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Effect of adenosine triphosphate on changes of fatty acids in harvested litchi fruit infected by *Peronophythora litchii*

Chun Yi[a,b], Yueming Jiang[a], John Shi[b], Hongxia Qu[a,*], Xuewu Duan[a], Bao Yang[a], Nagendra K. Prasad[a], Ting Liu[a]

[a] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China
[b] Food Research Center, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada N1G 5C9

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ABSTRACT

Recent investigations have shown that disease development of harvested horticultural crops may be attributed to a limited availability of energy or low energy production. In this study, litchi fruit were treated with 1.0 mM adenosine triphosphate (ATP) or 0.5 mM 2,4-dinitrophenol (DNP) and then half of the ATP-treated fruit were inoculated with *Peronophythora litchii*. The composition and contents of fatty acids (FAs) and esterase activity in litchi fruit during storage were investigated. Free fatty acids (FFAs) in all fruit increased over storage, especially in the *P. litchii*-inoculated fruit. In particular, the content of saturated FAs increased faster than unsaturated FAs. In polar lipids (PL), a decrease in the amount of C18:3 and an increase in the amount of C16:0 or C18:0 was found during storage, while the proportions of C16:0, C18:0 and C18:1 in neutral lipid (NL) gradually increased but the proportions of C18:3 decreased during storage. The proportion of C18:2 increased within the first four days and then decreased. Exogenous ATP treatment suppressed the release of FFAs and increased the contents of each FA in PL, indicating a slower hydrolysis of lipids. ATP treatment also delayed the increase in the proportion of C18:0 in NL. Further analysis showed that the double bond index (DBI) of litchi fruit decreased in all fractions of FAs and ATP treatment can slow the decrease in DBI. In addition, lower esterase enzyme activity was detected in all ATP-treated fruit. Treatment with DNP (a respiration uncoupler) increased esterase activity. *P. litchii*-inoculated fruit after ATP treatment also exhibited similar trends in delaying the release of FFAs. Enhanced disease resistance of litchi fruit by ATP could involve the levels of FAs and esterase activity.

1. Introduction

The onset of senescence of fruit either during natural senescence or resulting from environmental stress is initially characterized by irreversible loss of membrane integrity in connection with increased permeability, decreased fluidity, loss of unsaturated fatty acids, accumulation of peroxidation products and loss of cellular compartmentalization (Marangoni et al., 1996). As a natural cell barrier or location of signal receptor proteins, cell membranes are one of the determinant factors for the maintenance of host–pathogen balance (Marangoni et al., 1996). The changes in the composition of fatty acids (FAs) are associated with membrane alterations during ripening or senescence (Lurie et al., 1995) and are involved also in plant response to diverse environmental stresses, including pathogen attack (Palma et al., 1995; Feussner and Wasternack, 2002; Norman et al., 2008).

Involvement of adenylate nucleotides in FA synthesis and lipid metabolism in plants is well established (Pradet and Raymond, 1983; Harwood, 1988; Ohlrogge and Browse, 1995; Rawyler et al., 1999). Energy is essential for maintenance of membrane integrity. The depletion of adenosine triphosphate (ATP) reduces lipid synthesis ability and diminishes desaturation of acyl chains under anoxia (Brown and Beevers, 1987; Rawyler et al., 1999). Rawyler et al. (1999) calculated a threshold ATP production rate to preserve potato cell membrane integrity. Saquet et al. (2000, 2001, 2003) suggested a probable relationship between energy levels and the development of browning disorders in 'Conference' pears and 'Jonagold' apples. The lower levels of browning index and membrane ion leakage from pure oxygen treatment in litchi pericarp was also correlated with higher ATP and ADP levels (Duan et al., 2004).

Exogenous supply of ATP is an effective method to elevate energy levels in potato (Rawyler et al., 1999) and litchi (Song et al., 2006; Yi et al., 2008). Energy depletion could lead to fumonisin B1-induced cell death while the addition of ATP could prevent cell death (Chivasa et al., 2005). According to Loseva et al. (2004), exogenous ATP treatment has a positive influence on the defense reaction of *Chlorella* cells infected by *Mycoplasma*. Therefore, as the major

* Corresponding author. Tel.: +86 20 37252960; fax: +86 20 37252831.
E-mail address: q-hxia@scib.ac.cn (H. Qu).

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donor of free energy in biological systems, enough available ATP is vital for living cells to survive under environmental stress.

Litchi fruit are susceptible to downy mildew caused by *Peronophythora litchii* during storage. Our previous study (Yi et al., 2008), which focused on disease response to exogenously applied ATP, reported that exogenous ATP treatment could elevate endogenous ATP levels and thus maintain membrane integrity, delay browning and pathogen infection in litchi fruit. As a companion study, the objective of the present research was to examine whether increased endogenous ATP levels by the exogenous supply of ATP were related to the changes in FA composition and esterase activity in litchi pericarp during storage. The effect of the respiration uncoupler 2,4-dinitrophenol (DNP) on esterase activity was also investigated.

2. Materials and methods

2.1. Plant materials and treatments

Fruit of litchi (*Litchi chinensis* Sonn.) cv. Huaizhi were harvested fully colored (commercially mature stage) from a farm in Guangzhou, P.R. China. Fruit were selected for uniformity of shape, color and free of blemish or disease. A total of 1200 fruit was used for the experiment. The fruit were surface-sterilized in 0.5% sodium hypochlorite solution for 5–10 s, and washed with sterile distilled water. The fruit were divided into three groups (400 fruit/group) and infiltrated in a desiccator which contained sterile distilled water (control), 1.0 mM ATP or 0.5 mM DNP solution (200 fruit/L) under vacuum (75 kPa for 3 min). After air-drying, each group of the fruit was divided into two subgroups.

2.2. Pathogen inoculation

Spores of *P. litchii* were isolated from infected litchi fruit and then cultured on potato dextrose agar (PDA) medium at 28 °C. A suspension of 1×10^6 spores/L was prepared by counting the spores with a hemacytometer under a light microscope. One subgroup (200 fruit) of each of non-treated and ATP- or DNP-treated fruit were inoculated by making four equidistant 0.5-mm-deep punctures in the pericarp around the fruit equator with a 1-mm-wide sterile nail, then dipped into the spore suspension for 2 s. All the fruit were placed into covered plastic boxes (six fruit/box) and then stored at 25 °C and 90% relative humidity.

2.3. Lipid analysis

Lipids were extracted according to Saquet et al. (2003) with slight modifications. Fruit pericarp tissues (3 g) from 20 fruit were ground to powder in liquid nitrogen with a mortar and a pestle and then extracted with 9 mL hexane: isopropanol (3:2, v/v) for 30 min, and finally centrifuged at 6000 × *g* for 10 min. The residue was extracted and centrifuged again as mentioned above. According to Kaluzny et al. (1985), the extract was evaporated to dryness under N_2 and redissolved in 2 mL hexane, and then 0.5 mL was taken for total lipid analysis while other 1.5 mL was applied to an aminopropyl column (Supelco Inc. Bellefonte, PA) to separate neutral lipid (NL), free fatty acid (FFA), and polar lipid (PL). Elution was sequentially conducted by 4 mL of chloroform: isopropanol (2:1, v/v, NL), 4 mL of ether: acetic acid (99:1, v/v, FFA) and 4 mL of methanol (PL) and collected separately. All fractions were saponified with 0.2 mL of 25% ammonia solution for 60 min at 75 °C, dried under N_2 and methylated with 10 mL of 5% concentrated H_2SO_4–methanol (v/v) at 80 °C for 1 h. The fatty acid methyl esters were partitioned into hexane (2 mL) and then analyzed using a Packard FID GC (6890N, Aglient) equipped with an automatic sampler (Aglient 7683, USA) and HP–FFAP capillary column (30 m × 0.32 mm × 0.25 μm) programmed from 50 to 200 °C at 25 °C/min, and 200 to 230 °C at 5 °C/min with an initial hold for 1 min. Other performance conditions were as follows: injector temperature, 250 °C; detector temperature, 280 °C; splitter ratio, 50:1; inject volume: 1 μL and hydrogen flow, 40 mL/min. Identification of individual fatty acid methyl ester was made using known standards (Supelco Inc., Bellefonte, PA, US). Samples were quantified against a heptadecanoic acid (C17:0) as an internal standard, and the amount of each fatty acid was expressed as an absolute amount (μg) in 100 g fresh weight (FW) sample or a percentage of the total lipids present in the sample. The double bond index (DBI) was calculated using the following equation: DBI = ([18:1] + 2 × [18:2] + 3 × [18:3])/([16:0] + [18:0]).

2.4. Esterase activity

Esterase activity was assayed spectrophotometrically after hydrolyzing α-naphthyl acetate according to the method of Aspern (1962). Litchi pericarp tissues (2 g) from each treatment from 20 fruit were ground to a fine powder and then extracted with 10 mL of 0.2 M phosphate buffer (pH 7.8) containing 0.05 M mercaptoethanol. One unit of esterase activity was defined as the amount that caused a change of 0.001 in absorbance at 520 nm/min. Protein content was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

2.5. Data analyses

The experiment design was completely randomized. There were three replicates of each treatment. The measurements were carried out in at least one repeat experiment. Data were analyzed by ANOVA and means were compared using least significance difference (LSD) or Duncan's new multiple range test (DMRT) by SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Composition of lipids at harvest

The major fraction of lipids was determined to be NL (Table 1). The amount of NL was approximately three times higher than that of PL or FFA. PL, most of which are phospholipids, seems to share the equivalent FAs pool as FFAs. As FFAs are generally found to be released continuously during fruit ripening and senescence (Song and Bangerth, 2003), the comparable content of FFAs indicates that the freshly harvested litchi fruit may enter the senescence process. In this study, the major FAs were analyzed, with the following order: linoleic acid (18:2) (42–51%), palmitic acid (16:0) (16–25%), linolenic acid (18:3) (10–25%), oleic acid (18:1) (7–16%) and stearic acid (18:0) (4–8%).

3.2. Effect of ATP on FFAs

Proportional increases in contents of FFAs were observed (Table 2), which might be attributed to the degradation of lipids. Without inoculation with *P. litchii*, contents of C16:0, C18:0 and C18:1 in control fruit increased markedly within the first four days and then reached up to 2.46, 2.82 and 2.63 times by the end of storage, respectively, as compared with those of fruit at harvest. Content of C18:2 increased 1.41 times within the first four days and then stayed constant while C18:3 increased 1.38 times in the first four days but was not detected in the last two days. The ATP treatment delayed the increase in the content of each FA. After four days of storage, both the contents of saturated FAs (C16:0 and C18:0) and the polyunsaturated FAs (C18:2 and C18:3) were less than those in non-ATP-treated fruit (25% less than for C16:0, 59% for C18:0, 30% for C18:2 and 27% for C18:3) and the content