CBCS
HTTP://AGROBIOL.SGGW.WAW.PL/CBCS

COMMUNICATIONS IN BIOMETRY AND CROP SCIENCE
VOL. 11, NO. 2, 2016, PP. 105–113

INTERNATIONAL JOURNAL OF THE FACULTY OF AGRICULTURE AND BIOLOGY,
WARSAW UNIVERSITY OF LIFE SCIENCES – SGGW, POLAND

REGULAR ARTICLE

Solving yellow sap contamination problem in mangosteen (*Garcinia mangostana*) with Ca^{2+} application based on fruit growth stage

Odit F. Kurniadinata[1]*, Susi O.S. Depari[1], Roedhy Poerwanto[1,2]*, Darda Efendi[1], Ade Wachjar[1]

[1] Bogor Agricultural University (IPB), Bogor, Indonesia.
[2] Present address: Department of Agronomy and Horticultura, Bogor Agricultural University (IPB), Jl Meranti, Kampus IPB Darmaga, Bogor, Indonesia.
*Corresponding author: Odit F. Kurniadinata; E-mail: odit.ferry@gmail.com

CITATION: Kurniadinata O.F., Depari S.O.S., Poerwanto R., Efendi D., Wachjar A. (2016). Solving yellow sap contamination problem in mangosteen (*Garcinia mangostana*) with Ca^{2+} application based on fruit growth stage. *Communications in Biometry and Crop Science* 11, 105–113.

Received: 7 May 2015, Accepted: 16 May 2016, Published online: 30 May 2016

ABSTRACT

Yellow sap contamination causes poor quality of mangosteen fruits. Yellow sap is an issue when the sap contaminates the surface of the fruit or aryl. It is caused by the break of yellow sap duct in fruit rind. The break of yellow sap duct is connected with low concentration of Ca^{2+} in the fruit pericarp. Source of calcium and stage of fruit development affect uptake and translocation of Ca^{2+} to the fruit pericarp. This study aims to determine: (1) the best time for Ca application, (2) the frequently of Ca application, and (3) the effects of the presence of xylem and casparian strips on Ca^{2+} translocation in mangosteen plants. The study was conducted in Lampung (Sumatra) and Bogor (West Java), Indonesia between January 2011 to April 2013. Ca source is calcite ($CaCO_3$) at 17 kg calcite per tree for the experiments conducted in Lampung and 10 kg Calcite per tree in Bogor. The results showed that the blooming time was the best period for Ca application in both study location. The critical time of Ca uptake was between the first and the fourth weeks after blooming. Application of Ca twice could reduce yellow sap contamination on aril and pericarp. In Bogor, Ca^{2+} application at 4.5 kg $year^{-1}$ $tree^{-1}$ at anthesis stage reduced the percentage of yellow sap contaminated fruit on the aryl to 33% and 30% respectively. In Lampung, twice application of Ca at anthesis and 4 weeks after anthesis (WAA) reduced the percentage of yellow sap contaminated fruit on the aryl to 10% compared to 50% of that of control.

Key Words: *pedicel; xylem; casparian strip; calcium; pericarp; times application.*

INTRODUCTION

The main problem of mangosteen fruit production in Indonesia is the yellow sap contamination on arrylus and pericarp, leading to downgrade the appearance and taste of the mangosteen fruit. Yellow sap is produced naturally in every organ of mangosteen plant. Sap duct is present in all mangosteen plant tissues. Yellow sap ducts in the mangosteen fruit are elongated and branched channels, surrounded by epithelium cells (Dorly 2009). Yellow sap contamination occurs when the yellow sap comes out from broken duct and contaminates aryl or rind.

The disruption of duct in the mangosteen fruit occurs because of epithelium cells experience pressure. There are two kinds of this pressure: the first one comes from the increase in water potential in the duct while the second one comes from arryl and seeds that grow faster than the pericarp of the fruit. Both pressures will cause epithelial cells to break up when the cell wall is weak. The weak and easily broken cell walls are due to the lack of calcium (Ca) on the pericarp cells (Dorly 2009). Ca is the binding substance on the cell wall structure that binds pectin chains for stronger cell wall (Marschner 1995, Huang et al. 2005). However, several studies indicate that Ca fertilization might not be effective if applied under wrong time. Ca application before flowering cannot increase Ca content in fruit, but increases Ca content in leaves (Dorly, 2009). Ca application before fruit set will make Ca translocate into the leaf tissue because Ca is quite immobile in the floem and its distribution is influenced by transpiration. Since Ca is immobile in floem, Ca stored in leaves will not retranslocate to fruit or other new tissues.

Lampung and Bogor regencies are two mangosteen production centers in Indonesia with high yellow sap contamination levels. Lampung has the contamination occurence higher than that of Bogor. Martias (2012) reported that the incidence of the yellow sap contamination in West Java and Lampung mangosteen varies from 8.7-54.04% and 17.7-78.6% on arryl and rind, respectively.

Rapid fruit growth affected the uptake and translocation of Ca to the fruit tissue. Poovarodom (2009) divided mangosteen fruit developmental stage into three stages: the first stage is 1-4 weeks after anthesis (WAA), stage II is 5-13 WAA, and stage III is 14-15 WAA. Stage I is important to increase Ca^{2+} amount to the mangosteen fruit because at this stage the fruit develops rapidly and becomes the strong sink for nutrients absorbstion include Ca^{2+}. However, the best time for Ca application in the stage 1 to reduce yellow sap contamination is still unknown. This research was conducted in two different locations to determine (1) the best time for Ca application, (2) the frequently of Ca application, and (3) the effects of the presence of xylem and casparian strips on Ca^{2+} translocation in mangosteen plants.

MATERIALS AND METHODS

TIME AND PLACE

The study consist of three experiments conducted at different times and locations. The first experiment was conducted in January 2011 to June 2011 in Mulang Mayan village, Lampung, Indonesia. The second experiment was conducted in March 2011 to April 2013 in Cengal, Karacak Village, District Leuwiliang, Bogor, West Java, Indonesia. The third experiment was conducted in July 2014 in the Microtechnic Lab., Bogor Agricultural University (IPB), Bogor. Physical measurements of fruit were done in the Center for Tropical Horticulture Studies Lab., IPB. Photos of stem, fruit stalk and rind tissue morphology were taken in Microtechnic Lab., IPB. Pericarp Ca content was analyzed in Lab. of Science and Food Technology, Department of Nutrition and Food Technology, IPB and in the Soil Lab., Soil Research Institute, Bogor.

MATERIAL

50 productive mangosteen trees, approximately 20-year-old (see below for detailed information about the particular experiments). Calcite (45% of Ca) were used as source of Ca^{2+}.

RESEARCH METHODS

a. Experiment I. Ca Application in Lampung: The experiment used 17 kg calcite per tree, which is equivalent to 7.65 kg Ca^{2+} $year^{-1}$ $tree^{-1}$. Ca in soil was 4.18 me 100 g^{-1} (Soil Lab. Department of Soil Science and Land Resources, IPB 2011). This experiment used a randomized block design (RBD) with one factor with 8 treatments, with three replications, accounting for 24 sample trees. The treatments were:

1. no Ca (control, 4.18 me 100g^{-1} Ca in soil);
2. Application at anthesis stage:
3. Application at 1 week after anthesis (1 WAA):
4. Application at 4 WAA:
5. Application at anthesis and 1 WAA: (½ dose for each application)
6. Application at anthesis and 4 WAA (½ dose each application)
7. Application at 1 and 4 WAA: (½ dose each application)
8. Application at anthesis, 1 WAA and 4 WAA: (1/3 dose each application).

The data were analyzed by ANOVA using MINITAB program version 14, and the Duncan Multiple Range Test (DMRT) in case of it is significance at α=0.05 level.

Observation: Analyze the percentage of contaminated fruit by yellow sap on aryl and rind (100 fruits $tree^{-1}$).

b. Experiment II. Ca Applications in Bogor: The second experiment consisted of 7 treatments of Ca application time, arranged in a randomized block design (RBD) with tree replications. The treatments were:

1. no Ca (control, 4.59 me 100 g^{-1} Ca in soil)
2. Application before flowering (2 weeks after the rainy season begins)
3. Application when 80% of population flowering
4. Application at anthesis (80% of the population in blooming)
5. Application at 1 WAA
6. Application at 5 WAA
7. Application at 6 WAA

Ca fertilizer was applied to the furrow made 2 m around the mangosteen trunk under the tree canopy, and then the fertilizer was covered by soil. Calcite ($CaCO_3$) was used as source of Ca with dose of 10 kg Calcite per tree or equivalent to 4.5 kg Ca^{2+} $tree^{-1}$ $year^{-1}$. Based on soil chemistry analysis, Ca in soil was 4.59 me 100g^{-1} (Soil Lab. Department of Soil Science and Land Resources, IPB 2011). The data were analyzed by ANOVA using MINITAB program version 14, and the Duncan Multiple Range Test (DMRT) in case of it is significance at α=0.05 level.

Observation: Analyze the percentage of contaminated fruit by yellow sap on aryl and rind (100 fruits $tree^{-1}$).

c. Experiment III. The xylem vessel in branch, pedicel and fruit rind: The third experiment consisted of microscopic observations of the existence of xylem in branch, pedicel and rind at 30 days after anthesis (DAA) and 90 DAA. This experiment used 5 sample trees (10 fruits $tree^{-1}$).

RESULTS

EXPERIMENT 1. CA APPLICATION AT MULANG MAYAN VILLAGE, LAMPUNG

The results showed that Ca application at anthesis+4 WAA reduced the percentage of fruit with yellow sap contamination on aryl to only 10% whereas control fruits had 50% contamination. Similarly, the percentage of fruits with yellow sap contamination on pericarp reduced to 56% and 60% with Ca application at anthesis+4 WAA and at 1 MSA+4 WAA whereas control still high contamination, i.e. 90% (Table 1).

There was an increase in total Ca content in endocarp to 1.2% of ash weight with Ca application at anthesis+4 WAA. Control showed total Ca content in endocarp layer only 0.8% (Figure 1).

Table 1. Effect of Ca application on the percentage of fruit with yellow sap contamination on fruits segment, aryl, and rind, in Lampung and Bogor, Indonesia.

Ca treatment	Fruit with yellow sap contamination (%)		
	fruit segment *	aryl	rind
A. LAMPUNG			
Control (Without Ca)	16 a	50.0 a	90.0 a
Anthesis	12 ab	40.0 ab	86.7 a
1 WAA	19 a	53.3 a	96.7 a
4 WAA	4 bc	16.7 bc	83.3 a
Anthesis + 1 WAA	5 bc	16.7 bc	76.7 ab
Anthesis + 4 WAA	3 bc	10.0 c	60.0 b
1 WAA+ 4 WAA	3 c	16.7 bc	56.7 b
Anthesis + 1 WAA+ 4 WAA	4 bc	16.7 bc	76.7 ab
B. BOGOR			
Control (Without Ca)	24 a	56.7 a	100.0 a
Before flowering	14 ab	46.7 ab	73.3 bc
At 80% flowering	9 b	33.3 ab	63.3 cd
At Anthesis	15 ab	33.3 ab	50.0 d
At 1 WAA	8 b	30.0 b	90.0 ab
At 5 WAA	12 ab	40.0 ab	83.3 ab
At 6 WAA	17 ab	46.7 ab	90.0 ab

Note: The numbers are followed by the same letter in the same column showed no significant differences by the DMRT α=0.05. * Data are processed using the transformation $\sqrt{(x + 0.5)}$. WAA= weeks after anthesis

EXPERIMENT 2. CA APPLICATION IN BOGOR, WEST JAVA

Ca application at anthesis reduced the percentage of fruit with yellow sap contamination on the rind to only 50% whereas 100% of control fruits were contaminated (Table 1).

Ca application at 1 WAA also reduced the percentage of fruit with yellow sap contamination on the aryl to 30% in contrast to 56.7% in control (Table 1). Similarly, Ca application at 1 WAA reduced fruit segment contamination to only 8% whereas 27% of control fruits were contaminated. Ca application at anthesis increased Ca total content in fruit pericarp to 0.75% of ash weight, which was significantly higher than that of control (Figure 2). The highest Ca total content was obtained from Ca application at anthesis stage. Ca treatment also reduced the percentage of fruit rind contamination with yellow sap to only 50%, in contrast to 90% in control fruits.

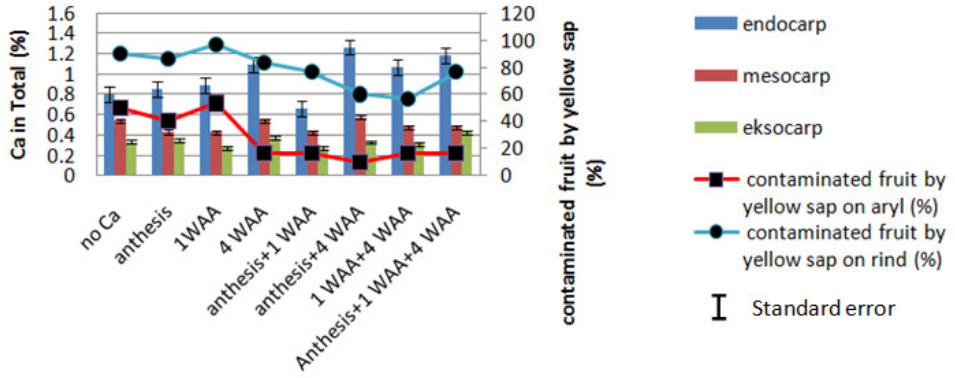

Figure 1. Relationship between the total Ca content in endocarp, mesocarp and fruit exocarp and the percentage of fruit with yellow sap contamination on aryl and fruit rind based on time application of Ca. The experiment was conducted in Lampung, Indonesia.

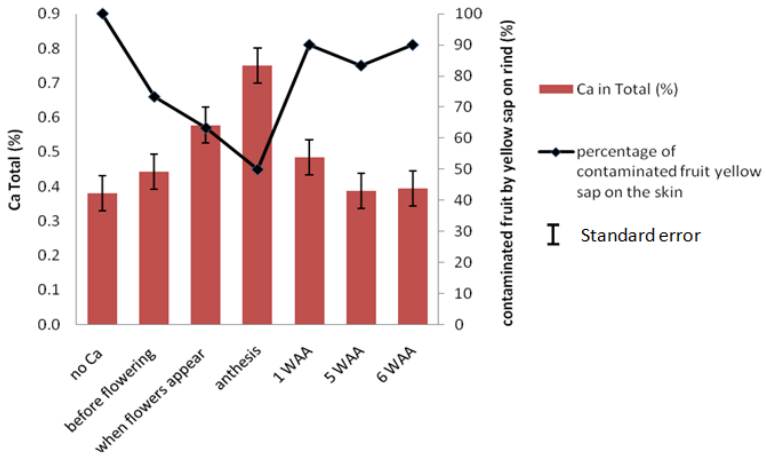

Figure 2. The percentage of fruit with contaminated skin and total Ca content in fruit pericarp based on Ca application time. The experiment was conducted in Bogor, Indonesia.

EXPERIMENT 3. THE XYLEM VESSEL IN BRANCH, PEDICEL AND FRUIT RIND

Xylem observations in branch, pedicel, and rind showed the different visual existance of xylem vessels at 30 and 90 DAA. In branch, pedicel, and rind of 30 DAA fruit, the xylem and phloem tissues were clearly visible and had clear pattern. On 90 DAA, fruit, xylem and phloem appeared only in tree branch while in pedicel and fruit rind only phloem was detected, without visible presence of xylem (Figure 3).

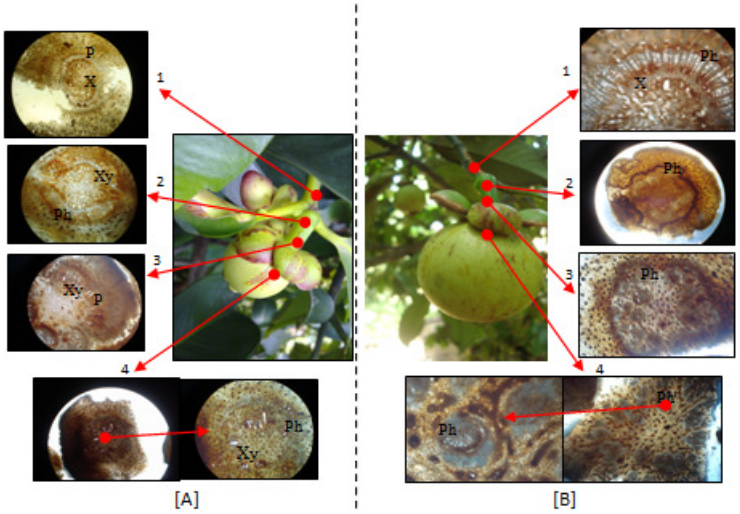

Figure 3. Xylem and phloem vessels in the branches [1], in the branch-end of the pedicel [2], in the fruit-end of the pedicel [3], and in the pedicel-end of the fruit [4] at young fruit stage (30 days after anthesis) [A] and at old fruit stage (90 DAA) [B]. ph = phloem; xy = xylem.

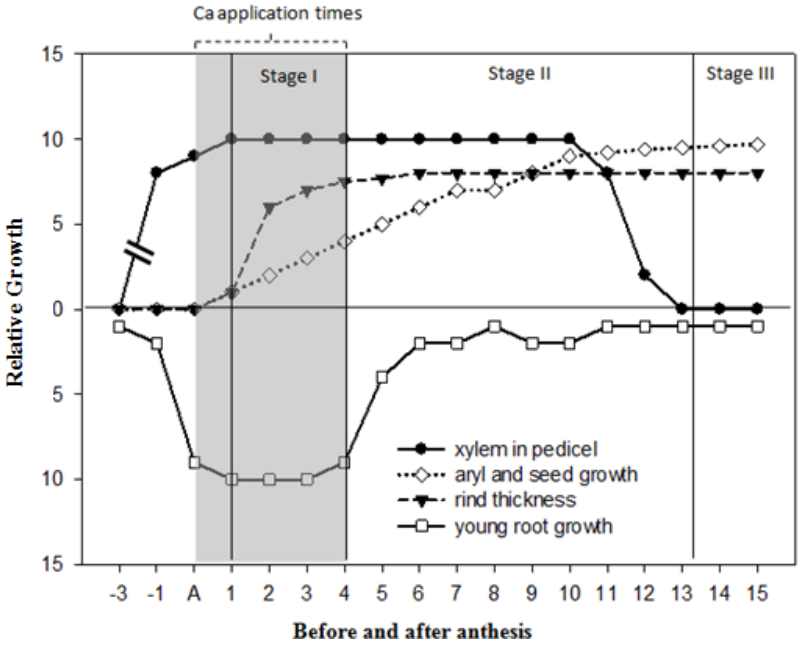

Figure 4. Relationship model of calcium absorbed and translocation based on young root growth, fruit growth, and xylem condition in pedicel. X axis ([-] = Weeks before anthesis; [A] = anthesis; [+] = weeks after anthesis). Y axis (xylem, aryl and seed, rin, and young root growth (not to scale).

DISCUSSION

APPLICATIONS OF CA BASED ON FRUIT GROWTH STAGE

Ca is one of immobile elements in the plant tissues, so it cannot be translocated from old tissues to young tissues, or from leaves to fruits. Unlike those elements that can be translocated through the xylem and phloem, such as nitrogen, phosphorus and potassium, Ca is supplied for fruits directly by root uptake through the xylem. Therefore, Ca^{2+} availability in soil during fruit growth is important. Fruits become a strong sink for Ca

during fruit growth phases, and this causes the increase in Ca uptake to the fruit tissues. The early stages of fruit growth are important because the fruit absorbs then high amount of nutrients, including Ca^{2+}, to support the rapid fruit growth (Poovarodom, 2009). Dorly (2009) reported that fruit rind (pericarp) thickness increased rapidly during their early stage of development. Marschner (1995), Pessarakli (2002), and Wilsdorf (2011) stated that at the time of fruit growth, all nutrients will be translocated to fruit tissue to support quick growth and development of the fruit, including Ca^{2+} that was uptaken by the roots and translocated through the xylem to the fruit pericarp.

Experiments in Lampung and Bogor showed that Ca application from anthesis to the first stage of fruit growth was able to reduce the percentage of contaminated fruit on the exocarp. Ca application at anthesis stage can reduce the percentage of fruit with yellow sap contamination on rind better than control. Similarly, Ca application at 1 WAA was able to reduce the percentage of contaminated fruit on aryl and fruit segment better than control (without Ca). The decrease in percentage of fruit with yellow sap contamination by Ca application at anthesis and at 1 WAA showed that the time between anthesis and stage 1 is critical for Ca^{2+} uptake. This critical time is correlated to Ca requirement for rapid cell division and growth in the pericarp at the early fruit growth.

The experiment in Lampung showed that Ca application twice during the stage 1 would ensure the availability of Ca^{2+} for mangosteen fruit development. With the abundance of Ca^{2+} in the rhizosphere, the plant can optimally absorb and translocate Ca^{2+} to the growing fruits. Ca^{2+} absorption and translocation to the fruit during rapid growth of the fruit can decrease the percentage of fruit with yellow sap contamination on aryl and rind (Table 1). To improve the efficiency of Ca in reducing yellow sap incidence, Ca application should be applied twice during rapid growth stage, i.e. at the anthesis and at 4WAA (end of stage I). A single application will likely be less efficient in absorbing and translocating Ca to the fruit tissue. At stage 1, mainly cell division occurs; at stage II, cell division and enlargement takes place, characterized by an increase in fresh weight of fruit linearly with age; however, growth begins to decline at stage III. Application of Ca at anthesis and at the end of the first stage (4 WAA) will supply Ca for the fruits. Ca^{2+} absorbed by roots and then translocated to fruit tissue hence decreases yellow sap contamination and increase the percentage of high quality fruits

RELATIONSHIP MODEL OF XYLEM IN PEDICEL AND YOUNG ROOTS GROWTH RATE

Stage 1 of fruit growth becomes a critical time for Ca uptake to support fruit growth and development. Based on the microscopic observations in Experiment 3 and literature review, we propose a model of the relationship among the presence of xylem in pedicel, fruit diameter, rind thickness and young root growth rate, to determine the relationship between xylem and young roots for Ca^{2+} uptake into the fruit tissue. The line of growth and development of young roots was made based on visual observations on young roots growth during anthesis and supported by the results by Hidayat (2002). The line of diameter and rind thicknes was made based on the results by Dorly et al. (2008). The xylem line in pedicel was made based on microscopic observation on xylem tissue in the mangosteen fruit pedicel at 30 DAA and 90 DAA (experiment 3) and based on Rigney and Wills (1981), Faust (1989), Ropiah (2009), and Poovarodom (2010). The model describes the state of xylem as the only transport duct for Ca^{2+} to get into the fruit tissue. The xylem is formed from the meristem tissue at differentiation phase to the fruit maturation phase while the young roots are initiated at the time of early anthesis stage. The growth rate of the young roots was optimal for approximately one month only, i.e. from starting of anthesis to 4 WAA (Figure 4).

The amount of Ca translocation to fruits varied with the stages of fruit growth and was influenced by physiological processes. Ca^{2+} translocation from branch into fruit tissue is very limited (Huang et al., 2005). This is because of the restriction of Ca in pedicel, which traps Ca in pedicel. This process is known as "Ca bottle neck" (Huang et al., 2005). Studies on the causes of the restricted Ca translocation in mangosteen are limited (Song et al., 2014). In

addition to the physiological mechanisms of Ca^{2+} restrictions on the pedicel, the damage of xylem tissue in pedicel also causing Ca^{2+} restrictions to the fruits. The microscopic observations in the third experiment illustrate the damage xylem in pedicel, especially at the time of fruit entering the ripening stage. Xylem tissue on the fruit pedicel functions optimally only in the early stages of fruit growth, and the xylem tissue is damaged afterwards (Drazeta et al., 2004). From the observations of branch, pedicel and mangosteen rind at 30 DAA and 90 DAA, it follows that there is a difference in the existence of xylem in the pedicel at 30 DAA and 90 DAA (Figure 3). In young mangosteen fruit (30 DAA), xylem and phloem is visible in the branch, pedicel, and fruit pericarp. In the old mangosteen fruit (90 DAA), xylem is just observed at the stem while in the mangosteen fruit pedicel and pericarp are detected only in old phloem tissue, with no visible existence of xylem. Rigney and Wills (1981) stated that Ca of the cell walls increases with the growth and development of the fruit, but then declines directly at the beginning of fruit ripening phase. Faust (1989) and Poovarodom (2009) clarified that after rapid fruit growth stage, Ca is still translocated to the fruit tissue even in very small amounts, and then this process stops when fruit enters the ripening phase.

Ca^{2+} absorbed in root especially in root elongation zone, located between the root meristem and root differentiation zone. At anthesis and Stage I, plants also initiates new roots (Marschner, 1995). Hidayat (2002) explained that the mangosteen rapid root growth occurred before bud break due to an increased need of assimilates to perform high rate of cell division. Development of new roots is an important factor in the mechanism of Ca uptake and translocation from the root to the xylem since Ca^{2+} is mainly absorbed by young root tissue (Himelrick and McDuffie 1983, Marschner 1995). Ca^{2+} can easily pass through endodermis of young roots. Ca translocation is more limited in older roots with well-formed casparian strips; casparian strip will shield the cells and block Ca^{2+} translocation into the xylem.

The experiments in Lampung and Bogor showed that anthesis is the best time for Ca application. At this stage, fruits become the strong sink, xylem tissues function optimally, and young roots are already formed and functioning. Application of Ca can be repeated at 4 WAA. Twice application of Ca at anthesis and 4 WAA will increase availability and uptake of Ca into fruit. Ca^{2+} that has been absorbed by young roots since anthesis and 4 WAA will be translocated into the fruit tissues throughout the growth and fruit development stage (Figure 4). The results of this study have provided important information to increase the quality of mangosteen by reducing yellow latex incidence.

CONCLUSION

From this research it can be concluded that:

1. Ca^{2+} application at 4.5 kg $tree^{-1}$ $year^{-1}$ in Bogor at anthesis stage or 1 WAA was able to reduce the percentage of yellow sap contaminated fruit on the aryl to 33% and 30%, respectively. In Lampung, twice application of Ca at anthesis and 4 WAA reduced the percentage of yellow sap contaminated fruit on the aryl to 10%, compared to 50% in control.
2. The critical timing of fruit needs for Ca^{2+} is the first stage of fruit growth, which is a rapid fruit growth stage. This period occurs approximately during 1-4 weeks after anthesis.
3. Anthesis until 4 WAA is the best time to apply Ca to reduce the percentage of yellow sap contaminated fruit on the aryl and rind.
4. Ca application at the anthesis and at 4 WAA will increase the efficiency of Ca uptake and translocation and reduce the percentage of contaminated fruit better than that of single Ca application.
5. The existence of xylem in pedicel of young fruit and formation of young roots at anthesis is important for supporting Ca^{2+} uptake and translocation from the root to the mangosteen fruit at its fast growing stage.

REFERENCES

Dorly, Tjitrosemito S., Poerwanto R., Juliarni (2008). Secretory duct structure and phytochemistry compounds of yellow latex in mangosteen fruit. *HAYATI Journal of Biosciences* 15, 99–104.

Dorly (2009). *Study of yellow latex secretory structure and the effect of calcium on yellow latex contamination on mangosteen fruits (Garcinia mangostana L.).* [Dissertation]. Bogor Agricultural University (IPB). Bogor, Indonesia.

Drazeta L., Lang A., Hall A.J., Volz R.K., Jameson P.E. (2004). Causes and effect of changes in xylem fungtionality in apple fruit. *Annals of Botany* 93, 275–282

Faust M. (1989). *Physiology of temperate zone fruits trees*. John Wiley & Sons, Inc. Canada.

Hidayat R. (2002). *Studies on growth rhythm of mangosteen (Garcinia mangostana L.) plants and it's influencing factors*. [Dissertation]. Bogor Agricultural University (IPB). Bogor, Indonesia.

Himelrick D.G., McDuffie R.F. (1983). The calcium cycle: uptake and distribution in apple trees. *HortScience* 18, 147–150.

Huang X., Wang H.C., Li J., Yin J., Yuan W., Lu J., Huang H.B. (2005). An overview of calcium's role in lychee fruit cracking. In: Chomchalow N., Sukhvibul N. (Eds.) *Proceedings of the Second International Symposium on Lychee, Longan, Rambutan and other Sapindaceae Plants*; Chiang Mai, Thailand, 25-28 Agt 2003. Belgium: Acta Horticulturae 665, 231–240.

Marschner H. (1995). *Mineral nutrition of higher plants. 2nd edition*. Academic Press. London.

Martias (2012). *The role of environmental study (soil chemical, physical and weather) on contamination of yellow sap on mangosteen (Garcinia mangostana).* [Dissertation]. Bogor Agricultural University (IPB). Bogor, Indonesia.

Pessarakli M. (2002). *Handbook of Plant & Crop Physiology. Revised & Expanded*. Marcel Dekker,Inc. NewYork

Poovarodom S. (2009). *Growth and nutrient uptake into mangosteen (Garcinia mangostana L.) fruit*. The Proceedings of International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis.

Poovarodom S. (2010). Calcium and physiological disorders of mangosteen fruits. *Proceedings of the 16th Asian Agriculture Symposium and 1st International Symposium on Agricultural Technology*. 25-27 August, 2010, Bangkok, Thailand.

Rigney C.J., Wills R.B.H. (1981). Calcium movement, a regulating factor in the initiation of tomato fruit ripening. *HortScience* 16, 550–551.

Ropiah S. (2009). *Morphological and physiological development of mangosteen fruit (Garcinia mangostana L.) during growth and ripening*. [Thesis]. Bogor Agricultural University (IPB). Bogor, Indonesia.

Song W., Chen W., Kurniadinata O. F., Wang H., Huang X. (2014). Application of electron probe to the observation of in situ calcium distributionin fruit tissues. *Journal of Fruit Science* 31, 730–732.

Wilsdorf (2011). *Evaluating the seasonal changes in calcium concentration and distribution in apple fruit after application of different Calcium fertilisation strategies*. [Thesis]. Stellenbosch University.