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Metabolic profiling of sapota fruit cv. Cricket ball grown under foliar nutrition, irrigation and water deficit stress

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ABSTRACT

The study was aimed at assessing the fruit quality of sapota (*Manilkara achras* L.,) grown under foliar nutrition, irrigation and water deficit stress. Metabolomics approach was employed to distinguish fruit quality characteristics and the data was subjected to Principal Component Analysis. Results showed that the metabolomics approach is a valuable tool in distinguishing fruit quality characteristics of sapota grown under different water and nutrient regimes and Principal Component Analysis was effective in identification of key factors determining fruit quality. Foliar application of mineral nutrients in the preharvest phase of fruit development was more effective, efficient and economical compared to conventional soil application of nutrients and irrigation for producing sapota fruits of superior quality. Fruits provided with foliar nutrition also showed increased levels of essential mineral nutrients and health promoting phytochemicals compared to fruits from control and irrigated trees. The study confirmed that foliar nutrition requiring minimum amount of nutrients, water and labor is an effective strategy which could be applied routinely by orchardists to improve fruit quality of sapota grown under water-limiting conditions.

1. Introduction

Sapota (*Manilkara achras* L.,) is a tropical tree bearing sweet delicious fruits rich in essential nutrients such as free sugars (15–20%), fat, protein, vitamins and minerals apart from many health benefitting phyto-nutrients (Gopalan et al., 1977). Among the commercial varieties of sapota cultivated in India, cv. "Cricket ball" is one of the most important in terms of area under the crop and production. Due to its high yield potential under rain-fed conditions, the cultivated area under "Cricket ball" has seen a rapid increase in many states of India where facilities for irrigation do not exist (Indian Horticulture Database, 2014). However, recent research has shown that drought stress during summer months induces a physiological disorder in cv."Cricket ball" known as Corky tissue adversely affecting fruit quality (Shivashankar et al., 2014). In view of this, it has become imperative to study the influence of pre-harvest practices on postharvest fruit quality in order to develop strategies to ensure superior fruit quality and enhanced profits to growers.

Past studies on other fruits have established that water stress affects the synthesis of several key metabolites linked to quality parameters in fruits (Gomez-Rico et al., 2007). Fallahi et al. (2010) reported enhancement in the composition and quality of apple fruits grown under irrigation. Studies by Sawan et al. (2001) highlighted the importance of application of essential mineral nutrients to soil for enhancing yield and quality of cotton. In recent times, foliar application of mineral nutrients is preferred to the conventional soil application due to the several benefits the method offers (Saadati et al., 2013) such as, low application rates, uniform distribution and quick plant responses to applied nutrients (Mengel, 2002). Foliar application is also found to be more efficient than soil application especially when soil conditions are not suitable for availability of certain minerals (Borowski and Michalek, 2011). Hence, in the present study, we have attempted to evaluate the quality attributes of sapota fruits raised under different growth environments.

Since plant metabolites represent the final 81 products formed at different regulation levels, metabolic profiling is being increasingly applied to assess fruit quality characteristics (Fernie and Stitt, 2012). Keeping in view the efficacy and versatility of the metabolic profiling approach, the present work was taken up with the aim of comparing the quality parameters of sapota fruits grown under water deficit stress (Control), irrigation (T1) and foliar application of nutrients (T2). Owing to the large number of metabolites involved in the study, Principal Component Analysis (PCA) was applied so as to reduce complexity of multivariate data and simplify the process of identification of key factors determining fruit quality.

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2. Materials and methods

2.1. Plant materials

Studies were conducted during the fruiting season of 2013–2014 on 20-year-old Sapota trees of Cv. Cricket ball planted at a spacing of 5m × 5 m and maintained under standard management conditions in the experimental orchards of the institute's research farm. Weather at the experimental site was characterized by a mild tropical climate throughout the year and a mean annual rainfall of 800 mm from July–November. The soil type in the experimental orchard site was a red loam with a pH of 6.7. NPK fertilizers were supplied at the rate of 1000:500:500 g per tree in two split doses during June and October. One set of experimental material consisting of 10 trees were maintained under rain-fed conditions (Control) while a second set of 10 trees were irrigated (T1). Irrigation water was applied to tree basins using a flow meter connected to irrigation pipe @ 200l/tree/week to achieve irrigation water (IW) to cumulative pan evaporation (CPE) ratio of 1.0. A third set of 10 rain-fed trees were given foliar nutrient spray (T2). Foliar nutrient application was initiated at 50% fruit maturity and repeated four times at 10-day intervals. Mineral nutrient medium containing 100 mg l^{-1} each of macronutrients, N, P K, Ca, and Mg, 10 mg l^{-1} each of the micronutrients, B, Cu, Zn, Fe and Mn with 5 mg l^{-1} of EDTA and 0.3 ml l^{-1} of All Purpose Spray Adjuvant (APSA) concentrate was used to spray the tree canopy, including the developing fruits, with a manually operated Knapsack sprayer. Weather at the experimental site was characterized by a mild tropical climate throughout the year and a mean annual rainfall of 800 mm from July-November. The soil type in the experimental orchard site was red loam with a pH of 6.7.

2.2. Determination of leaf water status

Plant water status was measured by determination of relative water content (RWC) of leaves. 10 leaf discs (5 mm dia) were collected per plant and floated on distilled water for 24 h at room temperature. The discs were removed from water, wiped gently with filter paper, weighed and dried in the oven for 48 h at 80 °C. The RWC was calculated following Barrs and Weatherley (1962) as follows:

$$RWC = [(freshweight\text{-}dryweight)/(turgidweight\text{-}dryweight)] \times 100$$

2.3. Biochemical determinations

Two plants per replicate were used for chlorophyll determination. One gram of fresh tissue was sampled from the youngest fully expanded leaf, extracted with 90% acetone and read using a UV/Visible Spectrophotometer (Bausch & Lomb, Belgium) at 663, 645 and 750 nm wavelengths. Absorbance at 750 nm was subtracted from absorbance at the other two wavelengths to correct for any turbidity in solution before chlorophyll concentrations were calculated according to Strain and Svec (1966).

$$Chl a(mg.ml^{-1}) = 11.64(A663) - 2.16(A645)$$

$$Chl b(mg.ml^{-1}) = 20.97(A645) - 3.94(A663)$$

where A663 and A645 represent absorbance values at 663 and 645 nm, respectively.

Electrolyte leakage was measured to assess membrane permeability using an electrical conductivity meter (Lutts et al., 1995). Leaf samples from second leaf above and below the shoot apex, representing developing and mature leaves, respectively were collected, washed and wiped clean. One cm leaf segments were cut and placed in individual stoppered vials containing 10 ml of distilled water and incubated on a rotating shaker at 100 rpm at 25 °C for 24 h. Electrical conductivity (EC) of the bathing solution (EC1) was determined after incubation. Samples were then placed in an autoclave at 120 °C for 20 min and EC was determined a second time (EC2) after cooling the bathing solutions to room temperature. Electrolyte leakage was expressed as percent of EC1/EC2.

Lipid peroxidation was monitored by measuring the conversion of lipids to malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) assay, as described by Draper and Hadley (1990). TBARS reagent (1 ml) was added to a 0.5 ml aliquot of tissue homogenate and heated for 20 min at 100 °C. The antioxidant, butylated hydroxytoluene (BHT), was added before heating the samples. After cooling on ice, samples were centrifuged at 840*g* for 15 min and absorbance of the supernatant was read at 532 nm. Blanks for each sample were prepared and assessed in the same way to correct for the contribution of A532 to the sample. TBARS results were expressed as MDA equivalents using 1,1,3,3-tetraethoxypropane as standard.

Total phenolic compounds were estimated in an 80% (v/v) methanolic extract of each tissue sample following Singleton and Rossi (1965), using gallic acid as standard. The content of total phenolic compounds was expressed as mg gallic acid equivalents g^{-1} 144 fresh weight (FW) of sample tissue.

For the analysis of nutrient elements, fruit and leaf tissues were separated dried to a constant weight at 70◦C in an oven, and ground to a fine powder. Nitrogen was determined by titrimetry after Kjeldahl digestion (Jackson, 1973). For other nutrient elements, 1 g of dry tissue was digested with nitric acid–perchloric acid (9:4) mixture. Phosphorous and potassium were estimated according to Jackson (1973). The other micro-nutrient elements were estimated using a Perkin Elmer A-Analyst-200 model atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA, USA) following Jones et al. (1991) for Ca and Mg elements and Jackson (1973) for Fe, Mn, Cu, and Zn.

2.4. LC–MS analysis

Sapota fruit sample was homogenized with 5 ml of 80% ethanol. The mixture was sonicated for 10 min followed by centrifugation at 4 °C at 10,000*g* for 10 min. The supernatant was evaporated to dryness, finally re-dissolved in mobile phase and filtered using 0.2 μm nylon filter paper prior to injecting 2 μl in Ultra Performance Liquid Chromatograph (UPLC) for LC–MS/MS analysis. Sugars were analyzed in the negative electrospray mode on a 2.1 × 100 mm UPLC analytical column packed with 1.7 μm particle size BEH-Amide (Waters, USA) and protected by vanguard BEH-Amide. A guard column (Waters, USA) was used with column temperature maintained at 25 °C. The mobile phase composed of Solvent (A) 80:20-Acetonitrile: water and Solvent (B) 30:70-Acetonitrile: water with 0.1% Ammonium hydroxide. A gradient program was used for running LC, starting with 100% of solvent (A) to 98% of (A) at end of 15 min andreturned to 100% (A) at 19 min. The flow rate was 0.1 ml min^{-1}. Elution was monitored using a Tandem Quadrupole Detector (TQD)-MS/MS (Waters, USA) system, optimized for sugar analysis. The specific precursor mass products, ions and collision energies applied are shown in Table 1.

2.5. Gas Chromatograph –Flame Ionization Detector (GC–FID) analysis

Fruit tissues were homogenized in a mixture of chloroform-methanol (2:1 v/v) and filtered through Whatman no.1 filter paper. The chloroform phase containing the lipids was separated, dried in a rotary vacuum evaporator at 40 °C and stored at −20 °C until further used (Folch et al., 1957). The extracted lipids were methy-