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Study on changes in ultrastructure of Banana cv. Grand Naine during ripening

P Mutharasu¹✉, M Kavino², I Muthuvel², KS Subramanian³ & K Venkatesan⁴

^{1, 2, 3}Department of Fruit Crops, HC&RI, Tamil Nadu Agricultural University-Coimbatore-03

²Department of Fruit Crops, HC&RI, Periyakulam-625 604

⁴ Department of Floriculture and Landscaping, HC&RI, Periyakulam-625 604

✉ Corresponding author: P Mutharasu, E-mail: mutharasupandian001@gmail.com

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Abstract

The periodical changes occurred at tissue level in fruit pulp and peel of banana cv. Grand Naine belongs to AAA genome was studied during storage period. In banana, structural modification in cell walls became apparent at advanced stages of ripening and showed predominantly dissolution of the middle lamella and gradual degradation of starch granules during ripening in fruit pulp and peel. In this study, four physiological stages of ripening of banana fruit peel and pulp were examined periodically under scanning electron microscope, elucidated degradation of starch granules at the time of ripening.

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Introduction

Banana is one of the most favorite fruit for all classes of people, because of its availability throughout the year, wider varietal range, high nutritive value, taste, easy affordability and also good export potential. India has an array of banana cultivars grown throughout the country depending upon preference, resource availability, production system and consumer preference. Being a climacteric fruit banana has usually harvested

at mature unripe stage and subsequently allowed to ripen. Harvested banana passes through four post-harvest physiological developmental stages such as pre-climacteric or 'green life', climacteric stage or ripe stage, eat-ripe and senescence or over-ripe stage (Mebratie et al. 2014; Saúco & Robinson, 2010). Banana, like other climacteric fruits, has a distinct ripening pattern with amplified ethylene biosynthesis and respiration rate during storage (Lelièvre et al. 1997). In

banana, post-harvest changes are important as they determine the fruit ripeness and storage life (Sakyi-Dawson et al. 2008). During ripening, changes that occur in physical, physiological and bio-chemical properties of fruits include alterations in pigment biosynthesis and accumulation, modification of cell wall texture and ultra-structure, breakdown of starch molecules, increased levels of flavour and aromatic volatiles and greater susceptibility to post-harvest pathogens (Saúco et al. 2010).

Rapid degradation of starch molecules occurred during ripening. On an average, around 25% starch molecules drops at pre-climacteric phase to less than 1% during climacteric phase. Subsequently, sucrose content increases 12 folds and leads the increase of hexoses (Areas et al. 1981). During the climacteric period, the phosphatase activity increased, while the incidence of increased phosphorylase activity take place before starch transformation began, thereafter decreased in a complex pattern (Areas et al. 1981). The starch was transformed into sucrose with later formation of glucose and fructose (Garcia & Iajolo, 1988). Starch conversion is one of the most conspicuous changes that characterized during ripening of climacteric fruits (Agravante et al. 1989). Hence, the study had been made to elucidate the ultra-structural changes occurred at tissue level in banana cv. Grand Naine using scanning electron microscope (SEM).

Materials and Methods

The ultra-structural changes of banana fruit tissue especially peel and pulp was

observed. This study was done at Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore. The fruit samples were collected from farmer's field at (9.31°N 77.20°E) Cumbum valley, Tamil Nadu and stored under ambient condition (Temperature: $27\pm 3^{\circ}\text{C}$, RH: $60\pm 5\%$) which periodically drawn at three days interval till the fruit ripening. The fruit pulp and peel was cut into 2-3 mm long segments with the help of a surgical blade. After lyophilization, the segments were observed under SEM. The SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimen. The signals that are generated from electron sample interactions revealed information about the sample including external morphology (texture), chemical composition and crystalline structure and orientation of materials making up the sample. Areas ranging from approximately 1cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20 X to approximately 30,000 X, spatial resolution of 50 to 100 nm). The surface morphology of pulp and skin were focused using scanning electron microscope (SEM-FEI-Quanta 250, Oregon, USA) at 500 X magnification. The fruit pulp and skin cut into small pieces and placed on aluminum stub. The stub was kept in sample holder of SEM and scanned for imaging.

Results and Discussion

Structural changes in starch granules during banana fruit ripening

SEM view showed compactly arranged cell in peel and pulp segment of unripe banana. The sample of ripe fruits showed prominent intercellular space and loss of starch granules. The illustrations of banana peel and pulp obtained through SEM revealed prominence of compactly arranged tissue and oval or round shaped starch granules immediately after harvest (Fig 1A). During process of ripening, rapid degradation of starch molecules and accumulation of soluble sugars takes place simultaneously (Purgatto et al. 2001). In our experiment, this process started around the 12th day of storage, maximum degradation of starch granules were observed then (Fig. 2). This was supported by the results obtained in Mango fruits ripening (Preethi et al. 2014). The amount of starch remains almost constant (around 20%) up to about 14 days, followed by a rapid degradation concomitant with accumulation of soluble sugars (Peroni-Okita et al. 2010).

Fig.1 SEM view of banana cv. Grand Naine pulp at (A) 0th day, (B) 3rd day, (C) 9th day and (D) 12th day

Fig. 2 SEM view of over ripe banana cv. Grand Naine fruit tissue

Amylase is the enzyme participates in starch hydrolysis but it is not linked to sucrose synthesis. In all stages of fruit ripening, several enzymes get activated mainly, α - and four β -amylase and α -1, 4- and α -1, 6-glucosidase. These enzyme activities were increased significantly during climacteric peak. Only β -amylase activity increased before onset of the respiration peak and parallel to starch decrease (Cordenunsi & Lajolo, 1995). ADP glucose pyrophosphorylases, starch branching enzymes, α - and β -amylases genes that were highly or specifically expressed in banana fruits for starch metabolism regulation (Jourda et al. 2016).

Structural changes in cell wall during banana fruit ripening

The arrangement of cell was compact in unripe fruits with few scales and cracks were also observed in banana peel immediately after harvest (Fig.3A) and it was

collapsed, as the fruits ripen (Fig 3D).

Fig. 3 SEM view of banana cv. Grand Naine peel at (A) 0th day, (B) 3rd day, (C) 9th day and (D) 12th day

Plant cell wall consists of mainly three types of polysaccharides, namely, cellulose, hemicellulose and pectic substances. These polysaccharides are made up by the polymers of sugar linked by glycosidic bond. In plant cell, various polysaccharides chains are integrated to form a network. During fruit ripening, cell wall polysaccharides are extensively modified by a variety of ripening-related enzymes synthesized from the symplast into the cell wall space mainly modifications occurs to the polysaccharide components of middle lamella and cell wall lead to weakening of the structure (Toivonen & Brummell, 2008). Subsequently, alteration of bonding between the polymers and degradation of polysaccharides, which combined with alteration in turgor resulting cell separation and softening of fruits. The modification of fruit cell wall is greatly

achieved by the activity of hydrolytic or cell wall degrading enzymes such as Polygalacturonase, Pectinmethylesterase, Pectate Lyase, Rhamnogalacturonase. Before ripening in cell wall, loss of pecticgalactan side chains and the depolymerisation of matrix glycans event occur followed by loss of pectin arabinoside chains and pectin solubilization changes occur. At the time of ripening starts, depolymerisation of pectin and cell wall swelling increased. These changes affect the structure and strength of the wall and ultimately bring about fruit softening. Both pectins and matrix glycans are degraded, although the nature of changes occurring is species-specific and even cultivar-specific (Brummell, 2006). Pectin methylesterase is one of the cell wall degrading enzymes, which acts on pectin, (a primary cell wall constituent) and releases methanol and hydrogen ions (Micheli, 2001).

Conclusion

The ultra-morphological changes occurred during ripening of banana cv. Grand Naine (AAA) were prominent at peel and pulp tissue level. The illustrations under scanning electron microscope visualize the sequential process of gradual degradation of starch molecules and increased in intercellular space while ripening.

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