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Application of calcium to decrease yellow sap contamination at different positions of *Garcinia mangostana* L.

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Abstract: The present research aimed at studying the effects of Ca application, through soil fertilization, on yellow sap contamination based on the position of the fruits on the canopy of the tree. The tree was divided into 6 sectors based on the differences in light exposure i.e. sector 1, 2, and 3 for shaded fruit positions and sector 4, 5, and 6 for well-exposed (to light) fruit positions. The present study used a Randomized Complete Block design (RCBD), consisting of 2 treatments i.e. 0 kg Ca/tree and 4.8 kg Ca/tree. The results revealed that Ca treatment lead to an increase in Ca-pectate content in pericarp. In addition, the exposed fruit position allegedly increase the absorption of Ca-pectate to the fruit. Thus, it is important to both apply Ca on the soil and ensure that the fruit, in the canopy, gets enough light to decrease the occurrence of yellow sap contamination. The well-exposed position of the fruit, in the 4.8 kg Ca/tree treatment during anthesis, had increased the Ca-pectate content of the pericarp which, in turn, resulted in a decrease in yellow sap contamination in segment, aryl, and rind of the mangosteen fruit.

1. Introduction

Yellow sap is a sap which is naturally produced in each organ of the mangosteen, excepts the root. Indeed, it constitutes the main constraint in the Indonesian mangosteen agribusiness industry due to the fact that it causes the fruit flesh (aryl) to have a bitter taste and a less attractive look (Osman and Milan, 2006). Statistical data (2015) revealed that only 14.8% of the total Indonesian mangosteen production is exported as a consequence of high percentage of yellow sap contamination.

Yellow sap is found in the yellow sap duct which is surrounded by typical epithelium cells (Dorly *et al*., 2008). It will contaminate the surface of the fruit (or aryl) if the epithelial cells of the secretory duct break as a result of Cadeficiency. The break of the epithelial cells is connected with the extreme changes in groundwater during the developmental process of the fruit (Pechkeo *et al.,* 2007), and the differences in growth rate

between the seed and the aryl with the fruit pericarp during the growing phase of the fruit (Poerwanto *et al*., 2010).

According to previous research, the break of yellow sap duct is related to the concentration of Ca. Indeed, Ca content of the pericarp of mangosteen contaminated fruit (by yellow sap) is lower compared to that of a normal fruit (Poovaradom, 2009; Kurniadinata *et al*., 2016). According to Marshner (2012), Ca structurally functions to strengthen the cell wall, plant tissues, and the stability of the membrane. Mortazavi *et al.* (2016) reported that Ca can minimize cell membrane injury. The increased effect of Ca application can be explained by its role in cell membrane structure. Meanwhile, Seligmann *et al.* (2009) stated that Ca in plant plays an important role regarding the strength of the mechanical tissue and the determination of the fruit quality. However, Ca is an immobile nutrient that could not be translocated from plant tissues. Thus, developing leaves and fruits fully depend on the transmission of Ca in the transpiration stream of the xylem. According to Qiang and Ling (2005), transpiration is the main factor that promotes Ca movements and a low transpiration rate will result in a low addition of Ca^{2+} to aerial organs.

Mangosteen is a small or medium height tree with a straight, symmetrically branched to form a conical and very tight canopy which leads to both a low light intensity and temperature in the shaded internal part compared to the well light-exposed parts (sector). The described architecture is believed to influence the microclimate of various parts of the plant canopy, causing differences in transpiration rate, which in turn, is believed to cause differences in Ca absorption rate to the fruit. Crisosto *et al*. (1995) report the greater the light interception by an individual fruit and its surrounding leaves the better its quality. Fruit that developed in the more shaded inner canopy positions have a greater incidence of internal breakdown than fruit from the high light, outer canopy positions. Erez and Flore (1986) reported that fruit exposed to light experienced a quality improvement by pigmentation of peach that is also assumed to relate to the sink strength of the fruit.

Research, on the relationship between both Ca and the fruit position in the canopy and the occurrence of yellow sap, become capital in determining the sector with a high contamination potential. The present research was aimed to determinate the effects of Ca on yellow sap contamination based on the position of the fruit in the tree canopy

2. Materials and Methods

Time and place

The present research was conducted in Tandolala village, Poso District, central Sulawesi, from October 2015 to April 2016. Tandolala village is situated at an altitude of 508 mdpl with a pH (soil) of 4.8, and a rainfall of 192.8 mm/month. The observations on yellow sap contamination were carried out in the laboratory of Natural science at the University of Sintuwu Maroso Poso. Analyses of total Ca and pericarp Ca-pectate contents were performed in the laboratory of soil chemistry and fertility, Bogor Agricultural University.

Materials

Ten productive plants, at an average 40 years old (with a planting distance ranging from of 4x4 m to 6x6 m), an average height of 16 meters and a canopy diameter of 4 m, were used in the present study. Dolomite [$CaMg(CO_3)_2$], containing 30% Ca, was used as Ca source.

The canopy of the tree was divided into 6 sectors as follows: sector 1 - inner bottom, sector 2 - outer bottom, sector 3 - inner middle, sector 4 - outer middle, sector 5 - inner top, and sector 6 - outer top. The tree was divided into sectors based on the modifications of Setiawan *et al*. (2012) techniques (Fig. 1).

Parameters, such as light intensity, temperature, and humidity, were measured on weeks 4, 8, and 12, on each sector by placing the measuring tool (thermohydrometer) in each sector. Afterwards, the light intensity was recorded as displayed on the measuring

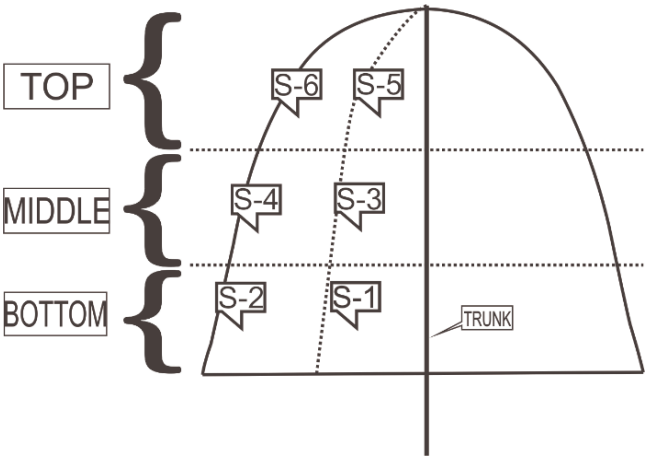

Fig. 1 - Fruit positions in the canopy of the tree. Adapted from Setiawan *et al*., 2012.
S= Sector.

tool. Light intensity, temperature and humidity averages for each sector are presented in Table 1.

Table 1 - Light intensity, temperature, humidity, and transpiration rate at different fruit positions (exposed and shaded)

Fruit positions	Light intensity (lux)	Temperature (°C)	Humidity (%)	Transpiration rate (Hpa/s)	
Sector 1	414.44	26.54	70.98	shaded	0.03
Sector 2	536.00	26.80	70.46		
Sector 3	492.76	26.08	71.54		
Sector 4	1581.51	27.04	69.77	well-exposed	0.06
Sector 5	944.60	26.74	72.04		
Sector 6	1163.11	27.26	70.97		

Experimental design

A Randomized Complete Block design (RCBD*),* consisting of 2 treatments i.e. 0 kg Ca/tree and 4.8 kg Ca/tree (equivalent to 16 kg dolomite/tree), was used as experimental design in the present study. Fertilization with Ca was carried out during anthesis by sowing in the path that was already made around the mangosteen tree (below the crown of the plant) and recovering it with soil.

Based on the light intensity measurement results in Table 1, the observations on yellow sap contamination were carried out on both shaded and well-exposed positions. A fruit was considered shaded if the perceived light intensity is low. Meanwhile, it was categorized as well-exposed if the average received light intensity is high. Shaded fruit positions were identified in sectors 1, 2 and 3, with an average light intensity of 481 lux, while well exposed fruit positions were identified in sectors 4, 5 and 6 with an average light intensity of 1229 lux. The total sample from each sector was 12 fruits (72 fruits per tree). Thus, a total of 720 mangosteen fruits were used as samples.

The transpiration rates, of the leaves, were measured at both shaded and well-exposed positions. The results of the above measurements will be used to estimate the transpiration rates of the fruits in various positions. The transpiration rate was measured by means of a lux meter.

Measurements

Harvesting was carried out 16 weeks post-anthesis (WPA). The observations on the percentage of yellow sap (PYS) were carried out in order to determine the percentage of contaminated fruit in the group of fruits that was observed. A fruit was considered contaminated although the yellow sap, that pollutes both the aryl and the rind just one spot (small patch). The percentages of contaminated aryl (PCA), contaminated rind (PCR), and contaminated segment (PCS) was calculated by means of the following equations:

PCA = (total yellow sap contaminated aryl /total fruit sample) x 100
PCR = (total yellow sap contaminated rind/total fruit sample) x 100
PCS = (total yellow sap contaminated segment/total fruit segment sample) x 100

Yellow sap contamination score shows the severity level of a fruit contaminated by yellow sap and ranges from 1 (very well) to 5 (very bad). Observations on aryl fruit and yellow sap contaminated rind scores referred to Kurniadinata *et al*. (2016) method as presented in Table 2 and 3.

Ca pectate analyses referred to an analysis method developed by Setyaningrum *et al*. (2011) which uses dry fruit sample. The samples were ground into parti-

Table 2 - Yellow sap contamination score on aryl

Score	Description
1	Very good, clean white aryl, no yellow sap between aryl and rind, and fruit vessels as well
2	Good, 1-2 yellow sap stains (small patch) on one end of the aryl, but does not make the fruit bitter
3	Good enough, the presence of some yellow sap stains (patch) on one end of the aryl or between the segments and the littering aryl
4	Bad, presence of yellow sap stains/blobs at the end of the segments, between the segments or the fruit vessels, making the fruit bitter
5	Very bad, the presence of large yellow sap stains/blobs at the end of the segments, between the segments or at the fruit vessels, making the fruit bitter with a clear colored aryl

Score 1= Very good/without contamination, up to score 5= very bad /high contamination score.

Table 3 - Yellow sap contamination score on rind

Score	Description
1	Very good, flawless rinds with no visible yellow sap.
2	Good, flawless rinds with 1-5 yellow sap stains (small patch) which dry without affecting the color of the fruit
3	Good enough, flawless rinds with 6-10 yellow sap drops which dry and do not affect the color of the fruit
4	Bad, flawed rinds due to medium/large yellow sap clumps, there are 1-2 yellowing streams
5	Very bad, flawed rinds with more than one large yellow sap clumps with lots of yellowing streams on the rind of the fruit and a dull fruit color

Score 1= Very good/without contamination, up to score 5= very bad /high contamination score.

cles, added with ion free water and shacked for 2 hours. Afterward, the solution was centrifuged for 15 minutes at a speed of 3000 rpm. The supernatant was then filtered to collect the pellet to which a 1 mol L^{-1} of NaCl was added, shacked for 2 hours and centrifuged for 15 minutes. The extraction result was analyzed Atomic Absorption Spectrophotometer (AAS) in order to obtain data on Ca pectate.

Statistical analysis

PCA, PCR, PCS, total Ca contents, and Ca-pectate were analyzed using SAS 9.1.3 program, which was followed by Duncan's post-hoc comparison (at a significance level of 5%) test. Meanwhile, data on fruit score, yellow sap contaminated aryl were analyzed by mean of a Kruskal Wallis test, which was followed by Dunn test.

3. Results

The application of Ca had an effect on the decrease in yellow sap contamination in mangosteen (Table 4). The lowest percentage of yellow sap contaminated segment was observed in the 4.8 kg Ca/tree (exposed fruit position), which significantly differed to those of other treatments. The percentage of yellow sap contaminated segment showed significant decline of 81% with application of 4.8 Ca/tree on exposed position compared to treatment with 0 kg Ca/tree on shaded position, 71% compared to treatment with 0 kg Ca/tree on exposed position and 73% compared to 4.8 kg Ca/tree on shaded position.

The percentage of yellow sap contaminated aryl, in the 4.8 kg Ca/tree treatment (exposed fruit position), showed an average percentage of 14.2%, which was not different to that of the 0 kg Ca/tree treatment (23.9%). No differences were observed among treatments application of 4.8 kg Ca/tree in terms of yellow sap contaminated rind percentage. The percentage of yellow sap contaminated rind was considerably high with treatment of 0 kg Ca/tree, which was 79.9% on shaded position and 73.9% on exposed position. While the application of 4.8 kg Ca/tree showed that yellow sap contamination was still considerably high at 61.3% on shaded position and 53.5% on shaded position. Although the numbers were still high, there was a decline of 33% with application of 4.8 Ca/tree compared to without Ca application on shaded position. According to Martias *et al.* (2012), percentage of yellow sap contaminated that was higher than 50% was considered very high.

Table 5 presents the yellow sap contamination scores of both aryl and rind. The best yellow sap contaminated aryl score was observed at the well-exposed fruit position with the application of 4.8 kg Ca/tree, and significantly differed to other treatments. Meanwhile, the yellow sap contaminated rind score in the 4.8 kg Ca/tree treatment revealed similar results for both shaded and well-exposed fruit positions (Table 5).

Accumulation of Ca in fruit pericarp was a representation of adsorbed soil Ca by plant. Total Ca content of the fruit pericarp did not significantly differ among treatments, but was significantly different to that of the Ca-pectate contents. The Ca-pectate con-

Table 5 - Score of yellow sap contamination in aryl and on rind (16 WPA)

Treatments	Yellow sap contaminated aryl and rind score in 1-5	
	Aryl	Rind
0 kg Ca/tree		
Shaded	1.99 a	2.38 a
Exposed	1.67 c	2.17 b
4.8 kg Ca/tree		
Shaded	1.77 b	1.90 c
Exposed	1.23 d	1.74 c
Dunn-test	*	*

Data were analyzed based on Kruskal Wallis test. Numbers followed by different letters within the same column showed significant differences based on Dunn test (1%). Score 1: Very good; score 2= Good; score 3= Good enough; score 4= Bad; score 5= Very bad.

Table 4 - Percentage yellow sap contamination in fruit segment, aryl and on rind (16 WPA)

Treatments	Fruits contaminated by yellow sap (%)			Pericarp Ca content (ppm)	
	Segment	Aryl	Rind	Total	Pectate
0 kg Ca/tree					
Shaded	21.3 a	45.7 a	79.9 a	1088.9	552.02 b
Exposed	13.6 a	23.9 b	73.9 ab	2100.0	528.87 b
4.8 kg Ca/tree					
Shaded	15.0 a	34.9 a	61.3 bc	1500.0	576.40 b
Exposed	4.0 b	14.2 b	53.5 c	1566.7	754.60 a
F-test	*		*	NS	*

Numbers followed by different letters within the same column showed significant differences in DMRT test (α= 5%). Shaded (sector 1, 2 and 3) with a light intensity of 481 lux, Exposed (sector 4, 5 and 6) with a light intensity of 1229 lux.

tent was higher in the Ca treated fruit (well-exposed position) (Table 6). The percentage of yellow sap contaminated rind and aryl was reduced because of increased Ca-pectate in fruit pericarp, proving that Ca-pectate had a role in strengthening cell wall epithelium which compile the yellow sap duct making the cell stronger and kept yellow sap from leaking and contaminate the aryl and rind.

Leaves transpiration rate measurement, on week 4, 8, and 12, resulted in average transpiration rates of 0.03 Hpa/s (shaded position) and 0.06 Hpa/s (well exposed position) (Table 1). This data supported the measurement result of light and temperature on Table 1, that the higher the temperature and light intensity, the higher the transpiration rate.

Table 6 - Calcium total and Ca-pectate content in pericarp and percentage ratio of Ca-pectate/total Ca at 16 WPA

Treatment	Ca content in pericarp (%)		Percentage pectate/total
	Total	Pectate	
0 kg Ca/tree			
Shaded	0.11	0.06 b	50.5
Exposed	0.21	0.05 b	23.8
4.8 kg Ca/tree			
Shaded	0.15	0.06 b	40.0
Exposed	0.17	0.08 a	47.1
F-test	NS	*	NS

Numbers followed by different letters within the same column showed significant differences in DMRT test (α= 5%). Shaded (sector 1, 2 and 3) with a light intensity of 481 lux, Exposed (sector 4, 5 and 6) with a light intensity of 1229 lux.

4. Discussion and Conclusions

Ca is an immobile nutrient, for its absorption rate follows the transpiration pathway in the xylem. Thus, a deficiency in Ca often occurs in fruits that do not transpire as much as the leaves. In the present study, the transpiration rate of the fruits was estimated by observing parameters such as light intensity, temperature and humidity in each sector, and also the transpiration rates of leaves at both the exposed and shaded positions.

Data shown in Table 4 showed that the percentage of yellow sap contamination on the rind was higher than aryl and segment. Yellow sap contamination was caused by the same factor which was the lack of Ca on epithelium cell of yellow duct sap, but triggered by different things. According to Dorly *et al*. (2008), yellow sap contamination on the aryl was caused by turgor and mechanical pressure, that was the pressure of aryl and seed growth outward during fruit enlargement. While contamination on the rind was caused by turgor pressure of pericarp cell, or by insect, fungal, or bacterial attack.

Fruits that were exposed to a high light intensity (Table 1) and treated with 4.8 kg Ca/tree had highest Ca-pectate content (Table 6) which was a consequence of the high light intensity received. Marschner (2012) stated that part of the pectin, in the leaves, is in the form of Ca-pectate in a high light intensity condition. Ca strengthens the main plant cell wall in a crosslinking with the pectin. Formation of calcium pectate due to the binding of calcium with pectins has been found beneficial to increase the strength of cell wall and middle lamella (Carpita and McCann, 2000). The high Ca-pectate content is believed to be a result of light intensity not only on the leaves but also on the fruit that possibly increases photosynthesis rate and transpiration rate, as demonstrated by the results of the present research.

Such fruits (well-exposed, treated with Ca, and showing highest Ca-pectate), showed also lowest yellow sap contamination levels. A high ratio of Ca-pectate over total Ca content (48% of the total Ca) (Table 6) could decrease yellow sap contamination through the strengthening of the epithelial cell walls in the yellow sap duct. A research, conducted by Setyaningrum *et al.* (2011), revealed that the Ca- pectate content represented 20% of the total Ca, which tend to reduce yellow sap contamination in mangosteen.

The improvement of fruits quality (low yellow sap contamination) is related to high light intensity of fruits exposition. Physiologically, light has a direct influence by photosynthesis and indirect influence throught plant's growth and development as the results of direct metabolic responses (Fitter and Hay, 1991). The improvement of fruits quality is caused by the distribution of photosynthate to the fruit exposed to light.

The high temperature and light intensity in the exposed tree canopy resulted in higher transpiration rate at exposed positions compared to the shaded ones (Table 1). Similar results were observed by Caleb *et al.* (2013) who demonstrated that temperature and humidity have significant effects on the transpiration rate. The high leaves transpiration rate at the exposed position is believed to be a consequence of higher Ca translocation from the root to parts of the leaf (including part of the exposed fruit), for the Ca is translocated along with water during the transpiration process. The high transpiration rate led to a high translocation of Ca from the root to the fruit, since Ca is translocated along with water during

the transpiration process. Hansen (1980) stated that Ca is transported in the fruit by means of water distribution through the xylem. Gilliham *et al.* (2011) demonstrated that the mechanism of Ca uptake is through the apoplast of the root along with the mass flow which follows the apoplast or the symplast pathway to the xylem. According to Qiang and Ling (2005) and White and Broadley (2003), the transport through the apoplastic pathway mainly depends on the transpiration, while the symplast pathway is selective in controlling Ca^{2+} to the xylem which depends on Ca^{2+} requirement at the canopy.

If there is a deficiency in Ca supply of the plant, the plant may experience damages at the cell level. Thus, the supplementation of Ca is required on soil with low Ca content. According to Martias *et al*. (2012), yellow sap contamination could be directly prevented by the Ca availability in the soil. Amor and Leo (2006) stated that the Ca concentration of the plant significantly decrease due to a low supply. In addition, Hocking *et al.* (2016) demonstrated that low supply and transport of Ca would result in a Ca deficiency, leading to damages of both membrane and cell walls of the fruit.

The results revealed that Ca treatment lead to an increase in Ca-pectate content in pericarp. In addition, the exposed fruit position allegedly increase the absorption of Ca-pectate to the fruit. Thus, it is important to both apply Ca on the soil and ensure that the fruit, in the canopy, gets enough light to decrease the occurrence of yellow sap contamination. The well-exposed position of the fruit, in the 4.8 kg Ca/tree treatment during anthesis, had increased the Ca-pectate content of the pericarp which, in turn, resulted in a decrease in yellow sap contamination in segment, aryl, and rind of the mangosteen fruit.

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