Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage

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This study was conducted to investigate the effect of postharvest salicylic acid (SA) treatment on alleviating chilling injury (CI), preserving bioactive compounds and extending shelf life of mango fruit during low-temperature storage. Physiologically mature mango fruit (cv. Chausa) were immersed in 1 mM and 2 mM SA solutions for 5 min and then stored at 8±0.5°C temperature and 90±5% relative humidity. Before taking observations, the fruit were subjected to exposure at 25±2°C temperature for 3 days to simulate shelf life. The results showed that SA treatments were highly effective in alleviating CI (11-25% lower) in mango fruit. Among the treatments, 2 mM SA proved best in lowering weight loss, fruit softening, disease incidence, pectin methylesterase and polygalacturonase activities over control. Bioactive compounds like carotenoids, phenolics and antioxidant capacity were also maintained higher in SA-treated fruit. The findings confirmed that, SA (2 mM) can be potentially used as a pre-storage treatment during low-temperature storage of mango fruit.

Keywords: Mango; Salicylic acid; Chilling injury; Antioxidant capacity; Enzyme activity.

Introduction

Mango (Mangifera indica L.) is one of the most popular fruit and commonly known as ‘King of fruits’ in Asian countries. Because of its delicious taste and pleasant flavor, it is ranked as one of the choicest fruits in the national and international market. Mangoes ripen and deteriorate very fast when stored at ambient temperature, which leads to reduction in shelf life. Therefore, low-temperature storage is necessary to slow down the metabolic processes and decay development but, when this fruit stored at temperature below 13°C, it develops chilling injury (CI) which further limits its shelf life during low-temperature storage. The symptoms manifest as discoloration and pitting of the peel, sunken lesions, lenticels spotting, shrivelling, uneven ripening, poor color, off-flavor development and increased susceptibility to decay. These lead to reduction in market value of the fruit. Salicylic acid (SA) belongs to a group of phenolic compounds that are ubiquitous in plants and is now considered as plant hormone. It plays pivotal role in regulating a variety of physiological processes in plants. Recently, SA has also been reported to act as a key signal molecule for expression of multiple stress resistance in plants. The effect of SA on delaying ripening, fruit softening and reducing disease incidence have been discussed by several researchers. In recent years, a few studies have reported that pre-storage SA treatment alleviates CI in fruit during cold storage. Considering the commercial importance of the fruit and its postharvest problem during low-temperature storage, we investigated the effect of SA in alleviating CI, extending shelf life and preserving fruit quality during cold storage.

Materials and methods

Plant materials and treatments

Mango fruit of cv. Chausa were harvested at physiological maturity stage (light cream pulp with 8–10°Brix total soluble solids) from an orchard located at IARI, New Delhi (India). After selecting uniform sized healthy fruit, they were randomly divided into 3 lots each of 216 fruit for the following treatments in triplicate (72 fruits per replicate): control (treated with distilled water), SA at 1mM and 2 mM concentrations. Fruit were treated by dipping in 20 l solution of SA containing Tween-20 (2 g L⁻¹) as surfactant, at 25°C for 5 min. They were then air-dried at room temperature and stored in a temperature-controlled chamber at 8±0.5°C along with 90±5% relative humidity. After 5, 10, 15, 20,
25 and 30 days of cold storage, fruit were moved from temperature-controlled chamber to ambient condition at 25±2°C for 3 days (simulating shelf life).

**Weight loss (WL) and fruit firmness**

To determine WL of the stored fruit, both treated and control fruit were weighed at different sampling intervals. Then WL was calculated as the difference between initial fruit weight and the fruit weight at the time of measurement and expressed in percentage (%). Fruit firmness was determined using a Texture Analyzer (TA+Di, Stable micros systems, UK) through penetrations (10 mm inside fruit) and the results were expressed in newton (N).

**Chilling injury (CI)**

To determine the incidence of CI, mango fruit were rated on 0-4 scale based on dark coloration on the peel of affected fruit. The scale used was: 0, no peel damage; 1, trace; 2, slight; 3, moderate and 4, severe. The CI index was calculated by multiplying the number of fruit in each category by the respective score, summing the products and dividing by the total number of fruit.

**Respiration and ethylene evolution rates**

Respiration rate was measured following the static headspace technique using a gas analyzer (Checkmate 9900 O2/CO2, PBI Dansensor, Denmark) and results were expressed in ml CO2 kg⁻¹ FW h⁻¹. Ethylene evolution rate was determined by a gas chromatograph (Hewlett Packard 5890 Series II, USA). The rate of ethylene evolution was expressed in μl ethylene kg⁻¹ FW h⁻¹.

**Total carotenoids, total phenolics content and antioxidant (AOX) capacity**

Total carotenoids content of fruit was determined by the method of Roy (1973) and expressed as mg 100g⁻¹ FW. Total phenolics content of the fruit extracts were determined by the method of Singleton and Rossi (1965) and expressed as µg Gallic acid equiv. g⁻¹ FW. AOX capacity was determined following cupric reducing antioxidant capacity (CUPRAC) method and expressed as μmol Trolox equiv. g⁻¹ FW.

**Polygalacturonase (PG) and pectin methyl esterase (PME) enzyme activities**

PG and PME activities were measured by following the method of Lazan et al. (1995) and expressed as μg galacturonic acid g⁻¹ FW h⁻¹ and μmol g⁻¹ FW min⁻¹, respectively.

**Decay incidence**

Fruit showing symptoms of rot irrespective of the severity, were considered a loss. The percent decay incidence was determined by the following formula: \( \frac{X}{Y} \times 100 \) where, \( X \) is the number of fruit decayed and \( Y \) is the total number of fruit kept at the beginning of storage.

**Statistical analysis**

The data obtained under different treatments in respect to various parameters during storage were subjected to analysis of variance (ANOVA) with treatment and storage time as sources of variation. Mean comparison among treatments were performed using the HSD Turkey’s test at a significance level of P<0.05. All analysis was performed with SPSS software package version 16.0 for windows.

**Results and discussion**

**Fruit weight loss (WL)**

The loss in weight of mango fruit found increased with the advancement of storage period under all the treatments (Table 1). Control fruit exhibited significantly higher WL from the initial days of storage. However, fruit treated with SA gave better results and were expressed in ml CO₂ (Checkmate 9900 O₂/CO₂, PBI Dansensor, Denmark) and results were expressed in ml CO₂ kg⁻¹ FW h⁻¹. Ethylene evolution rate was determined by a gas chromatograph (Hewlett Packard 5890 Series II, USA). The rate of ethylene evolution was expressed in μl ethylene kg⁻¹ FW h⁻¹.

<table>
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<th>Parameters</th>
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<th>10</th>
<th>Storage period (days)</th>
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Table 1: Effect of SA on weight loss, chilling injury and decay loss in mango fruits stored up to 30 days at 8°C plus 3 days at ambient condition (25±2°C and 60-65% RH). Data are means ± standard error of three replicate determinations (n=3)
result in terms of reduction in WL. Among the treatments, 2 mM SA was the most effective in reducing WL. At the end of the storage period, highest (17.63%) WL was recorded in control while it was lowest (15.35%) in 2 mM SA-treated mango fruit. Higher WL in control mango fruit was attributed to loss of water from the fruit and higher respiration rate. However, fruit treated with SA showed lower WL which might be due to the fact that SA suppressed the transpiration and respiration rates of mango fruit by closing the stomata of the treated fruit. Similarly, peach treated with SA also exhibited lower WL compared to control. Moreover, CI has also been reported to facilitate WL. Thus, lower incidence of CI in SA-treated fruit might also be another reason for lower WL.

Fruit firmness
Firmness of the control mango fruit declined sharply during cold storage (Fig. 1). However, the decrease in firmness was much slower in SA-treated fruit. After 30 day of storage, control mango fruit showed lowest (7.42 N) firmness, while SA 2 mM and 1 mM treatments retained about 40% and 23% higher firmness respectively, over control. Fruit firmness is one of the most important quality attribute determining product acceptability to the consumer. The higher fruit firmness in SA-treated fruit may be ascribed to reduced activities of cell wall and membrane degrading enzymes viz. PME and PG, which was caused by suppressed ethylene production. Previous study on mango has also confirmed the involvement of ethylene in increasing the activities of fruit softening enzymes. Retention of fruit firmness by SA treatment has also been reported earlier in several fruit.

Chilling injury (CI)
The incidence of CI developed in mango fruit irrespective of treatments and the severity increased with the advancement of storage period (Table 1). The highest CI (2.45 score) was recorded in control mango fruit while, the application of SA led to significant reduction in CI incidence. Among the treatments, 2 mM SA was found to be most effective and showed about 24% lower occurrence of CI compared to control fruit. Exposure of fruit to chilling temperature induces generation of reactive oxygen species (ROS), which causes oxidative stress and eventually CI to the fruit. In this experiment, fruit treated with SA developed significantly lower CI than control. When SA applied exogenously, it induced expression of ROS avoidance genes and ROS scavenging genes that increased the AOX capacity of the cells. Moreover, it has also been reported to induce synthesis and accumulation of heat shock proteins which confers protection against CI. Previous works on peach and pomegranate also support these findings.

Respiration rate
Control mango fruit showed onset of respiratory climacteric after 15 day of cold storage however, it was delayed up to 5 days in SA-treated fruit (Fig. 2). The respiratory climacteric peak was suppressed by 1.3-fold and 1.2-fold respectively, with SA 2 mM and 1 mM treatments. After 30 day of storage, highest respiration rate (166.58 ml CO\textsubscript{2} kg\textsuperscript{-1} FW h\textsuperscript{-1}) was recorded in control fruit while it was lowest (123.51 ml CO\textsubscript{2} kg\textsuperscript{-1} FW h\textsuperscript{-1}) in SA (2 mM). Exogenous application of SA highly suppressed the respiration rate in treated mango fruit. The suppression of respiration rate in SA-treated fruit might be due to

![Fig. 1- Effect of SA on fruit firmness and total carotenoids content in mango stored up to 30 days at 8ºC plus 3 days at ambient condition (25%±2°C and 60-65% RH). Vertical bars represent standard error of means (n=3).](image1)

![Fig. 2- Effect of SA on respiration and ethylene evolution rates in mango fruit stored up to 30 days at 8ºC plus 3 days at ambient condition (25%±2°C and 60-65% RH). Vertical bars represent standard error of means (n=3).](image2)
delaying the ripening process and lowering the incidence of CI. Increase in respiration rate as a consequence of CI could be due to damage of cell membrane and other cell organelles during storage at low-temperature \(^{13}\). Our finding is in accordance with the previous findings on pomegranate \(^{15}\) and banana \(^{16}\). Ethylene evolution rate

Irrespective of SA concentration, it significantly suppressed and delayed the ethylene evolution rate (Fig. 2). In this experiment, up to 5 day of cold storage, ethylene was not detected both in SA-treated and control fruit. Later, control mango fruit exhibited a sharp increase in ethylene evolution and climacteric peak (0.275 µl kg\(^{-1}\) FW h\(^{-1}\)) was recorded in fruits after 20 day of storage. However, SA treatment delayed the onset of peak up to 5 days. Finally, fruit treated with SA 2 mM and 1 mM showed about 32% and 21% reduction in ethylene evolution rate respectively, compared to control. Ethylene evolution rate of SA-treated fruit was significantly reduced up to 30 days of cold storage. This reduction was more pronounced in 2 mM SA-treated fruit. The suppression in ethylene evolution rate in SA-treated mango fruit might be associated with lower ACC oxidase and ACC synthase activities\(^{3}\). Similarly, inhibition of ethylene biosynthesis by SA treatment has been reported earlier in fruit crop like strawberry\(^{17}\). Total carotenoids content

The result showed that both the SA treatments delayed the formation of carotenoid pigments, compared to control (Fig. 1). However, control fruit showed rapid increase in total carotenoids content from 5 day of storage. At the end of the experiment, total carotenoids content of SA-treated fruit was not significantly different from that of control fruit. In this study, total carotenoids content initially increased rapidly in control fruit compared to SA-treated mangoes. Initial lower content of carotenoids in SA-treated fruit might be due to the fact that SA delayed the ripening process by suppressing the ethylene evolution and delaying the climacteric peak of ethylene\(^{17}\). However, at the end of the experiment, non-significant difference between treated and control fruit indicated that SA treatment did not hamper the synthesis of carotenoid pigments.

**Ethylene evolution rate**

Fig. 3- Effect of SA on total phenolics content and antioxidant capacity in mango fruit stored up to 30 days at 8°C plus 3 days at ambient condition (25%±2°C and 60-65% RH). Vertical bars represent standard error of means (n=3).

**Total carotenoids content**

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Decay incidence of CI was significantly reduced by lowering the enzyme activities and maintaining membrane fluidity at low temperature. Exogenous application of SA (2 mM) was found to be most effective in inhibiting postharvest decay of mango fruit. The activity of PG and PME enzymes in both the treated control fruit increased gradually with the advancement of storage period (Fig. 4). Among the applied treatments, SA (2 mM) was found to be most effective in maintaining lower activity of PG (55.57 µg galacturonic acid g\(^{-1}\)FW h\(^{-1}\)) and PME (0.39 µmol g\(^{-1}\)FW min\(^{-1}\)) enzymes. On the contrary, control fruit showed highest PG (72.67 µg galacturonic acid g\(^{-1}\)FW h\(^{-1}\)) and PME (0.54 µmol g\(^{-1}\)FW min\(^{-1}\)) activities at the end of storage. With the advancement of storage period, level of PG and PME enzymes increased, which is in agreement with the previous study in mango. Higher enzyme activities in control fruit might be attributed to decrease in fruit firmness and onset of senescence, owing to CI and decay. Due to occurrence of CI, cell membrane lipids undergo changes in physical state, which lead to an increase in membrane permeability and leakage of ions. During this period, cell wall degrading enzymes acted on cell wall, resulting in rapid decrease of firmness in control fruit. However, the exogenous application of SA maintained membrane fluidity at low temperature and thus reduced the enzyme activities by lowering the incidence of CI.

**Decay incidence**

SA treatments significantly reduced the incidence of decay compared to control (Table 1). Up to 5th day of storage, no symptom of decay was observed in any of the treatment; however at 10th day, symptoms appear only in control fruit. Fruit treated with SA 1 mM and 2 mM developed decay symptoms from 15th and 20th day onward respectively. At 30th day of storage, highest decay incidence (16%) was recorded in control fruit while, it was lowest (6.8%) in 2 mM SA-treated fruit. SA and its derivatives are known to trigger resistance system in plants against various diseases. Previous workers reported that SA increases the production of H\(_2\)O\(_2\) in plants, which acts as a signalling molecule and activates the plant’s systemic resistance against pathogens. The effect of SA in inhibiting postharvest decay of fruit has also been confirmed earlier.

**Conclusions**

The results of present study showed that oxenous application of SA (2 mM) had significant effect on alleviating CI in mango fruit cv. Chausa during storage at 8°C for 30 days. This treatment also retained higher firmness and reduced weight loss, ethylene evolution, respiration rate, enzyme activity and decay incidence throughout the storage.

**References**

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