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Inhibition of phospholipase D enzyme activity through hexanal leads to delayed mango (*Mangifera indica* L.) fruit ripening through changes in oxidants and antioxidant enzymes activity

M. Jincy[a], M. Djanaguiraman[a,b,*], P. Jeyakumar[a], K.S. Subramanian[b], S. Jayasankar[c,*], G. Paliyath[d]

[a] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India
[b] Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India
[c] Department of Plant Agriculture, University of Guelph, Vineland Station, Ontario, Canada
[d] Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

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ABSTRACT

Fruit ripening is a senescence process and phospholipase D (PLD) is a key enzyme causing degradation of membrane phospholipids during that process. Our earlier studies showed that hexanal, is known to inhibit the PLD activity. The objectives were (i) to quantify the effects of postharvest hexanal treatment in mango fruit on physiochemical traits, (ii) to assess the changes in oxidants, antioxidants, and antioxidant enzymes activity in mango fruit after hexanal treatment. Fully matured mango fruit, var. Neelum were harvested from the tree, dipped in 0.02% hexanal solution, and stored under ambient conditions to study the physio and biochemical changes during storage period. The results indicated that hexanal treatment significantly reduced ethylene evolution rate, oxidants content and PLD enzyme activity in the fruit compared with control, key factors to delay ripening and senescence in fruit. However, the activities of antioxidant enzymes like superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase and the contents of ascorbic acid were increased in response to hexanal treatment. The decreased ethylene evolution rate, PLD enzyme activity and oxidant production caused by hexanal treatment might have led to increased shelf life. Overall, the results suggest that post-harvest dip of mango fruits in 0.02% hexanal solution extended the shelf life of mango fruit under ambient storage conditions, without the loss of quality of fruits.

1. Introduction

Mango is a climacteric fruit and has to be harvested at physiologically mature green stage to reduce the postharvest loss. Fruit ripening is a highly regulated process with synchronized genetic and metabolic events leading to essential changes in gene expression, physiology, biochemistry and anatomy. Several major changes take place as fruits ripen are changes in carbohydrate composition, resulting in sugar accumulation and increased sweetness; change in colour; flesh softening and textural change; formation of aroma volatiles; and accumulation of organic acids with associated development of flavour. During the late stages of fruit ripening, senescence associated biochemical changes like membrane deterioration and programmed cell death (PCD) happen (Payasi and Sanwal, 2010). Hence, fruit ripening can be considered as an early step of a PCD process (Bouzayen et al., 2010). If ripening is an extended form of senescence, then the mechanisms of membrane deterioration during ripening may probably be similar to those characterized in true senescing systems. Earlier studies on pear (Brennan and Frenkel, 1977), peach (Qin et al., 2009), muskmelon (Lacan and Baccou, 1998; Lester, 2000), orange (Huang et al., 2007), and tomato (Jimenez et al., 2002; Mondal et al., 2004) showed that fruit senescence and/or ripening happens with concurrent increase in H_2O_2 levels in the fruit. It is proposed that high level of reactive oxygen species (ROS) may inactivate enzymes controlling cellular processes required for normal ripening (Fry et al., 2001) and accelerate the rate of metabolic changes associated with fruit senescence

* Corresponding authors.
E-mail addresses: jani@tnau.ac.in (M. Djanaguiraman), jsubrama@uoguelph.ca (S. Jayasankar).

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(Jimenez et al., 2002; Tian et al., 2013). As ROS plays a major role in senescence of fruit, their level might be regulated by the activity of antioxidant enzymes. The antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and ascorbate (APX) are involved in scavenging of the ROS produced under oxidative stress (Djanaguiraman et al., 2010). The enzyme SOD converts superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2), while, CAT, POX and APX convert H_2O_2 to water (Mittler, 2002). The natural antioxidants present in mango fruit such as ascorbate are also involved in quenching and neutralizing the free radicals produced during senescence leading to enhanced membrane stability (Kim et al., 2009).

Ripening is best considered as a set of coordinated biochemical pathways. These pathways are most likely to be under hormal control both for their initiation and for coordination. Ripening of the mango fruit is characterized by softening of the flesh by hydrolysis of various cell wall components by the enzymes namely polygalacturonase, cellulases, and pectinesterases. Similarly, the sucrose and starch metabolism is mediated by α-amylase, sucrose phosphate synthase, sucrose synthase and acid invertase (Gomez-Lim, 1993). Oxidative stress and ROS appear to be an integral part of postharvest deterioration of fruit quality involving elevated levels of ethylene; and it suggest that membrane lipid hydrolysis by phospholipase D (PLD) and other lipolytic enzymes can be an earlier, more important event during postharvest deterioration (Pavelic et al., 2000; Page et al., 2001). The enzyme PLD is gradually stimulated during fruit ripening process in an autocatalytic manner, which can result in membrane degradation and destabilization (Paliyath and Subramanian, 2008). The enzyme PLD hydrolyses the phospholipids to phosphatidic acid (PA) and a free head group, and it belongs to predominant family of phospholipases in plants (Wang, 2005). PA causes cell death of whole leaves and single cells through elevated levels of ROS (Park et al., 2004). Hence, there is an opportunity to delay the fruit ripening process by inhibiting the PLD activity. A primary alcohol such as hexanol and an aldehyde such as hexanal were potent inhibitors of PLD activity (Paliyath et al., 2003). Hexanal is naturally produced in plants during lipid peroxidation mediated by lipoxygenase and hydroperoxide lyases (Paliyath and Subramanian, 2008). Hexanal treatment as preharvest spray or postharvest dip have been found to extend the shelf life of both climacteric and non-climacteric fruit (apple, banana, cherry, strawberry, blue berry and tomato; Paliyath et al., 2003; Paliyath and Subramanian, 2008). The enhanced shelf life of fruit may be due to downregulation of PLD and it needs to be quantified. Both climacteric and non-climacteric fruit undergo similar biochemical changes such as sweetness, softness (cell wall softening), reduction in acidity and an increase in polyphenols and anthocyanins due to external hexanal treatment (Sharma et al., 2010; Cheema et al., 2014). Our recent study on mango have shown that preharvest sprays of 1.6 mM hexanal formulation on 30 and 15 days before harvest significantly delayed the fruit abscission process, reduced postharvest diseases, and extended shelf life under both ambient and cold storage conditions (Anusuya et al., 2016). However, the mechanism of delayed fruit ripening was not studied in detail. Hence, the present study was formulated to study the detailed mechanism of delayed fruit ripening after hexanal treatment. Mango being a climacteric fruit and having export market, its response to hexanal treatment to delay the ripening process will be of interest to growers and exporters.

Based on this background, the objectives were (i) to quantify the effects of postharvest hexanal treatment in mango fruit on physiochemical traits, (ii) to assess the changes in oxidants, antioxidants, and antioxidant enzymes activity in mango fruit after hexanal treatment.

2. Materials and methods

2.1. Fruit samples and treatments

The experiment was conducted during 2015–16 in the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India. The mango fruit var. Neelum was randomly hand harvested at hard green stage of maturity, from the mango orchard of Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar (11°29″N latitude and longitude of 77°80′E), Tamil Nadu, India.

Mango fruit were cleaned carefully by washing it with potable water. Maximum efforts were made to select the fruit of uniform in size and free from injuries or diseases. The fruit were divided into two groups. One group was dipped in 0.02% hexanal for 10 min (2 L of solution kg fruit^{-1}) and shade dried. The other group served as untreated control. Four mangoes (~2 kg) from each group were transferred to each airtight plastic container of 5.4 L capacity. There were totally 80 plastic containers for each group. Before the start of the experiment, the plastic containers were coated with wax to avoid the gas exchange with the environment. A cooling fan (80 × 80 × 25 mm) was fixed on the inner side of the plastic container lid to circulate the air evenly within the plastic container. The fan was powered using a 12 V switch mode power supply (SMPS) and was running constantly throughout the experiment. A brass nut and ferrule set (0.63 cm) containing replaceable and air-tight rubber septum was fixed on the lid of the plastic container. The leakage of gas from the sides and top of the plastic container was checked using soap solution to ensure that it is airtight. The plastic containers with mango fruit were maintained at a temperature of 25 °C, RH of 60% and light intensity (PPFD) of 200 μmol m^{-2} s^{-1}. Before the air sampling, the vial and cap were crimped and sealed with Teflon film and then flushed with nitrogen gas (99.9% purity) for five min. After 6 h of treatment imposition, the air samples were withdrawn from five plastic containers through the rubber septum and stored in 20 mL headspace vials at 4 °C until further analysis as described by El-Sharkawy et al. (2007). The air sample collected was considered as 0 day after treatment (DAT). Similarly, on 2, 4, 6, 8, 10, and 12 DAT, the air samples were collected at approximately the same time.

To quantify various physiological and biochemical traits, the two groups of mango fruit were maintained in two different rooms. The temperature, RH and light intensity (PPFD) of the rooms were 25 ± 0.8 °C, 60 ± 10% and 200 ± 50 μmol m^{-2} s^{-1}, respectively. There were five replicates for each treatment for each day of observation. Each replicate had four fruit (~2 kg). Mango fruit from each replicate were sampled on 0, 2, 4, 6, 8, 10, and 12 DAT from 10 to 11 AM by quick immersion in liquid nitrogen, sealed in zip lock cover and stored in −80 °C, until further analysis.

2.2. Physiochemical traits recorded during storage period

2.2.1. Ethylene evolution rate

Air samples from the headspace were withdrawn (15 mL) using a hypodermic syringe, and ethylene was quantified with a gas chromatograph (Nucon, New Delhi, India) equipped with an 3.175 mm outside diameter x 3 m stainless steel Poropak column and a flame ionising detector. The temperatures of the injector, column and detector were 160, 80 and 200 °C, respectively. The flow rate of the carrier N_2 gas was 1.8 kg cm^{-2}. The flow rates of oxygen and hydrogen were 2.1 and 1 kg cm^{-2}, respectively (Djanaguiraman and Prasad, 2014). The detector response was standardized by injecting known amounts of standard ethylene gas (Sigma-Aldrich, St Louis, MO, USA) by serial dilutions. The retention time was 2.3 min. The