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Hydrogen Peroxide Application Reduced Fruit Abscission on Chilling-Stressed *Syzygium samarangense* Merr. et Perry

Fruchtfallreduktion nach Kältestreß durch Applikation von H_2O_2

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Summary

We evaluated the effects of H_2O_2 pretreatment on the ability of 8 year old wax apple (*Syzygium samarangense* Merr. et Perry) trees to withstand chilling stress in Pingtung, Taiwan during the winter of 1998-1999. Plants pretreated with H_2O_2 at 10 mM exhibited the lowest rate of fruit abscission of 9.3 %, followed by 13 % and 28 % for 50 mM and 100 mM pretreatment, respectively, five days after a cold spell of 7.8 °C. The control plants had 26 % fruit abscission rate evaluated five days after the chilling stress. The theoretical lowest rate of fruit abscission was 2 % with 26 mM hydrogen peroxide treatment, estimated from the line of best fit of the rate of fruit abscission with the concentration of hydrogen peroxide. No statistical significance of the treatment effects on fruit parameters including length, width, weight, and total soluble solid (Brix) was found in this study. The potential economic benefits of hydrogen peroxide treatment on wax apple trees to reduce the loss of chilling-induced fruit abscission is profound, provided due care is taken with regard to public health and environmental safety.

Zusammenfassung

Gegenstand der Untersuchung ist es abzuschätzen, ob eine Vorbehandlung mit Wasserstoffperoxid (H_2O_2) die Widerstandskraft von acht Jahre alten Wachsäpfelbäumen (*Syzygium samarangense* Merr. et Perry) gegen Kältestress in Pingtung, Taiwan während des Winters 1998/1999 erhöhte. Pflanzen, die mit 10 mM H_2O_2 vorbehandelt wurden, zeigten fünf Tage nach einer Kältewelle mit 7.8 °C die niedrigste Fruchtfallrate mit 9.3 %, gefolgt von 13 % und 28 % bei einer Vorbehandlung mit 50 mM und 100 mM. Die Kontrollpflanzen wiesen zum selben Zeitpunkt 26 % Fruchtfall auf.

Die theoretisch mögliche niedrigste Fruchtfallrate von 2 % bei einer Behandlung mit 26 mM H_2O_2 ergibt sich aus der angepassten Funktionskurve aus Fruchtfallrate und H_2O_2-Konzentration. In dieser Untersuchung konnten keine signifikanten Behandlungseffekte auf Fruchtmerkmale wie Länge, Breite, Gewicht und Gehalt an löslichen Kohlenhydraten (Brix) festgestellt werden. Der mögliche wirtschaftliche Nutzen einer H_2O_2-Behandlung von Wachsäpfelbäumen zur Reduktion des kältestress-bedingten Fruchtfalls ist, unter der Voraussetzung, daß Volksgesundheit und Umweltbelange berücksichtigt werden, beträchtlich.

Introduction

Taiwan located in the subtropics between 22°–26° N latitude. The mean monthly temperature ranges from 17 °C to 27 °C. However, occasional cold spells with temperatures of 10 °C or lower occur. The wax apple (*Syzygium samarangense* Merr. et Perry), a tropical fruit native to South-east Asia, has been transplanted to Taiwan and is one of the top cash crops. This combination makes the wax apple unique for studying chilling injury and its prevention. According to the fruit grower, waxapple fruit can withstand 10 °C cold spells without symptoms of chilling injury including fruit abscission. However, the bell and pre-harvest stages are the most susceptible stages to chilling stress. The fruit usually start to abscise within 12 hours of initial exposure to cold temperatures. A drastic increase in temperature on the day following the chilling might cause severe abscission.

Several methods to minimize or alleviate chilling injuries to crops have been reported. Mefluidide has been reported to reduce chilling injuries in maize and rice (TSENG et al. 1986, ZHANG et al. 1987). Lab 173711, a terpenoid analogue of abscisic acid (ABA), increased chilling tolerance in mung beans (CHEN and LIN 1993), and GLK-8903 ameliorated *Phaseolus vulgaris* L, (FLORES-NIMEDEZ and LI 1993).

Most plants have developed scavenger systems to protect themselves from damage caused by superoxides and peroxides produced during exposure to low temperatures, including superoxide dismutase, catalase, ascorbate/ascorbate peroxidase (FOYER et al. 1994; ASADA et al. 1998) and total glutathione (LIN et al. 2000).

PRASAD et al. (1994a, 1994b) showed that a 3-day-old dark-grown maize seedlings developed a 4-fold increase in H_2O_2 content at 4 °C for 7 days before they died. However, when the seedlings were pretreated with 0.1 mM H_2O_2 at ambient temperature then chilled at 4 °C for 3 days (PRASAD et al. 1994a), or treated with 1 mM ABA for 24 h at 27 °C and chilled at 4 °C for 7 days (ANDERSON et al. 1994, 1995), they were able to survive. PRASAD et al. and ANDERSON et al. concluded

that H_2O_2 pretreatment imposed a transient signal that induced mitochondrial catalase isozyme-3 and peroxidase, and thus protected the mitochondria. Whether the same principle could be applied to chilling-sensitive tropical fruit such as the wax apple is yet to be evaluated. The object of this study is to test whether H_2O_2 pretreatment of wax apple leaf induced a radical scavenger system, thus reducing the chilling-induced fruit abscission in field conditions.

Materials and Methods

Eight year old wax apple trees grown in a commercial orchard near Pingtung city (22° N latitude) were used for this experiment. Trees planted in a 5 m x 5 m grid, with 60 cm diameter trunks at soil surface, had been grown in open field conditions without temperature or photoperiod controls. In preparation for the unpredictable arrival of cold spell that reached below 10 °C, five field plots representing the susceptible stage of fruit development were selected and readied for cold treatment. A simple random block design was used to assign each treatment; there were a total of four treatments, each with four trees. One day before a forecast of the arrival of a cold spell below 12 °C, the trees were sprayed with 10 mM, 50 mM or 100 mM H_2O_2 containing 0.05 % triton-x100 as a surfactant until the leaves were completely wet. Plants not sprayed served as the control. The trees were sprayed 15 hours before the night temperature reached the lowest point of 7.8 °C at 6:00 A.M.

In order to measure fruit abscission accurately, fruit bunches containing 5–6 fruits were bagged in lightweight white paper bags prior to treatment; 10 bags per tree were randomly selected and were assessed for the percentage of fruit abscission. Percent fruit abscission, fruit length, fruit width, fruit weight and total soluble solid (Brix) were recorded 5 and 9 days after spraying. Ten fruit samples were recorded for each tree. Fruit length and width were measured using a ruler, and total soluble solid was determined using a refractometer.

A statistical software package STATISTICA was used to perform the statistical analysis. We used Microsoft Excel 97 to prepare the graphic results. In order to reduce the field experimental error, the most outlying point was excluded from the data set and only three out of the four data points were entered for analysis.

Results and Discussion

There were three cold spells that reached 13 °C or below in the Pingtung area during the winter of 1998-1999. On February 3, 1999, the weather forecast indicated the temperature would reach 10 °C in Pingtung area. At a weather station within 2 km of Pingtung city, 9.7 °C was recorded at 6:00 A.M. on February 4; the temperature was below 10 °C for a total of 5 hours. At the open field test spot, 28 km south of Pintung city, the temperature was 7.8 °C at 6:00 A.M. on February 4, 1999. The data in this report represent the sole field plot that experienced a cold spell that reached below 10 °C during 1998 and 1999 in the area. As the fruit are susceptible to chilling stress below 10 °C, the temperature drop to 7.8 °C was deemed adequate for the purpose of this study.

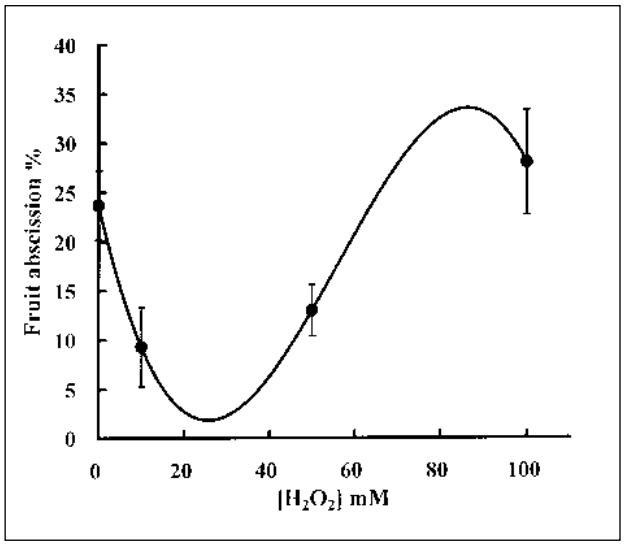


Fig.1. Polynomial regression plot for the rate of fruit abscission vs hydrogen peroxide concentration on waxapple plants.
Rate of fruit abscission, $y=-0.0003x^3+0.0477x^2-1.8882x+23.7$.
x: denote hydrogen peroxide concentration.
The vertical bars denotes standard deviation from average.
Polynominale Regressionskurve der Fruchtfallrate und der H_2O_2-Konzentration bei Wachsapfelpflanzen.
Fruchtfallrate, $y=-0.0003x^3+0.0477x^2-1.8882x+23.7$. x: steht für die H_2O_2-Konzentration.
Die vertikalen Striche stellen die Standardabweichung dar.

The mean rates of fruit abscission for H_2O_2 treatment at 10 mM, 50 mM and 100 mM were 9.3 %, 13 % and 28 %, respectively. Without H_2O_2 pretreatment, the control group had a 23.7 % fruit abscission rate. H_2O_2 pretreatment for 15 hours had a protective effect on wax apple trees from the damage of cold temperatures under the field conditions of 7.8 °C. The deviation data showed a difference between the mean fruit abscission rates of the control and both 10 mM H_2O_2 and 50 mM treated groups. However, no difference was found between the mean fruit abscission rates of control and 100 mM H_2O_2 treated groups. Therefore, the upper most threshold of H_2O_2 concentration on wax apple trees for protection against cold temperatures was between 50 and 100 mM (Fig. 1).

A significant correlation between the rate of fruit abscission and the concentration of hydrogen peroxide treatment was found in this study ($r=0.898$, $n=9$, $p<0.01$). Fig. 1 shows the estimated polynomial regression line, $y=-0.0003x^3+0.0477x^2-1.8882x+23.7$, where y is the rate of fruit abscission and x denotes the concentration of hydrogen peroxide treatment. The theoretical lowest possible rate of fruit abscission was 2 % under 7.8 °C temperature conditions with 26 mM hydrogen peroxide treatment. Although a second set of data was also collected at 9 days after the chilling stress, the fruit abscission rate remained the same as in the first fruit data collection. Hence, the experiment was terminated at 9 days after the cold spell.

Fruit parameters including length, width, weight and total soluble solid of the abscised fruits were not significantly different from that of intact fruits among the

Table 1. Comparison of H_2O_2 application at four rates on the percent fruit abscission, fruit length, fruit width, fruit weight and total soluble solid of waxapple plants under chilling stress. The numbers denote average±standard deviation. (I: denote intact unabscised fruit; A: denotes abscised fruit).
Wirkung von vier H_2O_2-Behandlungsstufen auf den Fruchtfall, die Fruchtlänge, die Fruchtbreite, das Fruchtgewicht und Gehalt an löslichen Kohlenhydraten (Brix) von Wachsäpfelpflanzen unter Kältestress. Numerisch dargestellt sind Mittelwert ± Standardabweichung. (I: bedeutet intakte Frucht; A: bedeutet abgestoßene Frucht)

H_2O_2		Fruit abscission %	Fruit Length mm	Fruit Width mm	Fruit Weight gm	Total Soluble Solid Brix
0 mM	I	76.3±3.5	66.3±1.5	66.6±1.1	110.0±1.9	9.0±0.1
	A	23.7±3.5	61.4±1.3	68.5±2.4	104.5±4.4	7.9±0.3
10 mM	I	90.7±4.0	64.1±0.3	68.3±2.0	104.7±7.4	8.7±0.6
	A	9.3±4.0	59.8±1.6	64.7±0.2	96.8±6.5	7.3±0.8
50 mM	I	87.0±2.6	60.1±1.8	66.8±4.1	113.3±3.8	9.1±0.3
	A	13.0±2.6	53.8±0.6	62.8±1.2	95.6±7.5	9.5±1.1
100 mM	I	72.0±5.3	63.8±1.0	67.1±2.0	111.0±9.7	8.5±0.3
	A	28.0±5.3	61.7±1.8	67.1±0.6	96.4±6.7	8.1±0.5

treated groups (Table 1). Since the duration between chilling stress and data collection was only 5 days apart, immense differences in growth parameters were not expected.

The plant material for this experiment is native to tropical South East Asia. The introduction of the plant to subtropical Taiwan poses a potential stress to the plants due to cold spells with temperature 10 °C or below during the winter season. Any treatment that can delay or reduce chilling symptoms indicates a significant physiological difference and potential use in agriculture. PRASAD et al. (1994a, 1994b) found that a transient oxidizing treatment with H_2O_2 induced an increased level of antioxidant, which is consistent with our result that pretreatment of wax apple trees with H_2O_2 produces a significant increase in tolerance to chilling. PRASAD et al. (1994a) and ANDERSON et al. (1994) found that ABA treatment of maize seedlings produced levels of catalase isozyme-3 in mitochondria similar to those produced by H_2O_2. CHEN and LIN (1993) reported acclimation effects in LAB173711, an ABA analogue, on mung bean. Whether this acclimation effect was mediated by catalase isozyme-3 in the maize seedling system has not been tested.

A transient increase in H_2O_2 might explain the acclimation effect of H_2O_2 induced chilling tolerance as conducted in this experiment. LIN et al. (2000) found that 8 °C treatment of two mung bean seedlings for 36 hour induced a higher glutathione level, a 32.8 % increase for TN 5, a more chilling-sensitive cultivar; and a 102.2 % increase for V3327, a less chilling-sensitive cultivar, respectively. The work of LIN et al. (2000) partly matched that of KOCSY et al. (1996, 1997), who found that a chilling tolerant variety of maize was characterized by a more rapid synthesis of glutathione at 12 °C, a more rapid induction of adenosine-5'-phosphosulfate sulfotransferase after chilling, and a higher level of glutathione reductase. As conducted in our experiment, the H_2O_2 pretreatment imposed a transient oxidative signal that induced the scavenger systems including a higher level of glutathione in the ascorbate-glutathione cycle. This theory is supported by the fact that 10 mM and 50 mM H_2O_2 provide better chilling protection over 100 mM H_2O_2 pretreatment, and are significantly different from the control group. Thus, artificial induction of the scavenger system by H_2O_2 pretreatment is probably an important factor in establishing chilling tolerance in this species.

Hydrogen peroxide is a colorless liquid with a slightly sharp odor. Since it is an oxidizer, the application of hydrogen peroxide in open fields may cause public health and environmental concerns. Exposure to hydrogen peroxide can irritate the eyes, nose and throat. Accidental ingestion may cause corneal ulcer or erythema, while direct contact may cause vesicles on skin and bleached hair. The IDLH (Immediate Danger to Life or Health) level is 75 ppm according the hand book of NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (1985). However because it is a non-toxic compound, hydrogen peroxide is commonly used as an external disinfecting reagent. The irritation hazards can be reduced by spraying in a confined orchard using an automatic device. Since it is a common practice of the grower to bag the fruits with paper to minimize insect damage, the residue of hydrogen peroxide on the fruit is avoided. Low concentrations of hydrogen peroxide decompose quickly in the field as it is a strong oxidizer. We recommend the orchard worker wears a respirator in the field while spraying the hydrogen peroxide. This will minimize the health hazard of applying hydrogen peroxide in the field.

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