[a]
Red	0.056[a]	0.027[a]	0.083[a]
Yellow	0.024[b]	0.010[b]	0.034[b]
Blue	0.027[b]	0.009[b]	0.035[b]
Interaction	tn	tn	tn

In each column, values followed by the same letter are not significantly different at DMRT α = 5%

Pigments and color of fruit peel: Pummelo peel's color raised due to the presence of two pigments, i.e., chlorophyll and carotenoids. Chlorophyll is the pigment that causes the green color generally higher in immature fruit and decreased at maturation stage due to chlorophyll degradation. Chlorophyll a is the major component of chlorophylls in citrus peel (Rodrigo *et al.*, 2013). Nagy *et al.* (1997) stated that the yellow color to orange on the fruit peel due to the content of carotenoids in the flavedo. Leaf-to-fruit ratio have not significant effect on the presence of peel chlorophylls, but the bagging bag color has very significant effect (Table 3).

Chlorophyll in the pummelo's fruit peel consisting of chlorophyll a and chlorophyll b. The concentration of chlorophyll a in pummelo peel almost twofold of chlorophyll b concentration. Transparent and red color bag produced highest chlorophyll concentration might because of high light absorption on the color bag. Lin and Jolliffe (1996) reported that high light intensity caused high chlorophyll content in the fruit peel.

Fruit peel color indicated by the lightness (L) and degree of hue (h°) (Table 4 and 5). The degree of hue (h°) showed the reflection color of fruit peel. Leaf-to-fruit ratio and fruit bagging bag color treatment have no significant effect on the lightness of the peel (L), but very significant effect on the degree of hue (h°). Interaction between L and h was significantly affected by carotenoid content and peel color.

The high carotenoids concentration in fruit peel at the lower leaf-to-fruit ratio presumably related with advance fruit maturation stage. Carotenoid concentration on fruit peel in blue color bagging bag was lower than other color, presumably because this bagging bag reflected a portion of the light at lower wavelengths that needed to synthesize carotenoids, i.e., at wavelengths 440-470 nm. Sims and Gamon (2002) reported that carotenoid synthesis occurred in the light absorption at a wavelength of 400-500 nm and the highest at wavelength 445 nm.

The highest peel lightness (L) was found on the combination treatment of leaf-to-fruit 50:1 (L1) and yellow color bag. The degree of hue in this treatment showed that peel color was yellow, whereas other treatments yellowish green. This is presumably due to the low chlorophyll but high carotenoid concentrations in the yellow color bag resulted in the brightest color and significantly different as compared to the other treatments.

The quality of fruit: Fruit quality consist of the external (Table 6) and internal (Table 7) quality. External quality fruit include smoothness, softness, fruit weight, peel thickness and edible portion. Internal quality include juice content, Total Soluble Solids (TSS), Total Titrable Acidity (TTA), TSS/TTA ratio and vitamin C. Leaf-to-fruit ratio treatment resulted in no significant effect on the smoothness, softness, peel thickness, edible portion, juice content, TSS and vitamin C but

Table 4: Carotenoid and fruit peel color

Treatments	Carotenoid (mg g^{-1})	Lightness L	Degree of hue (h°)
Leaf-to-fruit ratio			
50:1	0.020^{a}	52.5	105.2^{b}
75:1	0.017^{b}	51.9	110.5^{a}
100:1	0.015^{b}	51.6	111.6^{a}
Bag color			
Transparent	0.018^{a}	51.6	111.1^{a}
Red	0.018^{a}	51.9	110.4^{a}
Yellow	0.018^{a}	52.1	103.5^{b}
Blue	0.027^{b}	52.3	111.4^{a}
Interaction	*	**	**

In each column, values followed by the same letter are not significantly different at DMRT α = 5%. h°: Degree of hue (0° = Red purple, 45° = Orange, 90° = Yellow, 120° = Yellowish green, 150° = Green and 180° = Bluish green)

Table 5: Interaction effect of leaf-to-fruit ratio and bag color to fruit peel color

	Bag color			
Leaf-to-fruit ratio	Transparent	Red	Yellow	Blue
Carotenoid (mg g^{-1})				
50	0.022^{a}	0.018^{ab}	0.022^{a}	0.017^{b}
75	0.016^{b}	0.018^{ab}	0.016^{b}	0.016^{b}
100	0.016^{b}	0.018^{ab}	0.016^{b}	0.010^{c}
Lightness (L)				
50	50.6^{cd}	50.9^{bcd}	56.4^{a}	51.9^{bc}
75	52.2^{bc}	52.5^{b}	50.2^{d}	52.6^{b}
100	52.0^{bc}	52.1^{bc}	49.8^{d}	52.5^{b}
Degree of hue (h°)				
50	111.6^{a}	110.5^{a}	87.0^{b}	112.0^{a}
75	109.5^{a}	110.1^{a}	111.5^{a}	110.9^{a}
100	112.3^{a}	110.7^{a}	112.0^{a}	111.3^{a}

Values followed by the same letter are not significantly different at DMRT α = 5%. h°: Degree of hue (0° = Red purple, 45° = Orange, 90° = Yellow, 120° = Yellowish green, 150° = Green and 180° = Bluish green)

Table 6: External quality of pummelo fruit

Treatment	Smoothness (%)	Softness (mm 50 g^{-1} 5 sec^{-1})	Fruit weight (g)	Peel thickness (cm)	Edible portion (%)
Leaf-to-fruit ratio					
50:1	85.4	17.51	603.3^{b}	1.76	46.9
75:1	88.5	17.17	641.4^{b}	1.72	47.1
100:1	84.4	17.31	746.3^{a}	1.83	45.8
Bag color					
Transparent	84.7	17.30	665.3^{b}	1.80^{a}	46.1^{b}
Red	87.5	17.65	808.9^{a}	1.87^{a}	48.7^{a}
Yellow	86.1	17.26	543.8^{c}	1.62^{b}	46.9^{ab}
Blue	86.1	17.11	636.7^{bc}	1.80^{a}	44.7^{b}
Interaction	tn	tn	tn	tn	tn

In each column, values followed by the same letter are not significantly different at Duncan's Multiple Range Test (DMRT) level α = 5%

Table 7: Internal quality of pummelo fruit

Treatment	Juice content (mL/100 g)	TSS (°Brix)	TTA (%)	TSS/TTA ratio	Vitamin C (mg/100g)
Leaf-o-fruit ratio					
50:1	74.92	8.44	0.60^{b}	14.10^{a}	52.70
75:1	72.96	8.46	0.64^{ab}	13.35^{ab}	53.09
100:1	70.58	8.49	0.67^{a}	12.82^{b}	53.00
Bag color					
Transparent	72.61	8.63^{a}	0.63	13.83^{a}	54.10
Red	74.61	8.19^{b}	0.67	12.42^{b}	51.50
Yellow	71.21	8.73^{a}	0.64	13.70^{a}	53.97
Blue	72.85	8.30^{b}	0.61	13.74^{a}	52.15
Interaction	tn	tn	tn	tn	tn

In each column, values followed by the same letter are not significantly different at Duncan's Multiple Range Test (DMRT) level α = 5%, TSS: Total Soluble Solids, TTA: Total Titrable Acidity significantly affected TTA and TSS/TTA ratio and very significant on fruit weight. While the bagging bag colors treatment gave no significant effects on the smoothness, softness, juice content, TTA and vitamin C but having significant effect on TSS and TSS/TTA ratio and very significant on fruit weight and edible portion. The control fruit (without bagging) usually has lower appearance and could not be measured in this experiment because of all fruit dropped at fruitlet stage due to pest attack.

Fruit weight increased with increasing of leaf-to-fruit ratio. Similar condition also happened in persimmon (Choi *et al*., 2010) and cherry (Usenik *et al*., 2010). Red color bag resulted in highest fruit weight than other colors. Presumably, high temperature and light absorption in the red bag color acted as the trigger to increase sink strength. Yang *et al*. (2009) explained that increasing of temperature on bagging bag was a major factor that caused the enlargement of fruit size. Muchui *et al*. (2010) reported that the increasing of temperature (0.5°C) in the bagging bag increased the rate of fruit development and produced greater fruit size and weight equal to 10-16%. The leaf-to-fruit ratio have not significant effect on edible portion, but the red bag color resulted in the highest that reached approximately 50%. Mahardika and Susanto (2003) reported that edible portion of cultivar Nambangan approximately half of the fruit weight.

Yellow color bag has inversely microclimate conditions with red color bag. Lower temperatures on yellow color bag might caused lower sink strength resulted in smaller fruit than other treatments. Peel thickness on the yellow color bag was thinnest, presumably due to the smallest fruit size. The greater fruit size would have greater peel thickness. De Oliveira and Resende (2012) reported that fruit size and peel thickness have a linear relationship, where the larger fruit size would be followed by an increasing of peel thickness.

Sweetness of fruit indicated by TSS, it was not affected by leaf-to-fruit ratio. Bag color has significant effect on TSS. Red color bag produced fruit with the largest size but the lowest TSS. The low TSS on red color bag presumably because of large fruit size required a longer time to reach maturation stage. Gandin *et al*. (2011) found that soluble solids accumulation and maturation cell on a larger capacity sink required a longer time than smaller sink capacity.

Total Titrable Acidity (TTA) and vitamin C content of fruit was not affected by the bag color. Similar result was reported by Noorbaiti *et al*. (2013) in guava fruit. The TTA content in juice was higher with the increasing of leaf-to-fruit ratio. It might be related with fruit maturation stage, higher leaf-to-fruit ratio delayed fruit maturation stage. Lee and Kader (2000) explained that the vitamin C content in fruit accordance with fruit development. The vitamin C content was highest on the maximum fruit size and decreased on fruit maturation stage. Vitamin C on all treatments has no significant different, it's about 50 mg on each 100 mg flesh fruit.

Maturity index indicated by TSS/TTA ratio. The highest leaf-to-fruit ratio (100:1) and red color bag treatment has the low TSS/TTA ratio, it needed a longer time to fruit ripening stage. Transparent and yellow color bag produced fruit with the best internal quality indicated by high TSS, maturity index and vitamin C. TSS and maturity index was not significant different on yellow color bag with transparent color bag. This is presumably due to the small size of fruit on yellow bag. Gandin *et al*. (2011) explained that soluble solids accumulation and maturation cell required a shorter time at a smaller sink.

CONCLUSION

Each fruit of pummelo cv Nambangan required a minimum of 50 leaves to support the fruit development and produced better quality. In the pummelo cultivation, fruit bagging was important to suppress pests attack and produced fruit with high smoothness, which ranges from 84.72-88.54%. Bagging should used transparent and yellow color bag because it could increased the sweetness and maturity index of pummelo fruit. Interaction between leaf-to-fruit ratio and bag color affected carotenoid content and peel color, but have no effect to fruit internal quality.

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