Delayed ripening of banana fruit by salicylic acid

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Abstract

Salicylic acid treatment has been found to delay the ripening of banana fruits (Musa acuminata). Fruit softening, pulp:peel ratio, reducing sugar content, invertase and respiration rate have been found to decrease in salicylic acid treated fruits as compared with control ones. The activities of major cell wall degrading enzymes, viz. cellulase, polygalacturonase and xylanase were found to be decreased in presence of salicylic acid. The major enzymatic antioxidants namely, catalase and peroxidase, were also found to be decreased in presence of salicylic acid during banana fruit ripening. © 2000 Published by Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Ripening; Salicylic acid; Banana (Musa acuminata); Catalase

1. Introduction

Salicylic acid, a ubiquitous plant phenolic, has been reported to regulate a number of processes in plants [1]. One of its earliest known in vivo functions in plants is heat production during flower induction in some angiosperm species [2]. Later it was shown to regulate expression of pathogenesis related protein genes, which suggested its role as a signal molecule in providing resistance against pathogen attack. Salicylic acid mediated hypersensitive and systemic acquired resistance against pathogen attack are proposed to be mediated through the inhibition of catalase, which subsequently raises intracellular H₂O₂ concentration. This increased intracellular H₂O₂ concentration has been proposed to act as a second messenger in activation and expression of defense related genes [1]. Recently, it has been proposed to be a new kind of plant hormone [3]. Thus, salicylic acid has been shown to interfere with the biosynthesis and/or action of ethylene [1], abscisic acid [4] and cytokinins [5] in plants. Salicylic acid and its derivative acetyl salicylic acid (ASA) have been reported to inhibit ethylene production in pear [6,7], carrot cell suspension cultures [8], in apple and pear discs and mung bean hypocotyls [9] suggesting the role of salicylic acid as an antagonist to ethylene action. However, salicylic acid and ethylene have been reported to have some similar functions also. For example, both can induce certain pathogenesis-related proteins and the alternative pathway of respiration in many plant tissues [10]. Moreover, an increase in endogenous ethylene biosynthesis at low concentrations of salicylic acid has been reported in suspension cultures of carrot [11]. However, at higher salicylic acid concentration (＞10⁻⁴ M) an inhibition of ethylene production was observed in this study.

The plant hormone ethylene has been reported to affect a diverse array of plant growth and developmental processes including germination, senescence and abscission of both flowers and leaves, fruit ripening as well as the response to a wide variety of stresses such as pathogen attack and drought [12]. The induction of ethylene biosynthesis by a wide variety of stimuli, including wounding, pathogen attack, various stresses, mechanical stimulus and by hormones such as auxins,
cytokinins and ethylene itself during certain developmental processes is well documented [12–14]. Among all these effects of ethylene, its role in fruit ripening has been studied in great detail including isolation and characterization of gene encoding a key enzyme of ethylene biosynthesis [15] and development of transgenic tomato having delayed ripening [16]. Treatment of green banana fruits with ethylene during the preclimacteric phase, has been shown to accelerate their ripening. All the climacteric fruits are characterized by a transient increase in both ethylene synthesis and respiration at an early stage of ripening. Recently, Vendrell et al. [17] have attributed the ethylene as a trigger for ripening in banana.

As salicylic acid has been shown to affect the biosynthesis and action of ethylene, a well known fruit ripening hormone, in the present paper, we have investigated the effects of salicylic acid on banana fruit ripening by measuring respiration rate, pulp and peel contents and their ratio, reducing and non-reducing sugars and their ratio and invertase, antioxidant enzymes such as catalase and peroxidase, cell wall degrading enzymes such as cellulase, polygalacturonase and xylanase.

2. Materials and methods

2.1. Fruit tissue

Banana fingers (Musa acuminata cv Hari chhal) were purchased from local market.

2.2. Treatment of fruits

Banana fingers were randomized and divided into groups, containing ten fruits in each, according to the following treatments:

Group 1. Water
Group 2. Teepol (0.2%) [Control]
Group 3. Teepol (0.2%) + 500 μM salicylic acid
Group 4. Teepol (0.2%) + 1000 μM salicylic acid

Banana fingers were dipped in 2 l of distilled water (group 1) or teepol (group 2) or teepol containing salicylic acid (groups 3 and 4) under laboratory conditions for 6 h with occasional shaking. After draining the water/solution fingers were washed, air dried and kept in plastic containers at room temperature and covered with polythene sheets containing small holes.

2.3. Determination of pulp/peel ratio

Two banana fruits from each treatment were peeled off and the pulp and peel portions of each finger were weighed separately on a laboratory balance. The ratio of pulp to peel of each finger was calculated and mean value was recorded.

2.4. Determination of respiration rate

Respiration was measured as CO₂ production. A single banana fruit was taken in a chamber and air was passed through the chamber. The effluent air was connected to an ADC 225 MK3 Infrared Gas Analyzer (IRGA) and respiratory rate was measured. The IRGA was earlier calibrated with standard CO₂. The results were expressed as ml h⁻¹ kg⁻¹ fresh weight.

2.5. Determination of soluble sugars

Banana fruit pulp (1 g) was homogenized in 5.0 ml 90% ethanol by Ultra Turrax T25 for 2 min and then refluxed for 30 min. The sample was centrifuged at 10 000 × g for 30 min. The residue was again subjected to ethanol extraction. The extracts were combined and alcohol was removed by evaporation. An aliquot was taken and total sugars were measured by phenol–sulfuric acid method [18]. Reducing sugars were measured as described [19]. Non reducing sugar content was determined by the difference between total sugar and corresponding reducing sugar value. Glucose was used as standard for sugar estimation.

2.6. Enzyme extraction and assay

A 10% homogenate was prepared by homogenizing 2 g of pulp in 15 ml sodium phosphate buffer (20 mM, pH 7.0) containing cysteine–HCl (20 mM), EDTA (20 mM) and Triton X-100 (0.05%) by Ultra Turrax T25 for 2 min at high speed. The homogenate was passed through two layers of muslin cloth to remove cell debris and the volume was made to 20 ml with the homogenization medium. The homogenate was centrifuged at 15 000 × g for 30 min at 4°C in a Sorvall RC 5C refrigerated centrifuge. The clear supernatant was used for enzyme assay.
2.7. Assay of polygalacturonase (EC 3.2.1.15)

Polygalacturonase activity was assayed by measuring the formation of reducing groups using the methods of Nelson [20] and Somogyi [21]. The reaction mixture contained 0.2 ml sodium acetate buffer (200 mM, pH 4.5), 0.1 ml NaCl (200 mM), 0.3 ml polygalacturonic acid (PGA) (1%, pH 4.5), and an appropriate amount of enzyme in a total volume of 1.0 ml. The reaction was initiated by the addition of substrate. The reaction mixture was incubated at 37°C for 1 h. The reaction was terminated by heating the reaction mixture in a boiling waterbath. In the control tubes, the substrate was added after the heat treatment. The formation of reducing group was calculated using D-galacturonic acid as a standard. One unit of polygalacturonase activity is defined as the amount of enzyme producing 1 μmol of reducing groups per min at 37°C.

2.8. Assay of cellulase (EC 3.2.1.4)

Cellulase activity was measured as described by Ahmed and Labavitch [22] with slight modifications. The reaction mixture consisted of sodium acetate buffer (100 mM, pH 5.0), carboxy methyl cellulose (1.5% w/v) and enzyme in a final volume of 1.0 ml. The reaction mixture was incubated at 37°C for 16 h. After 16 h, the substrate was added to the control tubes and colour was developed according to Miller [19] using dinitrosalicylic acid (DNS). The tubes were boiled on a waterbath for 10 min and the colour was read at 540 nm using Spectronic 20D spectrophotometer. Amount of reducing sugar released was calculated from a calibration curve drawn using glucose as standard. One unit of cellulase activity was defined as the amount of enzyme liberating 1 μmol of reducing sugar per min at 37°C.

2.9. Assay of xylanase (EC 3.2.1.8)

Xylanase activity was assayed as described by Singh and Singh [23] with slight modifications. The assay mixture consisted of sodium acetate buffer (100 mM, pH 5.0), xylan (0.1%) and enzyme preparation in a total volume of 1.0 ml. The mixture was incubated for 1 h at 37°C. The released reducing sugars were measured using DNS as for cellulase (described above). One unit of xylanase activity was defined as 1 μmol of reducing sugar released per min at 37°C.

2.10. Assay of invertase (EC 3.2.1.26)

Invertase activity was measured as described by Moriguchi et al. [24] with slight modifications. The assay mixture contained acetate buffer (100 mM, pH 4.5), sucrose (100 mM) and enzyme preparation in a total volume of 1.0 ml. The reaction mixture was incubated for 1 h at 37°C. The substrate was added to control tubes after the incubation and colour was developed using DNS (as described for cellulase). Amount of reducing sugar released was calculated from the calibration graph. One unit of invertase activity was defined as micromoles of reducing sugars equivalent released per min at 37°C.

2.11. Assay of peroxidase (EC 1.11.1.7)

The peroxidase activity was assayed as described by Pütter [25] with slight modifications. The assay mixture consisted of sodium phosphate buffer (50 mM, pH 7.0), H₂O₂ (4 mM), guaiacol (3.33 mM) and 0.1 ml enzyme in a final volume of 3.0 ml. The increase in absorbance at 470 nm was measured using a Spectronic-2000 spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme catalyzing the production of 1 μmol tetraguaiacol per min at 30°C.

2.12. Assay of catalase (EC 1.11.1.6)

The catalase activity was assayed as described by Aebi [26] with some modifications. The assay medium consisted of sodium phosphate buffer (50 mM, pH 7.0), (20 mM) and 0.1 ml enzyme in a final volume of 3.0 ml. The disappearance of H₂O₂ was measured as decrease in absorbance at 240 nm using a Spectronic-2000 spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme catalyzing the decomposition of 1 μmol H₂O₂ per min at 30°C.

3. Results

3.1. Effect of salicylic acid on the rate of respiration during banana fruit ripening

The effects of salicylic acid on the rate of respi-
3.2. Effect of salicylic acid on softening of banana fruits during its ripening

Softening of fruits is one of the most common physical parameter to assess the progress of ripening. Therefore, the effect of salicylic acid on softening of banana fruits, as a physical parameter to assess the extent of ripening, has been investigated. It was observed that salicylic acid treatment inhibited the process of banana fruit softening during ripening. Another physical parameter, yellowing of peel was also found to be less in salicylic acid treated than those of control fruits. Thus, the control fruits were soft enough at day 8 of treatment while the salicylic acid treated fruits were still hard.

3.3. Effect of salicylic acid on pulp and peel contents of banana fruit during its ripening

Effect of salicylic acid on pulp and peel contents of banana fruits have been investigated during its ripening. Pulp content increased throughout the experimental period both in control and salicylic acid treated banana fruits. However, salicylic acid treatment resulted in decreased pulp content of fruits in a concentration dependent manner. On the other hand, peel content decreased throughout the ripening both in the absence as well as presence of salicylic acid. However, the decrease in peel content was less in salicylic acid treated fruits as compared with that of control. Because the pulp to peel ratio of fruits is taken as an important parameter for fruit ripening, we have presented the data in the form of their ratio. (Table 1). It is noteworthy that pulp to peel ratio increased with

<table>
<thead>
<tr>
<th>Concentration of salicylic acid (µM)</th>
<th>Pulp to peel ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0 (control)</td>
<td>1.40 ± 0.22</td>
</tr>
<tr>
<td>500</td>
<td>1.40 ± 0.22</td>
</tr>
<tr>
<td>1000</td>
<td>1.40 ± 0.22</td>
</tr>
</tbody>
</table>

*Each value represents a mean ± S.D. of three independent replicates.*
Fig. 2. Effect of salicylic acid on reducing (A), nonreducing (B) sugars and invertase (C) activity during ripening of banana fruit in the absence (■—■) and presence of 500 (■—■) and 1000 (▲—▲) μM salicylic acid. Each value represents a mean of three independent replicates.

3.4. Effect of salicylic acid on soluble sugar content of banana fruit during ripening

Effect of salicylic acid on reducing and nonreducing sugar contents of banana fruit has been studied during ripening. The results are shown in Fig. 2. Reducing sugar content increased both in control and salicylic acid treated fruits throughout the ripening (Fig. 2A). However, salicylic acid treatment resulted in, a concentration dependent manner, decreased levels of reducing sugar than those in the absence of salicylic acid (control) throughout the ripening period of banana fruits. On the other hand, the measurement of nonreducing sugar content of banana fruit revealed an opposite trend to that of reducing sugar, during ripening (Fig. 2B). Thus, on day 4 of ripening, salicylic acid treatment resulted in a decrease of total reducing sugar by 25 and 42% at 500 and 1000 μM salicylic acid concentration, respectively, while it resulted in an increase of nonreducing sugar by 9 and 20% at 500 and 1000 μM salicylic acid, respectively.

3.5. Effect of salicylic acid on invertase during banana fruit ripening

Effect of salicylic acid on invertase activity has been investigated during ripening of banana fruit. The results are shown in Fig. 2C. Invertase activity increased throughout the ripening both in control and in salicylic acid treated fruits. However, salicylic acid treatment resulted in decreased levels of invertase than those of controls, in a concentration dependent manner, during banana ripening. Thus, on day 4, salicylic acid treatment resulted in a decrease of invertase activity by 51 and 71% of the control value at 500 and 1000 μM salicylic acid concentrations, respectively, during the banana fruit ripening.

3.6. Effect of salicylic acid on cell wall degrading enzymes during banana fruit ripening

Effect of salicylic acid on the developmental profiles of major cell wall degrading enzymes namely, cellulase, polygalacturonase (PG) and xylanase in the absence and presence of salicylic acid have been investigated during ripening of banana fruits. Results are shown in Fig. 3. It is noteworthy that the activity of all the three major cell wall degrading enzymes increased gradually both in the absence and presence of salicylic acid during the ripening of banana fruits. However, the salicylic acid treatment resulted in decreased levels of all these cell wall degrading enzymes in a concentration dependent manner, during the ripening of banana. Thus, on day 6, the activities of cellulase, polygalacturonase and xylanase decreased by 55 and 82, 33 and 45, 35 and 50% of their respective
controls at 500 and 1000 μM salicylic acid, respectively during banana fruit ripening. Thus, out of the three cell wall degrading enzymes studied, cellulase seems to be most sensitive to salicylic acid inhibition. Furthermore, the two cell wall degrading enzymes namely, cellulase and polygalacturonase seems to play a major role in banana fruit ripening as their activities increased several folds during ripening in comparison to that of xylanase. Similarly, salicylic acid inhibited cellulase and polygalacturonase activity more strongly than that of xylanase.

3.7. Effect of salicylic acid on catalase and peroxidase during banana fruit ripening

As catalase and peroxidase have also been reported to be affected during fruit ripening, the effect of salicylic acid on these two enzymes has been investigated during banana fruit ripening. Results are shown in Table 2. Salicylic acid treatment resulted in decreased levels of catalase and peroxidase, than their respective controls, in a concentration dependent manner, during the ripening of banana fruits. Thus, the effect of salicylic acid on these two enzymes, during banana fruit ripening, revealed a similar response as observed for that of the pea during seedling growth [27].

4. Discussion

The data presented in this paper suggest that salicylic acid delays banana fruit ripening. Thus, the rapid rise in respiration during ripening of banana fruits, an observation typical of climacteric fruits, was found to be delayed by salicylic acid in a concentration dependent manner.

The ripening of banana fruit was accompanied by increase in pulp to peel ratio. Similar observation has been reported by others [28,29]. Rise in pulp to peel ratio during fruit ripening was suggested to be due to change in sugar concentration in the two tissues. A rapid increase in sugar contents in the pulp than those in the peel leads to a change in osmotic pressure, as a result of which water is withdrawn from the peel and hence pulp to peel ratio increases accordingly. Salicylic acid treatment reduced this increase in pulp to peel ratio, in a concentration dependent manner, leading to a delay in banana fruit ripening. Delay in ripening of banana is reported by ABA [30] and GA3 [31]. GA3 is also reported to delay ripening in tomato pericarp disc [31] and mango [32]. Post harvest dips of mature unripe Alphonso (variety of mango) fruits in solution of GA3 (10⁻⁶ M), IAA (10⁻⁶ M) and kinetin (10⁻⁵ M) are reported to delay ripening [33].

The data on soluble sugar content and invertase suggested that banana ripening was accompanied by an increase in the reducing sugar content and invertase activity concomitant with decrease in nonreducing sugar content. Salicylic acid treat-
Table 2
Effect of salicylic acid on catalase and peroxidase activities of banana fruits during ripening

<table>
<thead>
<tr>
<th>Concentration of salicylic acid (µM)</th>
<th>Catalase activity (U/ml)</th>
<th>Peroxidase activity (U/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number of days</td>
<td>Number of days</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>4th</td>
</tr>
<tr>
<td>0 (control)</td>
<td>0.091 ± 0.015</td>
<td>0.047 ± 0.011</td>
</tr>
<tr>
<td>500</td>
<td>0.091 ± 0.015</td>
<td>0.037 ± 0.007</td>
</tr>
<tr>
<td>1000</td>
<td>0.091 ± 0.015</td>
<td>0.029 ± 0.011</td>
</tr>
</tbody>
</table>

* Each value represents a mean ± S.D. of three independent replicates.
ment resulted in decreased levels of invertase and reducing sugar contents while an opposite effect on nonreducing sugar content, suggesting that salicylic acid delays banana fruit ripening. This accumulation of reducing sugars may be due to increased breakdown of starch during ripening as reported by Beaudry et al. [34]. Increased starch phosphorylase activity has been reported during development and ripening of banana fruits [35,36]. Recently, an increase in sucrose phosphate synthase and decrease in sucrose synthase activity have been reported during ripening of banana fruits [37]. Increase in invertase activity has been shown during papaya fruit ripening [38].

The data on cell wall degrading enzymes suggested that banana ripening was accompanied by an increase in all the three cell wall degrading enzymes namely, cellulase, polygalacturonase and xylanase. Levels of these cell wall degrading enzymes were found to be decreased, in a concentration dependent manner, suggesting that salicylic acid delays ripening of banana. Polygalacturonase is reported to be primarily responsible for ripening associated pectin degradation and fruit softening [39]. Level of polygalacturonase activity has been positively correlated with fruit ripening and softening in banana [29]. Polygalacturonase activity [40], mRNA [41] and gene transcription [42,43] are reported to increase at the onset of ripening of tomato fruits. Furthermore, tomato fruit ripening mutants, with delayed ripening exhibited decreased levels of polygalacturonase activity [44], mRNA [45] and gene transcription [43], while the activities of several other cell wall enzymes appeared to be affected to a lesser extent. Also, physical and chemical treatments which suppress ripening, inhibit polygalacturonase gene expression [46-48]. Carboxymethyl cellulase (Cm-cellulase) activity increases during ripening of tomato [49,50], strawberry, pear, peach [51] and avocado [52]. Modifications of hemicellulose structure of cell wall, during fruit ripening have been reported in tomato, pepper, strawberry and melons. The sizes of hemicellulose polymers are found to decrease during ripening of these fruits [39,53,54]. Ripening associated changes in hemicellulose structure are proposed to be responsible for the textural change in fruit.

Data on catalase and peroxidase revealed that ripening of banana fruit was accompanied by an increase in the activities of catalase and peroxidase while levels of these enzymes were found to be decreased in presence of salicylic acid, in a concentration dependent manner. Ripening of papaya fruit has been reported to be accompanied by an increase in the catalase and peroxidase activity [38]. Salicylic acid has been demonstrated to inhibit catalase and peroxidase in pea [26]. The inhibition of catalase by salicylic acid is suggested to be one of modes of action of salicylic acid in providing defense against pathogen attack [27,55].

Thus, the data presented here unequivocally suggest that salicylic acid delays banana fruit ripening. Based on these observations salicylic acid may be considered as antagonist of ethylene, a major fruit ripening hormone. Salicylic acid is reported to inhibit biosynthesis of ethylene [7,8]. Recently, salicylic acid has also been shown to inhibit ACC oxidase activity [56]. Even in the studies of Nissen [11] in which salicylic acid (at low concentration) has been shown to stimulate ethylene biosynthesis, an inhibition of ethylene production occurs at higher concentrations of salicylic acid ($>10^{-4}$ M). Therefore, in the present study since we have taken a much higher concentration of salicylic acid than those of Nissen [11] ($>10^{-4}$ M), only the inhibition of ethylene biosynthesis is expected to occur in our case. Ethylene synthesis is normally limited by the supply of the immediate precursor amino cyclopropane-1-carboxylic acid (ACC). There is a sharp rise in ACC synthase activity and the content of ACC during ripening [57]. Thus, present studies on the effect of salicylic acid in delaying the ripening of banana fruits may be thought of due to inhibition of ethylene biosynthesis and/or action. However, this needs further investigations.

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References


