

## Stimulation of Ethylene Production of 'Irwin' Mango Leaves by $\text{KH}_2\text{PO}_4$ Treatment under Light

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### Summary

The effects of  $\text{KH}_2\text{PO}_4$  with/without NaOH on ethylene production of 'Irwin' mango leaves under light and dark was investigated. The experiment was a completely randomized design (CRD) with 4 treatments; 1)  $\text{KH}_2\text{PO}_4$  (Light), 2)  $\text{KH}_2\text{PO}_4$ +NaOH (Light), 3)  $\text{KH}_2\text{PO}_4$  (Dark), 2)  $\text{KH}_2\text{PO}_4$ +NaOH (Dark). Five discs of leaf were immersed with these solution for 4 days. Results showed that ethylene production rate were clearly higher under light than dark conditions. The ethylene production rate tend to increase in all treatments, with significantly higher in  $\text{KH}_2\text{PO}_4$  and  $\text{KH}_2\text{PO}_4$ +NaOH under light when compared to dark conditions. In addition,  $\text{KH}_2\text{PO}_4$  applied alone had significantly higher  $\text{CO}_2$  production rate in the dark than the light conditions, this may due to dark respiration. This result indicates that  $\text{KH}_2\text{PO}_4$  stimulated ethylene production of 'Irwin' mango leaves under light condition.

### Introduction

Ethylene is a gaseous hormone which plays an important role of plant life cycle, including seed germination, root hair development, flower and leaf senescence, abscission, and fruit ripening (Johnson and Ecker, 1998). Previously, some researchers have been interested in observing ethylene biosynthesis in leaves tissue under light exposure (Bassi and Spencer, 1983; Grodzinski *et al.*, 1983). Although, several groups proposed that light inhibited the ethylene production in leaves due to the ethylene production was less under light than dark conditions (Bassi and Spencer, 1983; Grodzinski *et al.*, 1982). However, there have been reported that light

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can promote (Saltveit and Pharr, 1980) or inhibit (Kao and Yang, 1982) ethylene production depend on the tissue.

Carbon dioxide (CO<sub>2</sub>) has also been reported to promote, inhibit, or have no effects on the ethylene production rate at different stage of plant growth and development (Dhawan *et al.*, 1981). For example, CO<sub>2</sub> promotes the ethylene production resulting leaf senescence in tobacco (Aharoni and Lieberman, 1979). In addition, when CO<sub>2</sub> was removed from the atmosphere by KOH, the ethylene production on sweet potato roots was decreased (Imaseki *et al.*, 1968). CO<sub>2</sub> has been shown to inhibit ethylene production of pear fruit (Wild *et al.*, 2003). Till now, it is not clear how CO<sub>2</sub> affect the ethylene production on mango leaves.

It is possible that light and CO<sub>2</sub> concentrations are associated with the production of ethylene. Therefore, in the present study this possibility was further explored by comparing the effects of KH<sub>2</sub>PO<sub>4</sub> with/without NaOH (CO<sub>2</sub> absorbant) on ethylene production of 'Irwin' mango leaves under light or dark condition.

## **Materials and Methods**

### **1. Plant materials**

Mature leaves from 25 years old 'Irwin' mango tree were collected at the Horticultural Experiment Station of National Chung Hsing University in Wufeng district, Taichung city (latitude: 24°04'39.51"N; longitude: 120°43'21.22"E). Mature leaves with dark green color were sampled in the morning and placed in plastic bags. Then immediately transferred to the postharvest laboratory, National Chung Hsing University in Taichung city.

### **2. Experiment**

'Irwin' mango leaves were cleaned one time with tap water to remove the surface dirt. Leaf discs, 1.1 cm (in diameter) discs were cut from several leaves and distributed randomly between treatments. Five discs were incubated in a 2 mL 125 mM KH<sub>2</sub>PO<sub>4</sub> in the 25 mL Erlenmeyer flask and sealed with a rubber cap. For NaOH treatment, 1 mL of 2 N NaOH was added into a small tube and put into the Erlenmeyer flask (for absorbing CO<sub>2</sub> production inside the Erlenmeyer flask). Then, the samples were incubated at 25°C. For light treatments, the samples were illuminated from the top by a 14-W reflector fluorescent lamps with 2,800 Lux light intense measured by TES1332 Digital Lux Meter. For dark treatment, the Erlenmeyer flasks were wrapped with aluminum foil and covered with a black velvet. The production of ethylene and CO<sub>2</sub> were determined every day. The experiment had 4 replications per treatment and repeated for 3 times.

### **3. Ethylene determination**

Measurement of ethylene production of mango leaves followed the method by Shiesh (1990). 1 mL gas samples from the headspace were injected into a gas chromatograph (Shimadzu, Model GC-8A, Japan) fitted with a stainless steel column packed (6 mm x 2 m) activated alumina (mesh size 80/100) and with a Flame Ionization Detector (FID). The temperature of the injection port, column and detector were 130, 90 and 130°C, respectively. Ethylene production of mango leaves were expressed in  $\mu\text{LC}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ .

#### 4. CO<sub>2</sub> determination

The measurement of CO<sub>2</sub> production of mango leaves was followed the method by Shiesh (1990). 1 mL gas samples from the headspace were injected into an infrared gas analyzer (Model UNOR 610, Maihak, Japan). The concentration of the CO<sub>2</sub> standard gas was 1.07% CO<sub>2</sub> in N<sub>2</sub>. The CO<sub>2</sub> production was expressed in  $\text{mLCO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ .

#### 5. Statistical analysis

The data of the experiment were performed of statistical analysis by using SAS 9.4 (Institute Inc, 2002-2012) and subjected to one-way analysis of variance (ANOVA) for a completely randomized design (CRD) statistical model. Mean values among treatments were compared by t-Test (LSD) range test at the 5% ( $p \leq 0.05$ ) level of significance.

## Results

The effects of KH<sub>2</sub>PO<sub>4</sub> with/without NaOH on the ethylene production in mango leaves under light and dark conditions showed significantly different between treatments (Fig. 1). The ethylene production of leaves was clearly higher under light than dark conditions. Under light conditions, the ethylene production of leaves was dramatically increased after day 2 and reached its peak at day 3 in KH<sub>2</sub>PO<sub>4</sub> ( $47.0 \mu\text{LC}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and day 4 in KH<sub>2</sub>PO<sub>4</sub>+NaOH ( $33.1 \mu\text{LC}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), respectively. Under dark conditions, the ethylene production in leaves was low ( $0 - 0.42 \mu\text{LC}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and statistically no difference between KH<sub>2</sub>PO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>+NaOH treatment. However, the ethylene production in KH<sub>2</sub>PO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>+NaOH under light were 33 – 44 fold higher than under dark conditions.

The effects of KH<sub>2</sub>PO<sub>4</sub> with/without NaOH on CO<sub>2</sub> production in 'Irwin' mango leaves under light and dark conditions was compared (Fig. 2). The results showed that the CO<sub>2</sub> production in NaOH treatment under light and dark conditions were lower than without NaOH treatments. KH<sub>2</sub>PO<sub>4</sub> treatment under dark condition had higher CO<sub>2</sub> production than other treatment during treatment time. The CO<sub>2</sub> production of KH<sub>2</sub>PO<sub>4</sub> treatment under light condition was  $13.2 \text{ mLCO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  at day 1, then increased rapidly after day 2 and reached the highest peak at day 4

with  $107.2 \text{ mLCO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . In contrast, the  $\text{CO}_2$  production was low in  $\text{KH}_2\text{PO}_4 + \text{NaOH}$  under light ( $0 - 1.68 \text{ mLCO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and dark ( $1.32 - 2.56 \text{ mLCO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) conditions during treatment time.

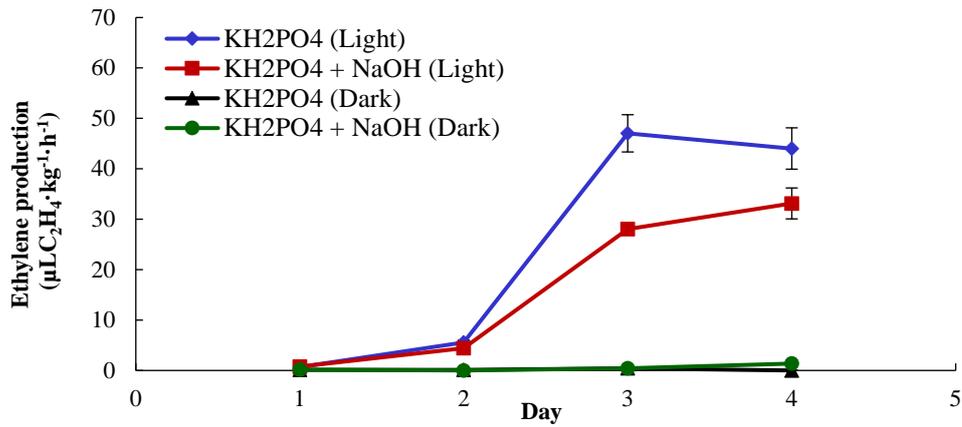


Fig. 1. Effects of  $\text{KH}_2\text{PO}_4$  with/without NaOH on ethylene production in 'Irwin' mango leaves under light and dark conditions.

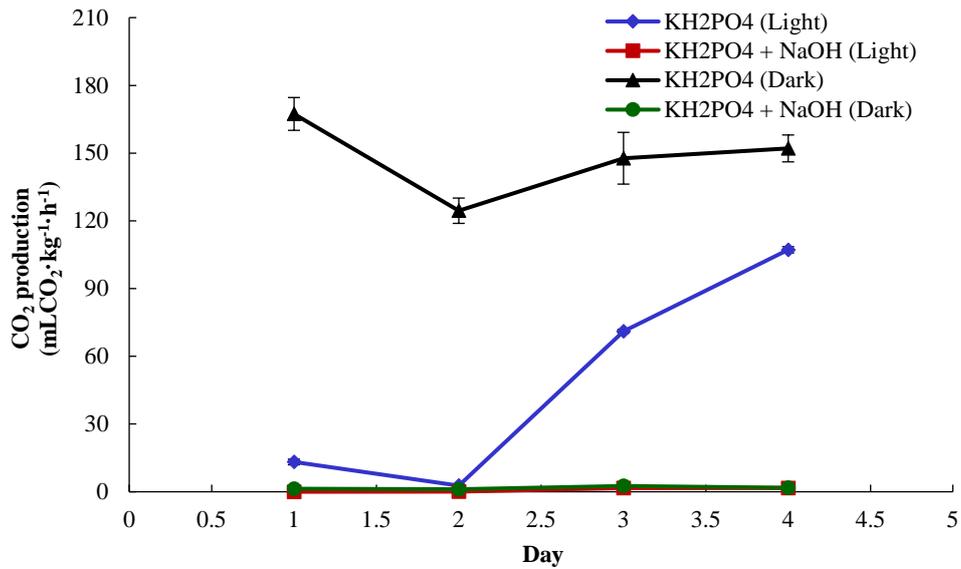


Fig. 2. Effects of  $\text{KH}_2\text{PO}_4$  with/without NaOH on  $\text{CO}_2$  production in 'Irwin' mango leaves under light and dark conditions.

## Discussion

There have been reported that light can promote (Saltveit and Pharr, 1980) or inhibit (Kao and Yang, 1982) ethylene production depending on the tissue. Dhawan *et al.* (1981) reported that carbon dioxide (CO<sub>2</sub>) can promote, inhibit, or have no effects on the ethylene production at different stage of plant growth and development.

The data presented in this study showed that ethylene production of leaves was clearly dependent on the light. The ethylene production of leaves tend to increase in all treatments, which there were significantly higher in KH<sub>2</sub>PO<sub>4</sub> (44-fold) and KH<sub>2</sub>PO<sub>4</sub>+NaOH (33-fold) under light when compared to under dark conditions. On the other hand, dark treatment showed very low rates of ethylene production in both KH<sub>2</sub>PO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>+NaOH. This stimulation of ethylene production by light is similar to the previous reports in cucumber seeds (Saltveit and Pharr, 1980) and leaves of *Gomphrena globose* L. (Grodzinski *et al.*, 1983). KH<sub>2</sub>PO<sub>4</sub>+NaOH had significantly lower in ethylene production than KH<sub>2</sub>PO<sub>4</sub> applied alone under light condition due to the CO<sub>2</sub> production was absorbed by NaOH and lead to the reduction of ethylene production. Similarly, when CO<sub>2</sub> was removed from the atmosphere with KOH, ethylene which was produced by sweet potato roots decreased (Imaseki *et al.*, 1968). Moreover, Dhawan *et al.* (1981) determined that an increasing of CO<sub>2</sub> concentration above the ambient level (0.033%) in the atmosphere surrounding the sunflower plants increased the ethylene production rate, and decreasing of CO<sub>2</sub> concentration resulted in a decrease in the ethylene production rate.

In this study can conclude that KH<sub>2</sub>PO<sub>4</sub> may induce ethylene production in 'Irwin' mango leaves after 3 days treatment under light condition.

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## 磷酸二氫鉀、光照對'愛文'芒果葉片乙烯 生合成之影響

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關鍵字: 磷酸二氫鉀、氫氧化鈉、乙烯生成、二氧化碳、光環境、暗環境

**摘要：**本試驗研究氫氧化鈉與磷酸二氫鉀處理對於'愛文'芒果葉片在光照環境及黑暗中的影響，試驗採完全隨機設計，共有四組處理：1)磷酸二氫鉀在光照下、2)磷酸二氫鉀+氫氧化鈉於光照下、3)磷酸二氫鉀在黑暗下、4)磷酸二氫鉀+氫氧化鈉於黑暗下。每片葉取5個葉圓片，於處理4天後進行測定。結果顯示'愛文'芒果葉圓片在照光環境下乙烯生成速率明顯較黑暗環境更高，乙烯生成速率在所有的處理組中都有增加的趨勢，且磷酸二氫鉀和磷酸二氫鉀+氫氧化鈉處理在光照環境下顯著高於黑暗條件組別；磷酸二氫鉀單獨施用時，黑暗處理組之二氧化碳生成速率顯著高於照光處理組，此現象有可能是由於暗呼吸導致。結果指出光線對於'愛文'芒果葉片乙烯生合成有促進的效果。

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