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Role of putrescine in regulating fruit softening and antioxidative enzyme systems in 'Samar Bahisht Chaunsa' mango

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ABSTRACT

The role of putrescine (PUT) in regulating fruit softening, antioxidative enzymes and biochemical changes in fruit quality was investigated during ripening and cold storage of mango (*Mangifera indica* cv. Samar Bahisht Chaunsa). Fruit were treated with various PUT concentrations (0.0, 0.1, 1.0 and 2.0 mM) and were allowed to ripen at 32 ± 2 °C for 7 days, or stored at 11 ± 1 °C for up to 28 days. Respiration rate and ethylene production were measured daily during ripening and cold storage. Cell wall degrading enzymes such as *exo*-polygalacturonase (*exo*-PG), *endo*-polygalacturonase (*endo*-PG), pectin esterase (PE), endo-1,4-β-D-glucanase (EGase), antioxidative enzymes including superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), fruit firmness as well as biochemical fruit quality characteristics were estimated during ripening and cold storage at 2 and 7 day intervals, respectively. PUT treatments reduced respiration rate, ethylene production and maintained higher fruit firmness during ripening as well as cold storage. PUT-treated fruit exhibited significantly suppressed activities of cell wall enzymes (*exo*-, *endo*-PG and EGase), but retained higher PE activity during ripening and cold storage. Total phenolic and antioxidant contents were significantly higher in PUT-treated fruit during ripening as well in the cold storage period than in the controls. Activities of antioxidative enzymes (CAT, POX and SOD) were also significantly higher in PUT-treated fruit during ripening as well as cold storage. SSC and SSC:TA were lower in PUT-treated fruit, while TA and ascorbic acid content showed the reverse trend. In conclusion, pre-storage 2.0 mM PUT treatment inhibited ethylene production and suppressed the activities of cell wall enzymes, while resulting in higher activities of antioxidative enzymes and maintaining better fruit quality during ripening and cold storage.

1. Introduction

Polyamines (PAs) are organic metabolites having low molecular weight polycations and are present in almost all living organisms (Kusano et al., 2007). The PAs putrescine^{2+} (PUT), cadaverine^{2+} (CAD), spermidine^{3+} (SPD) and spermine^{4+} (SPM) have high biological activity, and have been documented to be involved in cell division, fruit development, ripening, softening and senescence (Malik and Singh, 2005; Khan et al., 2007).

Level of endogenous PAs has been reported to change during ripening, which depends on maturity stage and cultivar. In general, endogenous PAs decrease during normal ripening in various fruit such as pear (Toumadje and Richardson, 1988) and peach (Liu et al., 2006). However, on the contrary, in 'Kensington Pride' mangoes, levels of PAs in pulp and skin tissues increased during fruit ripening (Malik and Singh, 2004). Due to a common precursor *S*-adenosyl methionine (SAM), both PAs and ethylene exhibit antagonistic effects during ripening of fruit (Pandey et al., 2000). Reduced levels of PAs have been correlated with increased ethylene production and vice versa (Kusano et al., 2007). Therefore, the level of these two opposing growth regulators is very important to delay or speed up these processes (Pandey et al., 2000). In general, PUT is the predominant PA and its endogenous level is closely linked with fruit ripening (Dibble et al., 1988). Pre-storage PUT application has been reported to significantly suppress ethylene production and delay ripening in plum (Khan et al., 2008), mango (Malik et al., 2003), and nectarine (Torrigiani et al., 2004) fruit.

During ripening, mango fruit undergo various qualitative and nutritional changes including change in colour, textural softening,

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accumulation of sugars, organic acids, development of taste, flavours, aroma and phytochemicals (Singh et al., 2013). Previously, pre-storage PA application has been reported to improve shelf-life of 'Kensington Pride' mango (Malik and Singh, 2005). Similarly, postharvest PUT application retarded fruit colour development, maintained firmness, and decreased sugar levels in 'Kensington Pride' mango (Malik et al., 2003). PUT (2 mM) treated fruit exhibited a high palatability rating, good SSC:TA ratio and low physiological weight loss and spoilage percentage in 'Langra' mango (Jawandha et al., 2012).

During ripening of fruit, excessive production and accumulation of reactive oxygen species (ROS) cause oxidative damage, which consequently reduce the ability of the antioxidant system to eliminate free radicals such as O^{2-}, H_2O_2 and superoxide anion (Jimenez et al., 2002). The generated ROS induce cell damage, including loss of membrane integrity in the tissue and oxidative damage to lipid, DNA and proteins (Hodges et al., 2004). Therefore, ripening can be considered a stressful process, with a progressive increase in oxidation (Singh et al., 2012). The formation of ROS is prevented by the stimulation of various antioxidative defence enzymes, such as CAT, POX and SOD and likewise by non-enzymatic low molecular mass antioxidants such as beta-carotene, ascorbic acid and glutathione (Hodges et al., 2004). PAs play a vital role in the antioxidative system and provide protection to membranes against the oxidative injury caused by ROS (Verma and Mishra, 2005). PAs exhibit antioxidant properties in relation to H_2O_2 and O^{2-} radicals (Li et al., 2004). Recently, postharvest PUT application had been found to increase the total antioxidant activity in 'Angelino' plum (Khan et al., 2008) and 'Lasgerdi' and 'Shahrodi' apricot (Davarynejad et al., 2013).

Ripening of the fruit is developmentally regulated programme involved modification of cell wall ultrastructure and texture in mango, which ultimately helps in defining the shelf-life of a fruit (Singh et al., 2013). Fruit softening in mango is triggered by various hydrolytic reactions which are controlled by enzymes such as *exo*-, *endo*-PG, EGase and PE (Razzaq et al., 2013a; Singh et al., 2013). Like hormones, PAs are involved in maintaining the cell membrane stability through binding with phospholipids or anionic sites on membranes (Slocum et al., 1984). PAs act as anti-senescence agents which delayed the softening in many fruit including plum (Khan et al., 2007) and mango (Malik and Singh, 2005). To the best of our knowledge, no information is available on a PUT role in modulating the activities of fruit softening as well as antioxidative enzymes in mango throughout ripening and cold storage, and this warrants further investigation. Therefore, the role of PUT on the activities of cell wall enzymes including *exo*-PG, *endo*-PG, EGase and PE, as well as antioxidative enzymes such as SOD, POX and CAT in the pulp tissues of 'Samar Bahisht Chaunsa' mangoes during ripening and cold storage was investigated.

2. Materials and methods

2.1. Fruit

Mango (*Mangifera indica* L. cv. Samar Bahisht Chaunsa) fruit at commercial maturity (163.27 N firmness and 7.36°Brix SSC) were sourced from a commercial orchard located at Lodhran (Latitude 29°32′ N; longitude 71°38′ E), Southern Punjab, Pakistan. All the fruit were dipped in 0.5% lime solution for 3 min to avoid sap burn injury. Following air-drying, fruit were packed in corrugated cardboard boxes, pre-cooled at 18 °C and transported to the Postharvest Laboratory, Institute of Horticultural Sciences, University of Agriculture Faisalabad, for further experiments. Uniform sized and mature (hard and green skin colour) fruit, without any disease symptoms and blemishes, were selected for both trials.

2.2. Treatments and experimental design

For postharvest PUT treatments, fruit were immersed in 0.0, 0.5, 1.0, 1.5 or 2.0 mM aqueous solutions of PUT with 0.01% Tween-20 as surfactant for 10 min at ambient temperature (32 ± 3 °C). Following treatments, fruit were dried under shade, packed in corrugated cardboard boxes and ripened in ambient conditions (Study I) and stored at low temperature (Study II).

2.3. Study I: influence of postharvest PUT application on fruit softening and antioxidative enzymes activities during ripening at room temperature

PUT-treated and control fruit were packed in corrugated cardboard boxes and allowed to ripen at room temperature (32 ± 3 °C; 53.2 ± 9.2% RH) until the eating soft stage. All treatments were replicated four times with six fruit as an experimental unit. The experimental design was two-factor factorial, including PUT treatments and ripening period. During the entire ripening period, respiration rate and ethylene production were quantified daily. Cell wall enzymes (*exo*-PG, *endo*-PG, PE and EGase), antioxidative enzymes (CAT, POX and SOD), biochemical fruit quality characteristics (SSC, TA, ascorbic acid, total phenolic and total antioxidant contents) were measured in pulp tissues only on days 1, 3, 5 and 7 of the ripening period.

2.4. Study II: influence of postharvest PUT application on fruit softening and antioxidative enzymes activities during cold storage

PUT-treated and untreated fruit were stored at 11 ± 1 °C and 80–85% RH for 28 days and removed every 7 days. The ethylene level and respiration amount were quantified daily. After removal, fruit were equilibrated for 2–3 h to room temperature prior to measuring respiration rate and ethylene production. Various cell wall and antioxidative enzymes were estimated in pulp tissues at 7-day intervals up to 28 days of cold storage. The experimental design was a two-factor factorial under CRD, including PUT treatments and storage period. Six fruit were used as an experimental unit with four replications.

2.5. Ethylene production and respiration rate

Ethylene production and respiration rates were estimated by ethylene meter (model ICA-56, International Controlled Atmosphere Ltd, UK) and CO_2 analyzer (Vaisala MI 70, Vaisala Inc., Helsinki, Finland) and expressed as μmol C_2H_4 kg^{-1} h^{-1} and mmol CO_2 kg^{-1} h^{-1}, respectively by using the method outlined by Razzaq et al. (2013a).

2.6. Physical fruit quality

Fruit weight was determined using digital balance (ELB 1200, Shimadzu, Kyoto, Japan) and weight loss percentage was calculated using the following equation:

$$\text{Fruit weight loss}\,(\%) = \frac{\text{Initial fruit weight} - \text{final fruit weight}}{\text{Initial fruit weight}} \times 100$$

A hand-held penetrometer (Model DFM50, Ametek Inc., USA) with an 8 mm tip was used to determine fruit firmness and was expressed as newtons (N).

2.7. Biochemical fruit quality

2.7.1. SSC, TA and ascorbic acid contents

A digital refractometer (ATAGO, RX5000, Atago Co. Ltd., Itabashi-Ku, Tokyo, Japan) was used for the determination of SSC