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The changes in physical, bio-chemical, physiological characteristics and enzyme activities of mango cv. Jinhwang during fruit growth and development

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ABSTRACT

The changes in the physical, bio-chemical and physiological characteristics, and enzyme activities of sucrose metabolism during growth and development in mango fruit cv. Jinhwang were investigated. Fruit was harvested at five stages i.e., 50, 80, 110 and 140 days after anthesis (DAA). Several changes in the fruits were analyzed. The physical parameters like the fruit weight, width and length increased throughout the growth. In turn, fruit firmness, titratable acidity (TA) and starch accumulation increased during the initial growth stage and later then decreased during maturity. Total soluble solids (TSS) tended to decrease throughout fruit development. Respiration rate and ethylene production were higher at 50 DAA compared to other growth stages. Sucrose accumulation occurred later in the fruit development, however fructose was the dominant of the soluble sugars. Starch accumulation was related to the reduction of sucrose phosphate synthase (SPS), acid invertase (AI) and neutral invertase (NI) activities, whereas sucrose synthase activity was increased. Moreover, the AI and NI are the dominate enzymes that plays a major role in sugar accumulation and quality of mango fruit. Therefore, mango fruit should be harvested after physiological maturity at 110 DAA, when the fruit reach the optimum size, weight and starch content including maximum value of firmness, TSS, fructose content, minimum value of TA, low respiration rate and the lowest of ethylene production.

1. Introduction

Mango (*Mangifera indica* L.) cv. Jinhwang, is a commercial variety in Taiwan which has a high value as export [1]. This cultivar is an average weight of 1,200 grams, is elongated and oval shape, small stone. In addition, this cultivar has highly resistant to diseases and insects, and also tolerates adverse weather conditions. All of these advantages value to the plant improvement [2]. It is necessary to measure the physical, bio-chemical and physiological variables that have been examined to define the optimal stage of maturity for harvest [3]. The measurement of maturity is important to harvest fruit to have good postharvest quality. There have been numerous studies investigating the physical, bio-chemical and physiological changes of several fruits during growth and development. Several mango fruit is harvested before the onset of the climacteric but, when physiologically mature stage at 105-112 days after fruit set, to get optimum fruit quality, whereas immature fruit do not ripen normally [4]. The suitable maturity for harvesting Jinhwang cultivar at 120 to 130 days after anthesis (DAA) [5]. TSS increases slightly at the mature-green stage. The firmness of the fruit remains almost constant throughout the growth period and fruit become less firm after maturity [4]. Starch accumulation is a major chemical change in mango fruit during growth and maturation [6].

Total sugars (sucrose, fructose, glucose) are one of the bio-chemical components of fruit quality [7] which is related to sink strength and is important in fruit development [8]. Increase in total sugar content during fruit maturity is dependent sucrose

Abbreviations: DAA, days after anthesis; TA, titratable acidity; TSS, total soluble solids; SPS, sucrose phosphate synthase; SS, sucrose synthase; AI, acid invertase; NI, neutral invertase.

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accumulation [9]. Several enzymes are involved in sucrose metabolism [10]; Sucrose phosphate synthase (SPS; EC 2.4.1.14) is a soluble enzyme located in the cytoplasm and plays a major role in control of sucrose biosynthesis because the hydrolysis of sucrose phosphate by an accompanying specific phosphatase renders the synthetic irreversible reaction in favour of sucrose accumulation [11]. Sucrose synthase (SS; EC 2.4.1.13) and invertases (AI, NI; EC 3.2.1.26) are sucrose-cleaving enzymes important for determining sink strength [10]. The major role of SS during the active growth phase is cleaved the sucrose moiety and also linked to cell wall biosynthesis by providing UDP-glucose for the cellulose synthase complex and substrates for starch synthesis during fruit growth and development. Fruit invertases are involved in the degradation of sucrose which catalyze the irreversible hydrolysis of sucrose to glucose and fructose [12]. Acid invertase (NI; EC 3.2.1.26) is compartmentalized in the vacuole, the site of sucrose accumulation, and neutral invertase (NI; EC 3.2.1.26) is located in the cytosol, the site of sucrose synthesis [13].

However, those studies rarely particularized the optimum stage of maturity for harvest. Moreover, authors have investigated sucrose metabolism in climacteric fruits during ripening such as banana [13] and mango [14] but studies of sucrose metabolism during growth and development of climacteric fruit such as mango has been rarely explored. Therefore, the purpose of this study was to investigate the changes in physical, bio-chemical and physiological at different stages of fruit growth and development. The study of the enzyme activities in sucrose metabolism of mango cv. Jinhwang during fruit growth and development stages are also included. These types of studies will help breeders to develop new mango breeding programs and determine the optimum harvest date for consumption and export.

2. Materials and Methods

2.1. Plant material

Mango fruit cv. Jinhwang (*M. indica* L.) harvested at four development stages [50,80, 110 and 140 days after anthesis (DAA)] during young fruit, intermediate and maturity stages from an orchard in Pintung, Taiwan ROC. Fruit used to determine physiological changes dipped in 1,000 μl l^{-1} 2-(4-thiazolyl) benzimidazole for 15 min to control a fungus (*Colletotrichum gloeosporioides* (Penz.) Sacc.).

2.2. Physical changes

Fresh weight measured using a precision balance model Mettler Toledo Classic light PL1501-S and was expressed in grams (g).

Fruit length and width were measured using a Vernier caliper model Mitutuyo 200 mm and were expressed in centimetres (cm).

Fruit firmness measured using a Shimadzu EZ test and a 0.5 mm-diameter plunger set to pierce 1 cm depth. Readings were taken in three positions of fruit area and averaged was recorded in kilogram force (Kgf).

2.3. Bio-chemical changes

Total soluble solids (TSS) measured by direct readings of mango juice using a digital hand refractometer (Atago Pocket refractometer PAL-1) with results expressed in °Brix. Measurement was taken in three positions of fruit area.

Titratable acidity (TA) and pH were measured from ten grams of mango pulp was homogenized with 100 ml of deionized water at speed 2 for 30 s with an homogenizer (model Heidolph DIAX 900). The homogenate was filtered using a Whatman No. 1 filter paper. 25 ml of extract was drawn from the filtrate in a titratable acid cup using a pipette. TA and pH were measured using a Titrator Mettler Toledo model DL53 and TA was expressed as a percentage (%) of citric acid following the procedure of Chen [15].

Soluble sugars (sucrose, fructose, glucose) were measured from one gram of mesocarp tissues with 5 ml of deionized water using a 1:5 tissue-to-deionized water ratio was ground in a chilled mortar and pestle. The homogenates were centrifuged at 12,000 x g for 10 min in a cooling centrifuge at 4 °C by using a high-speed micro centrifuge model Hitachi Himac CF 15RX. The solution were filtered manually through PVDF microfiltration membrane (hydrophilic PVDF, 0.22 μm pore size) model Millipore Millex-GV by using syringes. A 10 fold dilution of the soluble sugar solution (0.1 ml the soluble sugar solution with 0.9 ml of deionized water) was prepared. Thereafter, this solution (0.1 ml) was injected using a smaller syringe through a rubber septum in the Sugar Analyzer model DKK-TOA SU-300 Version 1.4 following the procedure as modified from Wang [16]. The total soluble sugar contents were expressed as mg g^{-1} fresh weight (mg g^{-1} fw).

Starch content was measured from about 0.05 g of dried mango sample which was dried by using a Freeze Dryer FD-series was added to 5 ml of deionized water into the test tube and shaken at 120 rpm, 30 °C for 3 h by using reciprocating shaker bath. Then the solution was centrifuged at 12,000 x g for 10 min (25 °C) and the residue retained. The residue was dried in precision ovens at 70 °C for 12 h. Then, 1 ml of deionized water was added to a centrifuge tube and the contents were boiled in hot water bath for 15 min. After cooling, 1 ml of 9.2 N $HClO_4$ (perchloric acid) was added to a centrifuge tube and was shaken at 150 rpm, for 15 min. After that the solution was centrifuged at 5000 x g, 25 °C for 10 min (25 °C). Afterwards, 20 μl of supernatants was added, plus 0.5 ml of 5% phenol and 2.5 ml of H_2SO_4 to each test tube and left for 30 min. The solution was measured at a wavelength 490 nm following the procedure as modified from Dubois et al. [17] and Sadasivam and Manickam [18] with glucose as the standard. Starch was expressed as percent dry weight (%DW).

2.4. Physiological changes

Respiration rate and ethylene production were measured from three fruit at each development stage. Fruit were weighed and individually placed in a 5.3 L plastic container for the duration of the trial and sealed with a plastic lid, and connected with flow boards by using the colorimetric method [19] at 20 °C. Then a 1 ml gas sample was withdrawn from the container by inserting the syringe through the rubber septum. The gas sample was then analysed using a gas chromatograph (GC, model Shimazu GC 8A) containing N_2 carrier gas, a Silica Gel (80/100) column and a thermal conductivity detector (TCD) with the operation of 80 °C column temperature, 100 °C injection temperature for determined respiration rate and expressed in mg CO_2 kg^{-1} h^{-1}. Ethylene production was determined using a GC containing N_2 carrier gas, a Propack T (80/100) column, and a flame ionization detector (FID) with the operation of 60 °C column temperature, 100 °C injection temperature and the results were expressed in μl C_2H_4 kg^{-1} h^{-1}. The respiration and ethylene production were measured once diary until fruit ripening and senescence.

2.5. Enzyme activity

2.5.1. Enzyme extraction

Mango mesocarp tissues were sliced into mid-section, and approximately 0.2-0.5 cm wide segment was frozen immediately in liquid N_2. The samples were maintained at -80 °C until they were used. The procedure of Castrillo et al. [14] was followed for enzyme extraction.