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EFFECT OF *TRANS*-2-HEXENAL VAPOR PRETREATMENT ON ALLEVIATTION OF HEAT SHOCK IN TOMATO SEEDLINGS (MICRO TOM)

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ABSTRACT

Trans-2-hexenal is a plant natural compound exhibiting a very safe, eco-friendly and strong antifungal activity. We investigated the effect of *trans*-2-hexenal in alleviating heat stress on tomato seedlings in trials conducted from November 28, 2016 to January 4, 2017 at the Tokyo University of Agriculture, Japan. Wilting in seedlings was alleviated through exposure to 0.001 or 0.010 ppm *trans*-2-hexenal, compared to untreated seedlings at 48°C. On the other hand, seedlings treated with 1.000 or 10.000 ppm *trans*-2-hexenal showed severe wilting. Electrolyte leakage and malondialdehyde (MDA) levels of heat-treated tomato leaves also showed alleviation from heat stress upon exposure to 0.001 ppm or 0.010 ppm *trans*-2-hexenal vapor. Results suggest that pre-treatment of tomato seedlings with *trans*-2-hexenal protected tomato seedlings from stress due to heat shock, indicating a possible practical application in tropical developing countries, as a cheap, energy-saving and environment friendly technique.

Key words: green leaf volatiles (GLVs), heat stress alleviation, natural compound

INTRODUCTION

Tomato, considered as one of the most important vegetables in the world, is conserved daily as a source of nutrients, like sugars, organic and amino acids, polyphenols, folic acid, lycopene (carotenoid), vitamin C, as well as minerals (Toor et al. 2005, Vinson et al. 2001 Giovanelli et al. 1999, Van Duyn and Pivonka, 2000). World tomato production has reached up to 170 million tons (FAOSTAT, 2014).

In developing countries, there is positive correlation between vegetable consumption and price, and a negative correlation between vegetable price and morbidity rate (Bouis 1991). Accordingly, an insufficient vegetable supply might affect negatively human health, leading to increased infant mortality and abortion rate (Bouis 1991). In many Southeast Asian countries, productivity of main crops has increased recently in a drastic manner, shifting the focus of food problems from quantity to quality, such as the intake of micronutrients to promote health (Imai 1998). Thus, fresh vegetables, including tomato, attract much attention and a year round stable supply is desirable. In addition, a low cost and simple technique for tomato production under high temperature is particularly required.

High temperature is one of the serious causes for decreased productivity of a variety of crops (Boyer 1982, Hall and Anthony 2001). To counteract the effects of increased temperature, agricultural equipment, such as air control facilities, are used. However, these require high investments for their introduction, and/or running costs (Hara 2016). Thus, there is a need for a simple and low-cost technology to alleviate the effects of stress due to high temperature.

Recently, carbonyl compounds derived from peroxidized linolenic acid are recognized as important signals involved in the environmental stress response (Yamauchi et al. 2008, Mueller and Berger 2009). There is therefore a need for new agricultural technologies which could alleviate the environmental stress caused by such chemicals. Thus, we focused on the possibility of using *trans*-2-hexenal to alleviate the stress caused by heat on tomato. *trans*-2-Hexenal belongs to a group of C6 carbonyl compounds, known as green leaf volatiles (GLVs), which are generated from linolenic and linoleic acid. Generally occurring in nature, *trans*-2-hexenal is well-known in the signaling of plant stress response (Hatanaka and Harada 1973; Mano 2012). This study sought to investigate the effects of *trans*-2-hexenal in alleviating stress in tomato seedlings due to heat by focusing on ethylene production.

MATERIALS AND METHODS

Plant material, pre-treatment with *trans*-2-hexenal vapor, and heat treatment

Experiments were conducted on tomato seeds (*Solanum lycopersicum* L. cv. Micro tom) grown at the Tokyo University of Agriculture from November 28, 2016 to January 4 2017. These were sown from a moist urethane sponge and then transferred to an incubator (MIR-253; SANYO, Japan) set at 25°C in dark conditions. After germination, the seedlings were transferred to a greenhouse at the Tokyo University of Agriculture and grown until reaching its 4th true leaf stage. Upon reaching this stage, the seedlings were transplanted into rockwool (10 × 10 × 5 cm) and grown for 6 weeks. The plants were grown hydroponically in a commercial nutrient solution (OAT house, OAT Agrio Co., Ltd, Japan). The seedlings were then placed inside a 38-L desiccator and exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm of *trans*-2-hexenal vapor which was vaporized by dropping on a filter paper for 1 h prior to heat treatment (untreated seedlings served as controls). After which, the seedlings were incubated at 48°C for 90 mins, and indicators were investigated as described below.

Ethylene production

To monitor the effect of the pre-treatment of *trans*-2-hexenal vapor on the time-dependent change of ethylene production upon heat treatment, a portable ethylene gas analyzer (CI-900FK, CID Bio-Science Inc., United State) was used. Heat-treated seedlings were placed in a 2L chamber set at 48°C and then ethylene concentration from the chamber was monitored every 20 minutes for 3 hours.

Stomatal conductivity and leaf temperature

Stomatal conductivity and leaf temperature were measured from the leaf abaxial surface using a leaf porometer (SC-1, Decagon, United States) after heat treatment (48°C) for 2 h.

Electrolyte leakage

Electrolyte leakage was determined by a conductivity method based on Lafuente et al. (1991). Tomato leaves were cut into discs (8 mm diam.) using a cork borer. The discs were soaked in a 2.0-mL tube filled with 1 mL de-ionized water. After incubation for 1 h at ambient temperature, the solution conductivity was measured using a portable electric conductivity meter (B-771, Horiba, Japan). The sample was then boiled at 100°C for 1h to allow maximum elution. Percentage electrolytes that originally diffused was calculated as follows:

$$\% \text{ electrolyte} = \frac{C1}{C2} \times 100,$$

where, C1 and C2 are solution conductivities before and after boiling, respectively.

MDA concentration

MDA concentration was measured based on the method of Zhang et al. (2010). A tomato leaf sample (0.2 g) was soaked in 6 mL 15% trichloroacetic acid (TCA) containing 0.25% thiobarbituric acid (2 TBA) and then incubated at 30°C for 24 h in the dark. The soaked extraction solution was measured the absorbance (A) at 532, 600, and 450 nm using a spectrophotometer (U-1100, Hitachi, Japan). The concentration of diffused MDA was calculated as follows:

$$\text{MDA concentration (nmol g-1FW)} = 6.45 \times (A_{532} \times A_{600}) \times 0.56 \times A_{450}$$

RESULTS and DISCUSSION

Visual observation

A concentration-dependent response was observed for tomato seedlings exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm of *trans*-2-hexenal vapor for 1 h and incubated at 48°C for 90 minutes. A concentration-dependent response was observed, Wilting of heat-treated seedlings was rather slight in seedlings pre-exposed to 0.001 and 0.010 ppm *trans*-2-hexenal vapor, compared to the control (no *trans*-2-hexenal vapor treatment 0 ppm). In contrast, seedlings exposed to 1 or 10 ppm *trans*-2-hexenal vapor were severely wilted.

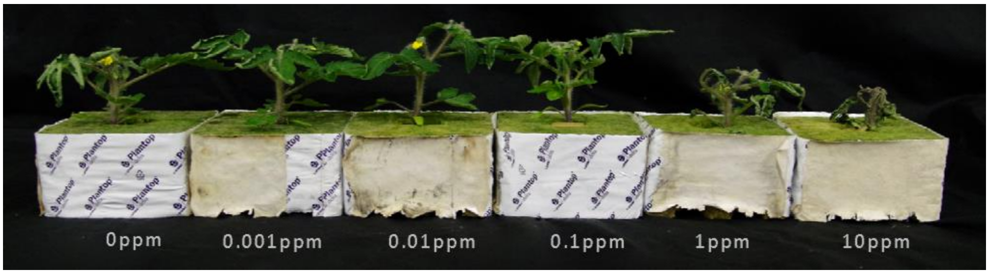

Fig. 1. *Trans*-2-hexenal vapor treatment on tomato 'Micro tom' seedlings. Tomato seedlings were exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm *trans*-2-hexenal vapor for 1 h and then incubated at 48°C for 90 mins.

Ethylene production

Ethylene production of tomato seedlings treated with *trans*-2-hexenal vapor and incubated at 48°C are shown in Fig. 2. An increase in ethylene production was observed for all treatments, with seedlings exposed to 1 and 10 ppm *trans*-2-hexenal vapor produced a remarkable amount of ethylene compared to the control.

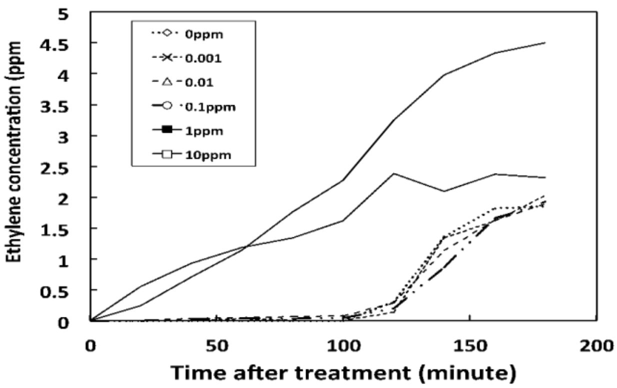

Fig. 2. Ethylene production from tomato 'Micro tom' seedlings pre-treated with *trans*-2-hexanal vapor at 48°C. Tomato seedlings were exposed to 0.001, 0.010, 0.100, 1.000 and 10 .000 ppm *trans*-2-hexenal vapor for 1 h and incubated at 48°C for 90 min. Tomato seedlings were kept in a 1-L plastic vessel and ethylene concentration was monitored every 20 min using a portable ethylene analyzer.

Ethylene is a gaseous plant hormone which sometimes has an important role in heat shock signaling (Abeles et al. 1992, Larkindale and Huang 2004, Jenks and Hasegawa 2005). In our case, the higher ethylene production from the severely-damaged tomato seedlings suggest a more sensitive response to strong heat shock.

Leaf stomatal conductance and leaf temperature

There was a variation in leaf stomatal conductance among seedlings exposed to *trans*-2-hexenal, depending on *trans*-2-hexenal concentration (Fig. 3). Seedlings exposed to 0.001 and 0.010 ppm *trans*-2-hexenal vapor exhibited a significantly greater stomatal conductance, while a significantly lower stomatal conductance was observed for seedlings treated with 10.000 ppm *trans*-2-hexenal. The temperature of seedling leaves for each treatment had no significant difference (data not shown). On peroxidase over produced tobacco, which wilt easily compared to a wild type, high level of stomatal conductance was reported (Lagrimini et al. 1990). Because our previous study showed that treatment with *trans*-2-hexenal reduces GSH levels in tomato fruit (Data not shown), wilting in tomato seedlings might be well attributed to a reduced cell turgor pressure (Bartling et al. 1993. *trans*-2-Hexenal induces abiotic stress tolerance, which is induced by several heat shock factors (HSFs) and heat shock proteins (HSPs) (Yamauchi et al. 2015).

Leaf electrolyte leakage and MDA

Electrolyte leakage of seedlings exposed to either 0.001 or 0.010 ppm *trans*-2-hexenal vapor was significantly lower than the control (Fig. 4). In contrast, seedlings exposed to 10.000 ppm *trans*-2-hexenal vapor had the highest electrolyte leakage, indicating the most severe cell membrane damage due to heat shock (Fig. 3). Heat stress was reported to cause cell membrane injury and membrane lipid peroxidation in many plant species (Van Rensburg and Krüger 1994; Gong et al. 1998; Liu and Huang 2000; Jiang and Huang 2001).

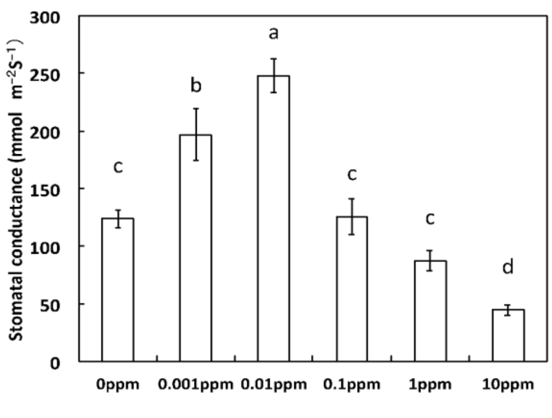

Fig. 3. Stomatal conductance of tomato 'Micro tom' seedlings pre-treated with *trans*-2-hexenal vapor at 48°C. Tomato seedlings were exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm *trans*-2-hexenal vapor for 1 h and then incubated for 90 min at 48°C. Stomatal conductance was measured with a leaf porometer. Bars represent means ± SE of three replicates. Treatments not sharing the same letter are significantly different based on Fisher's LSD test ($P < 0.05$)

The MDA content of seedlings exposed to either 0.001 or 0.010 ppm *trans*-2-hexenal vapor was significantly lower than the control (Fig. 5). On the other hand, MDA content of seedlings exposed to either 1.000 or 10.000 ppm *trans*-2-hexenal vapor was remarkably higher than the other treatments, showing the most severe level of membrane peroxidation (Fig. 4). MDA is a product derived from the peroxidation of unsaturated fatty acids, such as phospholipids, and causes cell membrane damage (Halliwell and Gutteridge 2015).

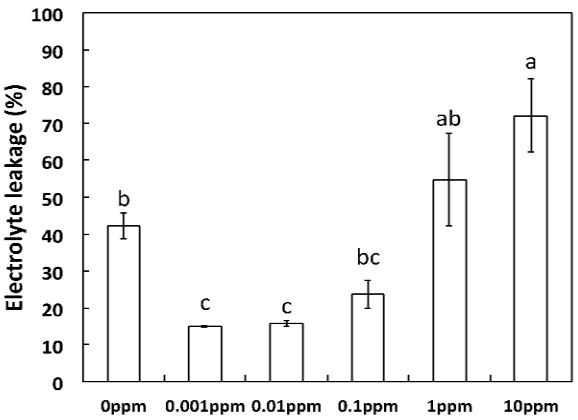

Fig. 4. Electrolyte leakage from tomato 'Micro tom' leaf discs pre-treated with *trans*-2-hexenal vapor at 48°C. Tomato seedlings were exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm *trans*-2-hexenal vapor for 1 h and then incubated at 48°C for 90 min. Leaf discs with a diameter of 1 cm were placed in 1 ml de-ionized water and electrolyte leakage was checked. Bars represent means ± SE of three replicates expressed as % electrolyte leakage against dead leaves. Treatments not sharing the same letter are significantly different based on Fisher's LSD test (P < 0.05).

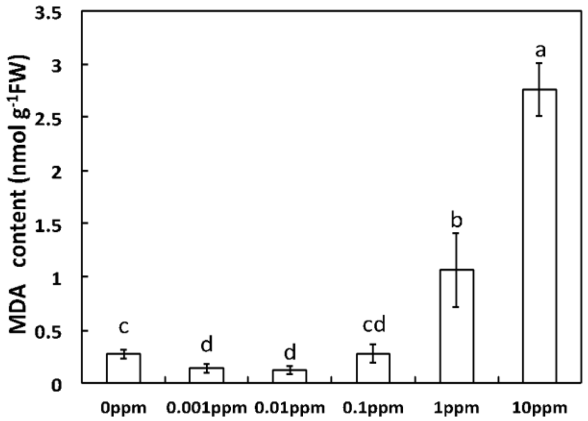

Fig. 5. MDA content of tomato 'Micro tom' seedlings pre-treated with *trans*-2-hexenal vapor at 48°C. Tomato seedlings were exposed to 0.001, 0.010, 0.100, 1.000 and 10.000 ppm *trans*-2-hexenal vapor for 1 h and then incubated at 48°C for 90 min. Bars represent means ± SE of three replicates expressed as % against dead leaves. Treatments not sharing the same letter are significantly different based Fisher's LSD test (P < 0.05).

CONCLUSION

The pre-exposure treatment of tomato seedlings with either 0.001 or 0.010 ppm *trans*-2-hexenal could alleviate damage caused by heat shock. It can be useful for producers and consumers in tropical countries, as pre-treatment could be applied as an energy-saving, environmentally friendly, and easy method of coping with high temperature conditions.

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