

# Effects of grafted eggplants on allelopathy of cinnamic acid and vanillin in root exudates

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## Abstract

Cinnamic acid and vanillin are the allelochemicals commonly exist in eggplant root exudates. With pot culture experiment, the effects of grafted eggplants on allelopathy of cinnamic acid and vanillin in eggplants root exudates were studied. The results showed that cinnamic acid and vanillin had allelopathic effects on eggplants, lower concentration of cinnamic acid and vanillin (0.1 mmol L<sup>-1</sup> or 0.5 mmol L<sup>-1</sup>) could promote the growth and physiological metabolism of eggplants, while higher concentration (from 1 mmol L<sup>-1</sup> to 4 mmol L<sup>-1</sup>) had slightly promotive or inhibitive effects on eggplants. Meanwhile, this study suggested grafting could relieve autotoxicity of cinnamic acid and vanillin, and significant difference in the regulation intensity for the autotoxicity was found between cinnamic acid and vanillin. Grafting decreased the amounts of cinnamic acid and vanillin, especially of vanillin. The maximum reduction amount of cinnamic acid reached 68.96%, and that of vanillin reached 100%. Under the stress of exotic cinnamic acid and vanillin, especially of exotic cinnamic acid, grafting relieved the autotoxicity of the two substances on eggplants. Compared with own-rooted eggplant, grafted eggplant had a higher plant height and a larger stem diameter, its leaf chlorophyll content increased by 5.26-13.12%, root electric conductivity and MDA content decreased, and root SOD activity enhanced. Grafting was found to be one of the most effective methods for relieving replanting problems caused by autotoxicity.

**Key words:** Grafting, root exudates, cinnamic acid, vanillin, autotoxicity

## Introduction

Eggplant (*Solanum melongena* L.) is a popular vegetable crop worldwide. However, yield and quality reduced sharply annually owing to continuous cropping in the same field, and the adverse effect is considered as continuous cropping obstacle and may become a key problem restraining eggplants sustainable production. There are many causes for continuous cropping obstacle, such as soil-borne diseases, nutrition unbalance and allelopathy (Zheng *et al.*, 2004).

Soil-borne diseases have been paid more attention in eggplant studies. Grafted eggplant root exudates could inhibit soil-borne diseases and induce beneficial microbe increase, which help to overcome the continuous cropping obstacle caused by soil-borne diseases (Zhou *et al.*, 2001; Wang *et al.*, 2005). When the previous crop root exudates or residues containing allelochemicals are in soil, allelochemicals accumulate in soil to a certain level, which inhibit the growth and yield of following crop in continuous cropping *i.e.* autotoxicity (Fisher, 1980; Yu and Matsui, 1993; Wu *et al.*, 1999; Yu and Matsui, 1999; Cao *et al.*, 2001). Eggplant root exudates have a key role to cause continuous cropping problem, and its autotoxicity (Yu and Matsui, 1999; Ruan *et al.*, 2003; Zheng *et al.*, 2004). The cinnamic acid and vanillin are general allelochemicals detected in eggplant root exudates (Zhou *et al.*, 2001; Wang *et al.*, 2005b; Zhang *et al.*, 2005), and they had allelopathic toxic effect on seed germination and seedling of eggplants (Wang *et al.*, 2006). Therefore, it is of importance to conduct study on technique which overcome continuous cropping obstacle caused by autotoxicity.

In present investigation, we studied the effect of grafted eggplants on the amount of cinnamic acid and vanillin in root exudates

to further understand the mechanism responsible for increased production by grafting by eliminating problems caused by continuous cropping

## Materials and methods

**Material preparation:** The experiment was carried out in greenhouses at Vegetable Experiment Station, Shenyang Agricultural University. The commonly grown eggplant (*Solanum melongena* L.) cv. 'Xi'anlv' and a kind of wild eggplant [*Solanum torvum* (*S.tor*)] were used as scion and rootstock, respectively. Rootstock and scion plants were sown on January 1<sup>st</sup> and February 12<sup>th</sup>, 2006, respectively. When the rootstock plants reached 4-5 leaf stage, cleft grafting was done (Wang *et al.*, 2005a). Then, some grafted eggplants and 'Xi'anlv' were transplanted into 20.5cm dia container with substrate of perlite, turf soil and vermiculite (3:2:1), and irrigated by Hoagland nutrient solution twice a week and used for collecting root exudates of different growth stage. Other grafted eggplants and 'Xi'anlv' were transplanted into 18 cm diameter container with substrate of turf soil, soil and chicken manure (1:1:1) and watered with exotic cinnamic acid and vanillin, which were used for the two substances stress treatment. The grafted eggplants and self-root eggplants were referred to as *S.tor* and control, respectively.

**Root exudates collection and cinnamic acid and vanillin contents determination:** Root exudates at different growth stages were collected by soaking root. The growth stages including phases of bud, first fruit expanding and second fruit expanding, were referred as earlier, mid and post stage, respectively. The root exudates solution was adsorbed with XAD-4 resin produced by Sigma Company, and concentrated after dissolving with alcohol,

then cinnamic acid and vanillin contents were determined with Waters High Pressure Liquid Chromatography made in America (HPLC) (Wang *et al.*, 2005).

**Cinnamic acid and vanillin solution:** Cinnamic acid and vanillin solutions were made with concentration of 0, 0.1, 0.5, 1, and 4 mmol L<sup>-1</sup>, respectively. Firstly, cinnamic acid was dissolved in alcohol, then diluted with distilled water, and finally regulated alcohol content to 1.5%. Vanillin was directly dissolved with distilled water, then also regulated alcohol content to 1.5%.

A week after transplanting, the plants were irrigated with cinnamic acid and vanillin solution respectively around root 10 cm once every 5 days. The irrigated amount was 100 mL each time. After a month, the growth and physiological metabolism indexes were investigated. Chlorophyll content, cell membrane permeability, MDA contents and SOD content was respectively determined with Zhaoshijie method (Xiong, 2003), relative electrical conductivity method (Zheng, 2006), Thiobarbituric acids method (TBA) (Omran, 1980) and nitroblue tetrazolium (NBT) Oxidation and Deoxidize Method (Li, 2000).

**Statistical analysis:** The data were statistically analyzed with Excel software and DPS software.

## Results

**Cinnamic acid and vanillin amounts:** The cinnamic acid content in *S.tor* root exudates was also less than control, whereas vanillin content was markedly lower than control (Table 1). Compared with control, vanillin content in *S.tor* root exudates decreased by 95.44% at earlier stage and 100% at mid and post stage. Cinnamic acid content in *S.tor* root exudates was significantly less than control, with 68.96% reduction at earlier stage and 50% reduction at post stage. The results showed grafting reduced release of the two substances by eggplant roots, in particular, significantly reduced vanillin amount.

**Growth response of eggplants:** The effect of cinnamic acid and vanillin on eggplant height and stem diameter were basically identical in action trend, whereas different in action character and degree (Fig. 1 and Fig. 2). The results showed lower concentration of cinnamic acid and vanillin had promotive effects, while higher concentration had inhibitive effects on eggplants height and stem diameter growth. The effect of cinnamic acid on plant height and stem diameter were stronger than vanillin, and impact of two substances was more plant height than on stem diameter.

**Physiological and biochemical responses of eggplants:** The chlorophyll content increased gradually with cinnamic acid and vanillin concentration ranging from 0 to 0.1 mmol L<sup>-1</sup>, and at 0.1 mmol L<sup>-1</sup> reached maximum, afterwards, decreased with the concentration increasing (Fig. 3). The results showed cinnamic

Table 1. Effects of grafting on the contents of cinnamic acid and vanillin in eggplant root exudates

Phenolic acids	Treatment	Content at different growth stage (μg g <sup>-1</sup> )		
		Early stage	Mid stage	Post stage
Cinnamic acid	<i>S.tor</i>	0.009bB	0.090aA	0.290bB
	Control	0.029aA	0.060aA	0.540aA
Vanillin	<i>S.tor</i>	0.115bB	0.0bB	0.0bB
	Control	2.520aA	4.430aA	12.260aA

Note: Data are mean values of three repetitions. Capital letter and small letter indicate significance at  $P=0.01$  and  $P=0.05$ , respectively

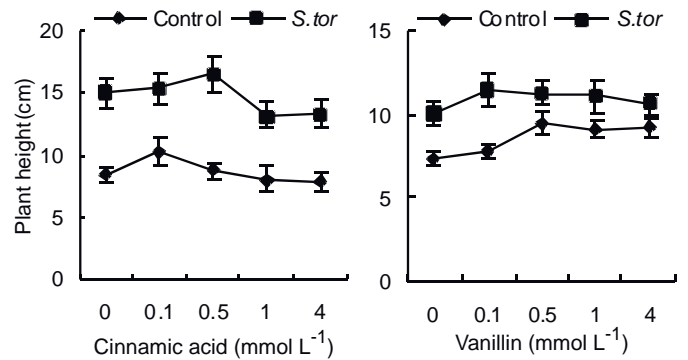


Fig. 1. Effect of cinnamic acid and vanillin on plant height of eggplant

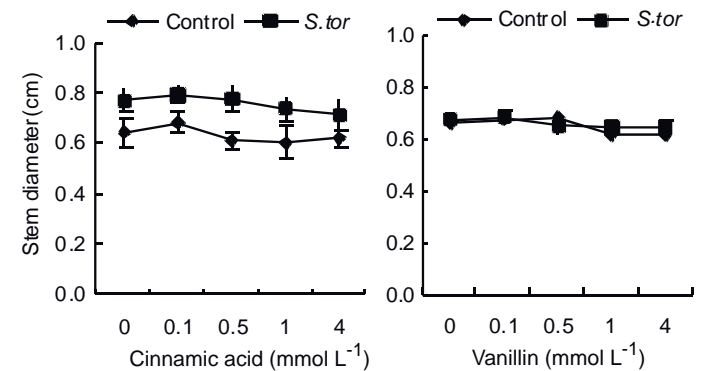


Fig. 2. Effect of cinnamic acid and vanillin on stem diameter of eggplant

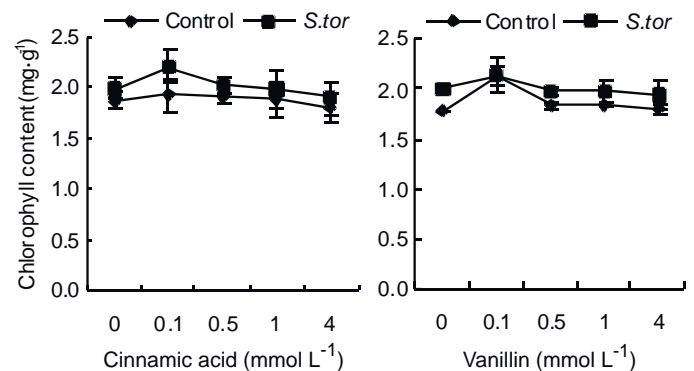


Fig. 3. Effect of cinnamic acid and vanillin on chlorophyll content of eggplant

acid and vanillin of lower concentration had promotive effects on chlorophyll content, whereas the higher concentration had inhibitory effect.

Compared with control, chlorophyll content in *S.tor* increased by 5.53, 13.12, 5.94, 5.53, 5.26% at 0, 0.1, 0.5, 1, 4 mmol L<sup>-1</sup> cinnamic acid treatment respectively, and increased by 11.05, -0.16, 6.60, 7.57, 7.73% at 0, 0.1, 0.5, 1, 4 mmol L<sup>-1</sup> vanillin treatment, respectively. The result showed chlorophyll in grafted eggplant was generally higher than control, and grafting could maintain better chlorophyll content under cinnamic acid stress than vanillin stress.

The relative electrical conductivity in eggplant root decreased with cinnamic acid and vanillin concentration increasing from 0 to 0.1 mmol L<sup>-1</sup>, and reached the minimum at 0.1 mmol L<sup>-1</sup>, afterwards, the relative electrical conductivity increased as the concentration increasing (Fig. 4). MDA contents sharply increased at 0.5, 1, 4 mmol L<sup>-1</sup> cinnamic acid treatment (Fig. 5). It reached

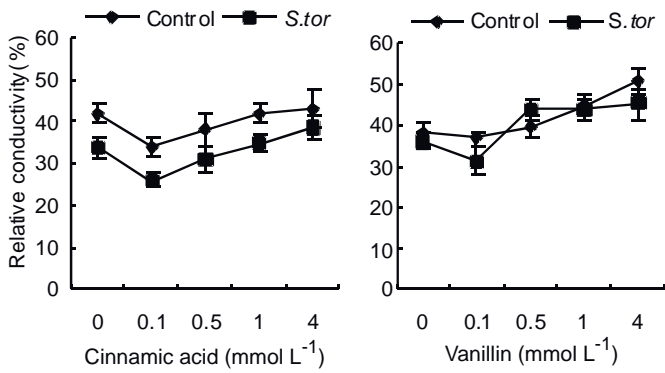


Fig. 4. Effect of cinnamic acid and vanillin on relative conductivity in root system of eggplant

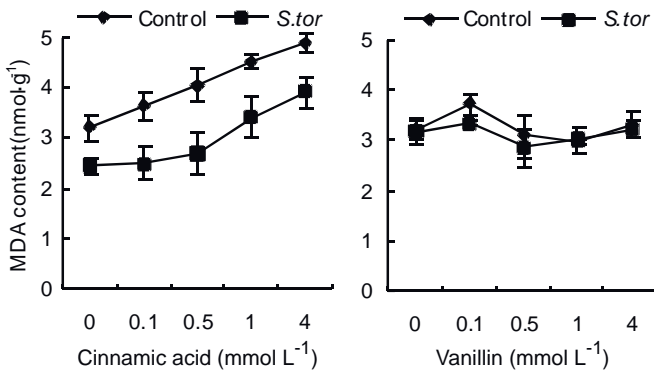


Fig. 5. Effect of cinnamic acid and vanillin on MDA content of eggplant

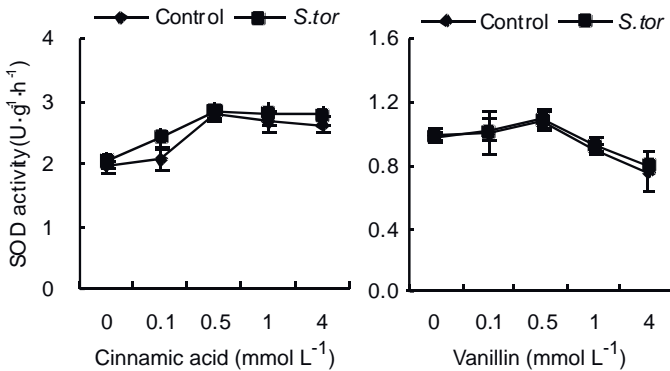


Fig. 6. Effect of cinnamic acid and vanillin on SOD activity in eggplant

the maximum at 0.1 mmol L<sup>-1</sup> vanillin, and dropped significantly at 0.5 mmol L<sup>-1</sup>, then increased again with concentration going up.

Compared with control, the relative electrical conductivity in *S.tor* reduced by 19.90, 23.63, 18.56, 16.97, 10.02%, and MDA content lowered by 23.19, 31.86, 33.17, 24.77, 20.04% at 0, 0.1, 0.5, 1, 4 mmol L<sup>-1</sup> cinnamic acid treatment, respectively. Under the vanillin stress, the variation regularity of two indexes was not obvious. The results showed the relative electrical conductivity and MDA contents in grafted eggplants was markedly lower than control, which suggested that grafting could maintain or enhance resistance to cinnamic acid stress.

SOD enzyme activity increased with cinnamic acid and vanillin concentration ranging from 0 to 0.5 mmol L<sup>-1</sup>, and began to decline as the concentration going up from 1 to 4 mmol L<sup>-1</sup>, with more reduction under cinnamic acid than vanillin stress (Fig. 6). It indicated that the action of cinnamic acid on SOD enzyme activity was stronger than that of vanillin.

Compared with the control, the enzyme activity in *S.tor* increased by 1.99, 15.29, 1.06, 4.67, 5.45% at 0, 0.1, 0.5, 1, 4 mmol L<sup>-1</sup> cinnamic acid treatment, respectively. Under vanillin stress, the enzyme activity in *S.tor* was similar to that in control. It showed that effect of grafting on SOD enzyme activity under cinnamic acid was stronger than vanillin.

## Discussion

The action intensity of autotoxic substances on receptor plants had close relation with its concentration. The study showed cinnamic acid and vanillin also had allelopathic effect on eggplants, lower concentration (0.1 mmol L<sup>-1</sup> or 0.5 mmol L<sup>-1</sup>) could promote the growth and physiological and biochemical metabolism, while higher concentration (from 1 mmol L<sup>-1</sup> to 4 mmol L<sup>-1</sup>) had slightly promotive or inhibitive effects on eggplants. The result was accorded with the conclusion of cucumber and fir (Chen *et al.*, 2002; Wu *et al.*, 2002).

Our results suggested that autotoxicity mechanism of cinnamic acid and vanillin on eggplants were basically identical, while allelopathic intensity of cinnamic acid was stronger than vanillin. With the enhancing of cinnamic acid stress, MDA content and electrical conductivity increased remarkably, SOD enzyme activity and chlorophyll content initially increased and then decreased, plant height decreased gradually, but the change in stem diameter was not apparent. Under the action of vanillin, the change of MDA content was not apparent, electrical conductivity increased, SOD enzyme activity slightly increased initially, and then decreased remarkably, chlorophyll content also followed the same trend. Plant height showed a gradual rise and the change in stem diameter was not conspicuous. From the above, cinnamic acid and vanillin disturbed cell membrane firstly and enhanced membrane permeability, after that influenced some enzyme metabolism, and finally growth and development. The result was in accordance with the conclusion drawn by Einhellig (1995) and Cao *et al.* (2001).

The allelochemicals variation and amounts belonged to quantitative inheritance controlled by polygene (Kong *et al.*, 2002), which depended on the common action of plant oneself and environment (Einhellig, 1996). Allelochemicals were affected by environment, with increase in quantity and enhanced intensity at adverse circumstances, while greatly reduced at favourable circumstances (Kong *et al.*, 1996; Kong *et al.*, 1997). Graft union is a new individual plant, and the interaction between rootstock and scion changes metabolism of the two parts such as substance synthesis, translocation, and transformation and so on (Zheng *et al.*, 2005; Qi *et al.*, 2006; Xu *et al.*, 2006). Through in grafting, eggplant oneself and ecological environment of rhizosphere improved (Wang *et al.*, 2005), which might influence the roots secondary metabolism, change the capability to release allelochemicals. The study showed grafting decreased the amounts of cinnamic acid and vanillin released from eggplants root, especially of vanillin. The former studies suggested grafted eggplant had better advantage than own-rooted eggplant in growth vigour, diseases resistance, increased production etc (Gao and Zang, 1998). In this study, we found grafted eggplants could maintain or enhance advantage in the growth and physiological metabolism under stress, especially for cinnamic acid stress.

The study suggested that grafting could overcome autotoxicity of cinnamic acid and vanillin, whereas there was difference between them. For vanillin, grafting significantly decreased the vanillin exudation amount from root, and for cinnamic acid, grafting relieved stress by maintaining or enhancing advantage in the growth and metabolism. Grafting is one of the effective methods for relieving replanting problems caused by autotoxicity.

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