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Cell Ultrastructure and Peel Nutrient Content of Neck Zone in Six Cultivars of *Musa* sp. Fruit During Ripening

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Abstract: A study was carried out to determine the cause of weak neck in *Musa* sp. by observing the cell structure and peel nutrient content. Two cultivars with weak neck problem were compared to four cultivars without weak neck problem. For this study, cultivars Rastali and Awak were identified as cultivars which have weak neck problem whereas cultivars Lemak Manis, Abu, Berangan and Tanduk were without weak neck problem. Fruits with the same level of maturation were obtained from the local market. Scanning Electron Microscope (SEM) was used to observe the cellular structures. Ten elements N, P, K, S, Ca, Mg, Fe, Mn, Zn and B were analyzed from the peel. Cell Width (CW), cell Wall Thickness (WT) and CW/WT ratio were determined. Cells of Rastali were found to collapse completely after ripening while cells of Awak became thinner, making these two cultivars prone to weak neck. The cells of the other four cultivars became elongated and cell wall became thicker after ripening, thus not showing weak neck symptom. Concentrations of all the 10 elements in the six *Musa* sp. cultivars were found to be above the critical levels. It was concluded that weak neck in cultivars Rastali and Awak was caused by disintegration, collapse and thinning out of cells along the neck region and not due to nutritional deprivation. Calcium deficiency was not the cause of weak neck in these *Musa* sp. cultivars.

Key words: *Musa* sp., ultrastructure, weak neck, SEM

INTRODUCTION

Banana is the fourth most important food crop in the world, after rice, wheat and maize. Many people in the tropical and sub-tropical regions consumed this fruit on a daily basis (FAO, 2008). However, there are many obstacles faced by the farmers which are interrelated with the development of banana as superior commodity, particularly the productivity and the post harvest handling of the fruit.

One of the problems faced by the farmers is the packaging and transportation of some banana cultivars to the market. During these long duration and distance, the fingers are easily separated from its hand. This problem is known as weak neck. Weak neck was thought to be controlled by complex factors, such as nutrients, hormones, enzymes and their interactions. According to Sairam *et al.* (2008) and Reddy and Raghavendra (2006), the abscission process of flower and leaf on the plant are caused by the combined activity of hormones and enzymes, such as abscisic acid (ABA), ethylene, catalase (CAT) and ascorbate peroxidase (APX). The hormonal and enzymatic activities in papaya during maturation

period caused changes in their chemical composition and this will change the strength of cell structure and tissue texture. This is because during the maturation period the quantity of galacturonic acid and non-glucose monosaccharide in the middle of the lamella decreased and therefore caused disassembly of the primary cell and middle lamella structures (Jackman and Stanley, 1995).

Musa sp. is in the group of climacteric fruits where cell structure rigidity and texture are lost quickly when maturation begins (Cheng *et al.*, 2008). The dissolving and depolymerization of pectin, hemicellulose and cellulose are primary causes of cell reduction or tissue rigidity (Brummell and Harpster, 2001; Lohani *et al.*, 2004). The strength of cell or tissue decreases because cell wall and middle lamella collapsed. Miller and Fry (2001) showed that the activity of hydrolase will increase the damages in fruit cell wall. In addition, the involvement of polygalacturonase (PG) and Pectin Methyl Esterase (PME) in cell wall damages were also reported (Verlent *et al.*, 2005; Nikolic and Mojovic, 2007). The reduction of cell strength was not only caused by the enzyme activity. Several results showed that non-enzymatic activity was involved in the destruction of

tissue (Schopfer *et al.*, 2001; Schweikert *et al.*, 2002; Dumville and Fry, 2003; Cheng *et al.*, 2008). There was a positive correlation between the maturation of fruit, cell wall damage and concentration of radical hydroxyl. When fruits matured, the concentration of radical hydroxyl will increase and caused a corresponding increase in cell wall damage (Schopfer *et al.*, 2001; Schweikert *et al.*, 2002; Cheng *et al.*, 2008).

The rigidity of tissue is determined by the cell structure and texture. Cell walls are constructed of celluloses and hemicelluloses which bind to form fibre bundles. Strong bonds between these fibres are created by the presence of pectin, lignin and suberin (Fengel and Wegner, 1984). Cell wall also has low level of protein and pectin content (Agarwal, 2006). Cellulose is the main component of cell wall in plant; therefore cellulose is the primary decisive factor in cell wall rigidity and mechanical strength (Appenzeller *et al.*, 2004; Ching *et al.*, 2006). Lignin is primarily a strengthening agent in the wall. It also resists fungal or pathogen attack (Salerno *et al.*, 2004). Beside lignin, the other organic materials in cells which are important for rigidity are suberin, wax and cutin. Suberin, wax and cutin are the varieties of lipids that are associated with the wall for waterproofing (Richard, 2008). Cell wall is strong if it has high lignin and suberin content.

The damage to cell wall at the neck zone occurs more quickly compared to other parts of the fruit. This was obvious in cultivars Rastali and Awak which is why these cultivars are mainly used for local consumption and not for export. Therefore, studies of cell wall structure at the neck zone are needed to understand the mechanism of weak neck problem in cultivars Rastali and Awak particularly, to make them favourable for export purposes. Currently there is no report on the cellular basis of the weak neck problem. Preliminary studies were carried out to describe the cell ultrastructures of the weak neck zone and to relate nutrient content to the cell wall rigidity at the weak neck zone. The objectives of this study were : (1) to explain the alterations of cells and tissue structure in the neck zone before and during maturation of six cultivars of *Musa* sp. fruits, (2) to compare the cell structure in neck zone among these cultivars and (3) to determine the nutrient composition of the weak neck zone of the fruit.

MATERIALS AND METHODS

The research was done in Plant Breeding and Genetics Laboratory, Plant Microstructure and Anatomy Laboratory and Plant Physiology Laboratory, Crop Science Department, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia on September-December 2008. Six cultivars of *Musa* sp., i.e.,

Rastali, Berangan, Abu, Awak, Tanduk and Lemak Manis were used in the evaluation. Fruits with uniform maturation level were obtained from the market. Two fruit hands were used for each cultivar. All of the samples were from the first hand of the bunch. Fruits from the upper row were collected before ripening and those from the lower row were taken one day after ripening in order to compare the cell structures before and after ripening.

To observe the cell morphology, Scanning Electron Microscope (SEM) was used. Tissue samples from the abscission layer of the neck zone were collected before ripening and one day after ripening. The samples were fixed in 70% formaldehyde-acetic acid alcohol (FAA) and dehydrated using CO₂ in Critical Point Dryer (CPD). Fixed and hydrated samples were mounted on aluminium stubs, coated with gold for 2 min, twice and finally viewed under the SEM. From the SEM micrographs Cell Width (CW), Cell Wall thickness (WT) and CW/WT ratio were gathered. CW/WT ratio described the relative cell wall thickness for each cultivar. The higher CW/WT ratio indicated thinner cell wall relative to cell width. On the other hand, lower CW/WT ratio indicated thicker cell wall relative to cell width. For comparison of the CW, WT and CW/WT means, the Student's t-test was employed.

The nutrient level in the fruit peel was also determined. Peel from three fruit samples were obtained from each cultivar in order to analyze the levels of N, P, K, S, Ca, Mg, Fe, Mn, Zn and B. The peel sample was then oven dried, granulated and weighed to achieve 0.25 g per sample. After that, the sample was placed in a digestion flask where 5 mL of H₂SO₄ was then added then heated on a hot plate at 450°C for 7 min. Then, 10 mL of H₂O₂ (50%) was added into the digestion set with a small funnel. The digestion flask was removed from the hot plate using a glove when cool (i.e., ± 4 min) and a clear concentrated solution produced. Next, the solution made up to 100 mL with deionized water (Hermans *et al.*, 2004). The solution was then analyzed using the Atomic Absorption Spectrometry (AAS) Perkin Elmer Model 3110 to determine the content of K, Ca, Mg, Fe, Mn and Zn in the samples. Nitrogen and phosphorus was analyzed using the Auto Analyzer (AA) Perkin Elmer Model 403. To measure B concentration in tissues, the azomethine-H⁺ method was used and the absorbance was read using spectrophotometry at 410 nm (Wolf, 1974). The nutrient level data were analyzed using Analysis of Variance (ANOVA) at 5%, if significant to be continued with Least Significant Differences (LSD) to know the differences among cultivars. All the analysis was performed using the General Linear Model Procedure (PROC GLM) (SAS Institute, 1990).

RESULTS

Micrographs from SEM showing abscission layer of six *Musa* sp. cultivars were presented in Fig. 1A-L. Cell structures before and after ripening were compared. It was known that among the *Musa* sp. cultivars, Rastali has the weakest neck zone. Cells at the abscission layer of Rastali before ripening (Fig. 1A) consisted of elongated parenchyma cells, including metaxylems (shown by arrow). One day after ripening, the fingers detached from the hand and the whole regions of cells disintegrated and collapsed (Fig. 1B). Figures C and D showed the cells in the neck zone of Awak cultivar one day after ripening. Cells remained firm before and after ripening. This was also observed with the rest of the cultivars. Parenchyma cells of cultivars Lemak Manis (Fig. E, F) and Abu

(Fig. 1G, H) became very thick after ripening. Cells were found to be elongated and polygonal in shape.

Table 1 showed Cell Width (CW) and cell Wall Thickness (WT) of six *Musa* sp. cultivars before and after ripening. Weak neck problem occurred in cultivars Rastali and Awak. Fingers of Rastali dropped one day after ripening. As for Awak, fingers dropped three days after ripening. Since, the cells of cultivar Rastali collapsed after ripening, data for CW and WT were not available. Comparison of cell wall thickness for Rastali to other cultivars was not available as the wall completely collapsed.

The CW of Lemak Manis increased from 8.13 ± 1.70 to 45.97 ± 1.73 μm , while the WT increased from 0.58 ± 0.21 to 6.23 ± 2.79 μm (Table 1). The CW of cultivars Abu,

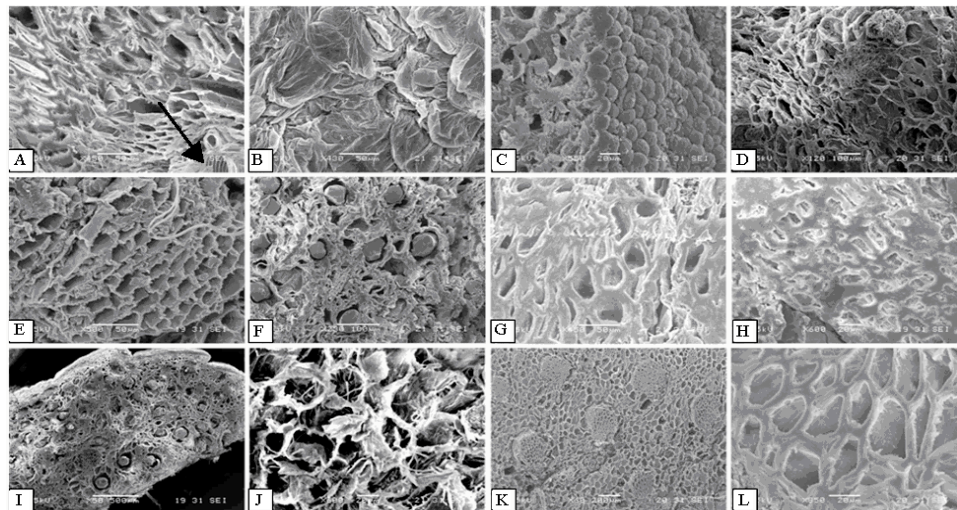


Fig. 1: SEM of abscission layer in banana neck zone; (A-B) Cell structures of abscission layer in *Musa* sp. cv. Rastali before and after abscission, respectively; (C-D) Cell structures of abscission layer in *Musa* sp. cv. Awak before and after abscission, respectively; (E-F) Cell structures of abscission layer in *Musa* sp. cv. Lemak Manis before and after abscission, respectively; (G-H) Cell structures of abscission layer in *Musa* sp. cv. Abu before and after abscission, respectively; (I-J) Cell structures of abscission layer in *Musa* sp. cv. Berangan before and after abscission, respectively; (K-L) Cell structures of abscission layer in *Musa* sp. cv. Tanduk before and after abscission, respectively. Bars: A, B, E and G, 50 μm ; C, H, J and L, 20 μm ; D and F, 100 μm ; I, 500 μm ; K, 200 μm

Table 1: Cell width and cell wall thickness of cells in the abscission layer of fruits of six *Musa* sp. cultivars

Characteristics	<i>Musa</i> sp. cv. Rastali	<i>Musa</i> sp. cv. Awak	<i>Musa</i> sp. cv. Lemak Manis	<i>Musa</i> sp. cv. Abu	<i>Musa</i> sp. cv. Berangan	<i>Musa</i> sp. cv. Tanduk
Cell Width (CW)(μm)						
Before	30.40 ± 9.91	19.53 ± 7.50	8.13 ± 1.71	42.67 ± 7.90	140.48 ± 51.68	64.00 ± 13.50
After	cells collapse	72.86 ± 25.60	45.97 ± 1.73	15.10 ± 5.28	26.87 ± 8.30	23.80 ± 5.94
t-test	-	*	*	*	*	*
Cell Wall Thickness (WT) (μm)						
Before	3.78 ± 2.37	5.46 ± 0.13	0.58 ± 0.21	9.07 ± 2.42	13.10 ± 4.70	8.00 ± 2.58
After	cells collapse	10.36 ± 4.60	6.23 ± 2.79	4.45 ± 1.32	3.93 ± 0.91	4.10 ± 1.02
t-test	-	*	*	*	*	*
CW/WT ratio						
Before	8.04	3.50	14.02	4.70	10.72	8.00
After	cells collapse	7.03	7.38	3.40	6.84	5.81
t-test	-	*	*	*	*	*

N = 10, $\pm$ SE, *Significant differences at $p \leq 0.05$ using Student's t-test

Table 2: Nutrient content in the fruit peel tissue of six *Musa* sp. cultivars

Elements (%)	<i>Musa</i> sp. cv. Rastali	<i>Musa</i> sp. cv. Awak	<i>Musa</i> sp. cv. Lemak Manis	<i>Musa</i> sp. cv. Abu	<i>Musa</i> sp. cv. Berangan	<i>Musa</i> sp. cv. Tanduk	Significance	Critical concentration (deficiency)
N	2.75	2.74	2.70	2.79	2.83	2.84	ns	2.60
P	0.53	0.48	0.51	0.47	0.46	0.45	ns	0.20
K	5.03	4.96	4.37	4.47	4.65	4.51	ns	3.00
Ca	0.62	0.59	0.58	0.61	0.56	0.56	ns	0.50
Mg	0.35	0.39	0.42	0.40	0.36	0.44	ns	0.30
S	0.30	0.28	0.33	0.31	0.32	0.27	ns	0.23
Fe (ppm)	77.42	88.01	84.65	85.78	86.54	88.12	ns	80.00
Zn (ppm)	19.05	18.97	18.42	18.45	18.27	18.21	ns	18.00
Mn (ppm)	25.45	26.03	26.19	25.62	26.07	25.21	ns	25.00
B (ppm)	11.31	11.03	11.51	12.11	11.05	11.27	ns	11.00

Not significant (ns) differences according to LSD test ($p = 0.05$). The critical nutrient concentration of banana from Memon *et al.* (2001)

Berangan and Tanduk decreased from $42.67 \pm 7.90 \mu\text{m}$ to 15.10 ± 5.28 , 140.48 ± 51.68 to $26.87 \pm 8.30 \mu\text{m}$ and 64.00 ± 13.50 to $23.80 \pm 5.94 \mu\text{m}$, respectively, whereas the WT decreased from 9.07 ± 2.42 to 4.45 ± 1.32 , 13.10 ± 4.70 to $3.93 \pm 0.91 \mu\text{m}$ and 8.00 ± 2.58 to $4.10 \pm 1.02 \mu\text{m}$, respectively. The CW/WT ratio of cultivars Lemak Manis, Abu, Berangan and Tanduk decreased from 14.02 to 7.38, 4.70 to 3.40, 10.72 to 6.84 and 8.00 to 5.81, respectively. The CW/WT ratio was lower for cultivars Lemak Manis, Abu, Berangan and Tanduk, indicating that their cell walls were thickened when ripe. The CW/WT ratio increased in cultivar Awak, indicating that the cell wall was relatively thinner. The CW of cultivar Awak increased from 19.53 ± 7.50 to $72.86 \pm 25.60 \mu\text{m}$, even as the WT increased from 5.46 ± 0.13 to $10.36 \pm 4.60 \mu\text{m}$. However, the CW/WT ratio increased from 3.50 to 7.03 indicating that the cell wall became thinner.

Table 2 showed the nutrient content in the fruit peel tissue. The analysis showed that content of all the 10 nutrients were higher than the critical concentration (Memon *et al.*, 2001) in all the six cultivars evaluated. This indicated that the cultivars used as samples in this study had sufficient amount of nutrients and therefore no nutrient deficiency has occurred.

DISCUSSION

As previously reported, cultivar Rastali has the weakest neck zone when compared to other cultivars. This is because after one day of the ripening stage, fingers dropped and the cell along the abscission region had completely collapsed, leading to detachment of fingers from the hands. In cultivar Awak, although, the WT decreased (CW/WT ratio increased), the separation of fingers from the hand happened later (at three days after ripening) than Rastali because of the rigidity of the cell wall that apparently prevented the cell from collapsing easily. Although, the CW and WT increased during abscission for Awak, weak neck occurred because the cell walls were thinning as shown by the increased CW/WT ratio from 3.50 to 7.03. Increased in CW/WT ratio

indicates that the wall became thinner. The decreased CW/WT ratio for other cultivars during ripening stage indicated that the thickened cell wall was the main reason for the absence of weak neck problem on them (other cultivars apart from Rastali and Awak). Jiang *et al.* (2000) and Ahmad *et al.* (2001) said that hydrolysis in cell wall due to the thinner of cell wall. It causes the tissues become soft. If this problem prevails in abscission layer, thus the finger of banana fruit will experienced to weak neck. However, abscission layer does exist i.e. on the region where the cells will lose the rigidity and break when the fruits are pulled from its hand.

Although, the CW of cultivar Awak increased, it does not always mean that the cell will be rigid and strong. This is because when CW/WT increased the cell wall becomes thin. Therefore, when the cell became wider and the WT became relatively thinner, the cell at the abscission region became weak and easier to collapse when compared to cultivars Lemak Manis, Abu, Berangan and Tanduk. In these four cultivars, CW, WT and CW/WT ratio decreased indicating that the cell wall was relatively thicker. Decrease in WT indicated that the cells became elongated as the fruit ripens. Elongated cells with thicker cell wall resulted in cells that are more rigid and are able to withstand abscission. The tissues that were arranged by thicker cell wall were rigid and strong, thus they were not experienced to breakdown (Imsabai *et al.*, 2006; Saengpook *et al.*, 2007). The cells in the abscission layers will be broken when there is a force such as pulling or collapsing of cell through slicing.

In Table 2, calcium content for all six cultivars was higher than the critical level. When compared among cultivars, cultivars Berangan and Tanduk had the lowest calcium content while Rastali had the highest. However, Rastali has the weakest neck region while cultivars Abu and Tanduk had the strongest (base to the CW/WT ratio data before and one day after ripening in Table 1). This suggests that calcium do not determine the cell rigidity of the neck zone. In fact, the content of the 10 elements was more than the critical concentration. This condition

contradictive with the result from Matoh and Kobayashi (1998) that shown calcium and boron were two essential elements which determined the rigidity of cells.

CONCLUSION

The cells and cell structures of cultivar Rastali fruits completely collapsed after ripening. Awak cultivar, however, was able to maintain cell rigidity much longer than Rastali cultivar but collapsed three days after ripening. The cell wall became wider but thinner causing the cell along the neck zone to be weaker and leading to finger detachment from the hand. Cells became elongated, polygonal in shape but WT increased for cultivars Lemak Manis, Abu, Berangan and Tanduk apparently weak neck was absent for these cultivars. From the peel nutrient content analysis, the 10 elements were more than the critical concentration, indicating that deficiency did not occur. It can be concluded that weak neck was apparently caused by thinning of the cell wall at the weak neck zone and not because of the calcium deficiency. Since calcium was found not to be the main cause of cell wall weakness, further studies were proposed to investigate the effect of pectin, lignin and suberin content on the weak neck problem using Transmission Electron Microscopy (TEM).

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