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Research article

The influence of hydrogen peroxide on the growth, development and quality of wax apple (*Syzygium samarangense*, [Blume] Merrill & L.M. Perry var. *jambu madu*) fruits

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ABSTRACT

The present study represents the first report of the effect of hydrogen peroxide (H_2O_2) on the growth, development and quality of the wax apple fruit, a widely cultivated fruit tree in South East Asia. The wax apple trees were spray treated with 0, 5, 20 and 50 mM H_2O_2 under field conditions. Photosynthetic rates, stomatal conductance, transpiration, chlorophyll and dry matter content of the leaves and total soluble solids and total sugar content of the fruits of wax apple *(Syzygium samarangense, var. jambu madu)* were significantly increased after treatment with 5 mM H_2O_2. The application of 20 mM H_2O_2 significantly reduced bud drop and enhanced fruit growth, resulting in larger fruit size, increased fruit set, fruit number, fruit biomass and yield compared to the control. In addition, the endogenous level of H_2O_2 in wax apple leaves increased significantly with H_2O_2 treatments. With regard to fruit quality, 20 mM H_2O_2 treatment increased the K^+, anthocyanin and carotene contents of the fruits by 65%, 67%, and 41%, respectively. In addition, higher flavonoid, phenol and soluble protein content, sucrose phosphate synthase (SPS), phenylalanine ammonia lyase (PAL) and antioxidant activities were recorded in the treated fruits. There was a positive correlation between peel colour (hue) and TSS, between net photosynthesis and SPS activity and between phenol and flavonoid content with antioxidant activity in H_2O_2-treated fruits. It is concluded that spraying with 5 and 20 mM H_2O_2 once a week produced better fruit growth, maximising the yield and quality of wax apple fruits under field conditions.

1. Introduction

The wax apple or *jambu air madu*, is a nonclimacteric tropical fruit from the Myrtaceae family and botanically identified as *Syzygium samarangense* [1]. Wax apple is widely cultivated throughout Malaysia, mainly as smallholdings ranging from 1 to 5 ha, with a total hectarage estimated at 1500 ha in 2005 [2]. The fruits are pear shaped, usually pink, light red or red, sometimes greenish-white or cream-coloured, often crisp, with a subtle sweet taste and an aromatic flavour. In Malaysia, *jambu air madu* fruits are eaten raw with salt or cooked as a sauce. Fruit production is non seasonal and almost all of the fruit is edible. The fruit pulp is a rich source of phenolics, flavonoids and several antioxidant compounds and as a result it is believed to have great potential benefits for human health. It has become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3USD per kilogramme and has the potential to bring great benefits to local farmers and the country's economy.

Hydrogen peroxide (H_2O_2) is a stable, partially reduced form of oxygen, and its rapid turnover is characteristically mediated by enzyme action. H_2O_2 plays a dual role in plants. At low or normal concentrations (1–5 μmol g^{-1} FW), it acts as a messenger molecule involved in adaptive signalling, triggering tolerance against various abiotic stresses and at high concentrations (above 7 μmol g^{-1} FW), it orchestrates programmed cell death [3,4]. H_2O_2 provides a host of benefits by cleansing water of harmful substances such as spores, dead organic material and disease-causing organisms while preventing new infections from occurring. H_2O_2 is of great use in hydroponics and soilless gardening and is sometimes used for root initiation in cuttings. Recently, Chun-yanl et al. [5] reported that spraying H_2O_2 on *Brassica campestris* plants increased their antioxidant levels. An important factor determining fruit quality is sweetness, and Ozaki et al. [6] recently reported that the application of H_2O_2 enhanced sweetening in melon fruits. They investigated the effect of H_2O_2 on photosynthetic activity and its effects on selected Calvin cycle enzymes.

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Currently, no information is available in the literature on the effects of H_2O_2 on wax apple fruit growth. In this study, the effects of H_2O_2, applied by spraying, on the growth and development as well as the quality of the wax apple fruit was investigated under field conditions. It is proposed that the application of H_2O_2 can affect or promote the growth, development and quality of the wax apple fruit.

2. Results

2.1. Leaf dry matter and chlorophyll contents

As shown by the results listed in Table 1, hydrogen peroxide (H_2O_2) treatment had a significant effect on leaf dry matter contents in all seasons. In the second season, we found that the branches treated with 5 mM H_2O_2 appeared healthier than those of the control and exhibited a higher leaf dry matter content, 1.2-fold that of the control. This was followed by 20 and 50 mM H_2O_2 treatments. Leaves from the control treatment showed the lowest leaf dry matter content. Similar findings were recorded in the first and third seasons. Regarding the chlorophyll content, which indirectly indicates the health status of a plant, chlorophyll content was significantly higher in the leaves of the treated branches. As shown in Table 1, in the second season, the chlorophyll levels in all treated leaves were higher than in the control, up to 24% higher for the 5 mM H_2O_2 treatment. The differences between the treatments and the control were statistically significant in all three seasons.

2.2. Photosynthesis, stomatal conductance and leaf transpiration

To measure the activity level of photosynthetic carbon metabolism, we determined the photosynthetic activity in terms of μmol CO_2 fixed m^{-2} s^{-1}. H_2O_2 treatments increased the leaf photosynthetic activity considerably; this effect was statistically significant in the observations of the 2010–2011 season. The activities were 1.6-, 2.4- and 2.4-fold higher than the control at 350 ppm CO_2 and light intensities of 400, 800 and 2000 μmol m^{-2} s^{-1}, respectively, in the leaves treated with 20 mM H_2O_2 (Fig. 1A). Leaf photosynthesis was the highest with the 20 mM treatment, followed by the 5 and 50 mM treatments, in that order, whereas the control leaves evidenced the least photosynthesis. The photosynthetic activity of H_2O_2-treated leaves was also significantly higher than the control in the 1st and 2nd seasons (data not shown). H_2O_2 treatment produced a significant effect on the stomatal conductance of the leaves. The highest stomatal conductance was observed with the 50 mM treatment, followed by the 5 and 20 mM treatments, with values of 0.09, 0.08 and 0.07 mol H_2O m^{-2} s^{-1}, respectively, at a light intensity of 400 μmol m^{-2} s^{-1}. The lowest stomatal conductance was recorded in the control treatment (Fig. 1B). Similar to results obtained in photosynthesis measurements, leaf transpiration was also affected by H_2O_2 treatment. The transpiration rates of the H_2O_2-treated leaves were significantly higher than that of the untreated leaves (Fig. 1C). The leaf transpiration rates were 3.30-, 3.90- and 3.70-fold higher than the control with the 5, 20 and 50 mM treatments, respectively, at a light intensity of 800 μmol m^{-2} s^{-1} (Fig. 1C). As shown in Fig. 1D, we observed that transpiration had a strong relationship (R^2=0.80) with the stomatal conductance of the leaves: transpiration increased proportionally with stomatal conductance.

2.3. Bud drop, fruit set and fruit drop (%)

In the 1st season, H_2O_2 treatment significantly reduced bud abscission numbers, averaging approximately 36% (Table 1). In the three successive growing seasons, treatment with 20 mM H_2O_2 yielded the best results, followed by in order, the 50 and 5 mM H_2O_2 treatments. In the 2010–2011 season, almost 1.7 times as many buds dropped from the untreated branches as the treated branches, where the control branches had a bud drop of approximately 48%. This result showed that H_2O_2 had a positive effect on the bud development and reduced bud drop. Furthermore, the data in Table 1 shows that fruit set was increased almost 1.5- fold on the 20 mM H_2O_2-treated branches compared to the control, followed by in order, the 50 and 5 mM H_2O_2 treatments in the 2nd season; this effect was statistically significant in all the three seasons. H_2O_2 treatment had a significant effect on fruit drop in all seasons. For the 2009–2010 season, our results showed that the control branches experienced the highest (40%) premature fruit drop whereas the 5 mM H_2O_2 treatment had the lowest (28%) percentage of fruit drop, followed by 20 and 50 mM H_2O_2 treatments. Similar observations were recorded in the 2008–2009 and 2010–2011 seasons (Table 1).

Table 1
Effects of hydrogen peroxide treatment on leaf dry weight, chlorophyll content, bud drop, fruit set, fruit drop, yield and dry matter content in wax apple based on harvest values for first (conducted in Malaysian Agricultural Research and Development Institute station) and 2nd and 3rd (conducted in Banting, Selangor) seasons.

Treatment (mM)	Leaf (g/DW/leaf)	Chlorophyll SPAD value	Bud drop (%)	Fruit set (%)	Fruit drop (%)	Yield (kg)	% dry matter in fruits (g/100 g)
Season 1 (December 2008/April 2009)							
Control	1.12 ± 0.08c	55 ± 3.1c	42 ± 2.4a	27 ± 2.0d	43 ± 4.9a	0.25 ± 0.02c	2.22 ± 0.03c
H_2O_2 5	1.53 ± 0.04a	68 ± 2.5a	40 ± 3.0a	30 ± 2.4c	30 ± 3.2d	0.36 ± 0.03a	2.94 ± 0.05ab
H_2O_2 20	1.51 ± 0.03a	64 ± 4.6b	31 ± 1.7c	33 ± 2.5a	35 ± 3.6c	0.39 ± 0.02a	3.16 ± 0.07a
H_2O_2 50	1.38 ± 0.03b	65 ± 2.7ab	34 ± 2.7b	31 ± 3.7b	39 ± 3.0b	0.30 ± 0.02b	2.86 ± 0.06b
	**	**	*	*	**	**	**
Season 2 (May 2009/October 2010)							
Control	1.06 ± 0.08c	53 ± 3.1b	40 ± 0.9a	26 ± 4.40c	40 ± 4.58a	0.26 ± 0.06c	2.28 ± 0.04c
$H_2O_2$5	1.30 ± 0.12a	67 ± 4.5a	39 ± 2.0a	29 ± 5.85b	28 ± 2.18b	0.38 ± 0.04b	2.78 ± 0.08b
$H_2O_2$20	1.24 ± 1.24ab	66 ± 1.8a	33 ± 1.5a	38 ± 3.80a	34 ± 6.00a	0.43 ± 0.01a	3.07 ± 0.11a
$H_2O_2$50	1.15 ± 1.15bc	65 ± 2.8a	35 ± 3.1a	32 ± 4.60a	36 ± 5.00a	0.39 ± 0.13b	2.42 ± 0.13c
	*	**	ns	*	**	**	**
Season 3 (December 2010/ May 2011)							
Control	1.07 ± 0.06c	56 ± 3.2c	48 ± 2.9a	25 ± 4.40b	42 ± 6.00a	0.28 ± 0.02c	1.97 ± 0.06c
H_2O_2 5	1.41 ± 0.03b	75 ± 4.5a	39 ± 2.bb	27 ± 2.88b	33 ± 2.30c	0.36 ± 0.02ab	2.69 ± 0.09a
H_2O_2 20	1.46 ± 0.04a	67 ± 6.2b	29 ± 2.9d	37 ± 5.76a	35 ± 5.80bc	0.41 ± 0.01a	2.70 ± 0.14a
H_2O_2 50	1.44 ± 0.03ab	68 ± 6.1b	33 ± 2.5c	34 ± 6.09a	38 ± 5.36b	0.34 ± 0.02b	2.53 ± 0.10b
	**	**	**	**	**	**	**

Means (±S.E) within the same column followed by the same letter do not differ significantly according to the LSD test at $\alpha = 0.05$; ns, not significant; *, significant at the 0.05 levels; **, significant at the 0.01 level.