JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Preharvest L-Arginine Treatment Induced Postharvest Disease Resistance to *Botrysis cinerea* in Tomato Fruits

Yang Zheng, Jiping Sheng, Ruirui Zhao, Jian Zhang, Shengnan Lv, Lingyi Liu, and Lin Shen*

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

ABSTRACT: L-Arginine is the precursor of nitric oxide (NO). In order to examine the influence of L-arginine on tomato fruit resistance, preharvest green mature tomato fruits (*Solanum lycopersicum* cv. No. 4 Zhongshu) were treated with 0.5, 1, and 5 mM L-arginine. The reduced lesion size (in diameter) on fruit caused by *Botrytis cinerea*, as well as activities of phenylalanine ammonia-lyase (PAL), Chitinase (CHI), β-1,3-glucanase (GLU), and polyphenoloxidase (PPO), was compared between L-arginine treated fruits and untreated fruits. We found that induced resistance increased and reached the highest level at 3−6 days after treatment. Endogenous NO concentrations were positively correlated with PAL, PPO, CHI, and GLU activities after treatment with Pearson coefficients of 0.71, 0.94, 0.97, and 0.87, respectively. These results indicate that arginine induces disease resistance via its effects on NO biosynthesis and defensive enzyme activity.

KEYWORDS: Preharvest treatment, L-arginine, nitric oxide, disease resistance, tomato fruit

INTRODUCTION

Gray mold disease caused by *Botrytis cinerea* is considered an important pathogen that results in substantial postharvest losses of fruits and vegetables around the world. Although postharvest diseases can be controlled by synthetic fungicides,[1] their use in postharvest treatment has been restricted because of reduced efficiency and growing public awareness of the negative impact on human health and the environment. Relatively broad-spectrum, bioefficacious, economical, and environmentally safe substitutes of fungicides are required.[2,3] Among the viable alternatives, enhanced protection of host plant tissue through induced/acquired disease resistance is considered a preferred strategy.

Preformed and/or inducible defense mechanisms are important in enhancing or boosting natural disease resistance in horticultural crops.[4,5] After harvest, the resistance of fruits and vegetables to natural disease usually declines, leading to infection by pathogens.[6] Field applications of some biotic or abiotic antimicrobial compounds before harvest would enable the fruit to resist pathogen infection. Preharvest application of salicylic acid or methyl jasmonate on sweet cherry could induce disease resistance in storage.[7] Preharvest chitosan sprays significantly reduced postharvest fungal rot and maintained the keeping quality of the fruit from subsequent picks.[8] It suggested that preharvest treatment with these naturally occurring compounds may open promising opportunities, such as disease resistance and the extension of shelf life. Thus, priming holds promise to be a novel and applicable means for crop and food improvement in commercial plant production.[4]

Evidence has shown that nitric oxide plays an important role in inducing disease resistance in postharvest plants. A rapid accumulation of NO in response to avirulent bacteria has been observed in soybean and *Arabidopsis* suspension-cultured cells,[9,10] as well as in *Arabidopsis* plants.[11] The production of NO at the early stage was induced because of the direct contact of avirulent crown rust fungus with oat plant in the defense response.[12] In addition, exogenous application of NO activated the expression of a series of resistance-related genes in *Arabidopsis*, soybean, and tobacco and promoted the activities of defensive enzymes.[13−15] These results point to a strong association between NO level and disease resistance in plants.

L-Arginine is one of the most functionally diverse amino acids in living cells. Meanwhile, it has physiological roles, for example, as a precursor for NO and polyamines (PAs). Recently an arginine-dependent mechanism was described in plants. Some studies indicate that both endogenous and exogenous arginine have roles in the plant stress response, such as from salt and wounding response.[16,17] Exogenous arginine improved salt resistance of rice via modification of the put/spd+spm ratio.[16] Decreased arginine levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impaired nitric oxide synthesis and inhibited the hypersensitive response to *Pseudomonas syringae*.[18] However, it remains unclear whether the unique roles of arginine in plants are attributable to the biosyntheses of polyamines and/or nitric oxide.

In a previous study, we discussed the function of L-arginine in postharvest fruit and found that L-arginine could induce disease resistance in tomato fruits and found that the increased endogenous NO levels were involved in L-arginine-induced disease resistance. We hypothesize that L-arginine plays the same role in pretreatment compared with that in postharvest treatment. To test this hypothesis, tomato fruits were used, and research was focused on investigating the role of preharvest L-arginine treatment in adjustment of induced disease resistance. The regulatory effects of L-arginine on NO level and defensive enzyme activities during the whole treatment period were also studied.

MATERIALS AND METHODS

Treatment and Sampling of Fruit Material. Tomato fruit (*Solanum lycopersicum* cv. No. 4 Zhongshu) were picked at the mature

Received: October 2, 2010
Accepted: May 16, 2011
Revised: May 8, 2011
Published: May 17, 2011

 © 2011 American Chemical Society

green ripening stage[19] in a greenhouse at the Xiaotangshan Geothermal Special Vegetable Base, Beijing.

When the first spike fruit florescence reached 50 days, the tomato plants were cut to nearly 0.5 cm^2 near the root area, injected with 5 mL of 0.5, 1, or 5 mM L-arginine solution. The same volume of distilled water was injected as the control. Each treatment contained 30 plants. At 0, 3, 6, 9, and 12 days, respectively, after treatment, fruits whose florescence were reached at 50 days were picked and selected for uniformity of shape, color, and size. All fruits selected for experiments were unblemished and free of disease.

Fruits were stored at 25 ± 1 °C (90−95% RH) for 12 h to remove field heat. The mesocarp from the fruit equator area was cut into small pieces, frozen in liquid nitrogen, and stored at −80 °C.

Pathogen Inoculation and Disease Symptom Measurement. *B. cinerea* was isolated from decayed tomato fruit and incubated on potato dextrose agar medium in the dark at 24 °C. A conidial suspension was prepared by flooding 7-day-old cultures with sterile distilled water containing 0.5 g/L Tween-20. The spore suspension was adjusted to 2×10^5 conidia mL^{-1}. Inoculations were carried out 24 h after fruit were treated. For each replicate (three replicates per treatment), 30 fruits were sterilized with 70% ethanol for 1 min and then punctured with a sterile nail (4 mm deep × 2 mm wide) at three points on the equator of each fruit. Then, 10 μL of spore suspension was injected into each wound site, and the fruits were incubated at 25 ± 1 °C and 90−95% RH.

Disease incidence and lesion diameter (where symptoms were visible and countable) on each fruit were recorded at 4 days after inoculation. Disease incidence is expressed as the percentage of fruit showing disease symptoms. Lesion size was calculated as $3.14 \times (\text{lesion diameter}/2)^2$.

Determination of Defensive Enzyme Activities. To determine activities of the defensive enzyme, 1 g of frozen mesocarp tissue was homogenized with 5 mL of extraction buffer [0.05 mol L^{-1} phosphate buffer (pH 6.8) for phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO); 0.1 mol L^{-1} acetic acid buffer (pH 5) for Chitinase (CHI) and β-1,3-glucanase (GLU)] and centrifuged at 10,000*g* for 5 min at 4 °C. The supernatant was collected and stored at −80 °C until analysis.

PAL (EC 4.3.1.5) activity was determined as described by Koukol and Conn[20] with minor modifications. An aliquot (500 μL) of the extract was incubated with 1 mL of 0.02 mol L^{-1} L-phenylalanine and 2 mL of 0.2 mol L^{-1} boric acid buffer (pH 8.8) at 30 °C for 1 h, after which absorbance at 290 nm was measured. PAL activity was expressed as U g^{-1} fresh weight (FW), where one unit is defined as a change of 1 at OD_{290} per hour ($U = \Delta A_{290} h^{-1}$).

PPO (EC 1.10.3.2) activity was determined as described by Liu et al.[21] with some modifications. An aliquot (200 μL) of the extract was reacted with 2 mL of buffered substrate (0.05 mol L^{-1} phosphate buffer, pH 6.8) and 1 mL of 0.1 M catechol, and the change in absorbance at 420 nm was recorded over 3 min. Specific activity is expressed as U g^{-1} fresh weight (FW), where one unit is defined as an increase of 1 at OD_{420} per min.

CHI (EC 3.2.1.14) activity was measured according to Boller et al.[22] with some modifications. An aliquot (500 μL) of the extract was mixed with 0.5 mL of colloidal chitin (Sigma) and incubated at 40 °C for 1 h. Then, 0.1 mL of 20 g L^{-1} desalted snailase (Sigma) was added, and the mixture was incubated at 37 °C for 1 h. The reaction was stopped by the addition of 0.3 mL of 0.6 mol L^{-1} potassium tetraborate and boiling for 5 min. After cooling, 2 mL of 100 g L^{-1} 4-(dimethylamino)benzaldehyde reagent diluted with glacial acetic acid (1:5 v/v) was added, and the mixture was incubated at 37 °C for 20 min, and then absorbance was measured at 585 nm. CHI activity is expressed as U g^{-1} FW, where one unit is defined as 10^{-9} mol *N*-acetyl-D-glucosamine produced per h under these assay conditions.

GLU (EC 3.2.1.39) activity was assayed with laminarin as the substrate as described by Siefert et al.[23] and Hinton and Pressey,[24] with some modifications. Crude extract (100 μL) was mixed with 50 μL of 0.4% laminarin, and the mixture was incubated for 1 h with shaking at 37 °C. The reaction was stopped by the addition of 200 μL of 3,5-dinitrosalicylic acid and boiling for 5 min. The mixture was then cooled, and absorbance at 500 nm was determined. Glucanase activity was determined as the amount of reducing glucose released from laminarin using glucose as the standard. Enzyme activity is expressed as U g^{-1} FW, where a unit is defined as 1 mg of glucose released per min under these assay conditions.

Determination of Total Phenolics and Total Flavonoids Content. Total phenols were quantified using the Folin−Ciocalteu reagent.[25] Phenol content was determined according to McCue et al.[26] with some modifications. Frozen tissue (1 g) was homogenized with 2.5 mL of 95% ethanol. The mixture was incubated at 4 °C for 24−48 h and then centrifuged at 10,000*g* for 10 min. An aliquot (1 mL) of the supernatant was combined with 1 mL of 95% ethanol, 5 mL ofdistilled water, and 0.5 mL of 50% Folin−Ciocalteu phenol reagent, and the mixture was incubated for 5 min at room temperature. Then, 1 mL of 5% (w/v) sodium carbonate was added, and the mixture was vortexed briefly before incubation for 1 h in the dark at room temperature. The absorbance at 685 nm was determined, and phenols were quantified by comparison to a standard curve of gallic acid. Results are expressed as catechin equivalents (mg/100 g FW). Reported values are averages of three replicates.

Total flavonoid content was determined as described by Lamaison and Carnat[27] with some modifications. Frozen tissue (1 g) was homogenized with 2 mL of 70% ethanol and then centrifuged at 10,000*g* for 10 min. An aliquot (1 mL) of the supernatant was mixed with 0.3 mL of $AlCl_3$ reagent. After 5 min, 0.3 mL of $Al(NO_3)$ reagent was added, and the mixture was shaken gently. The reaction was terminated by the addition of 2 mL of 1 M NaOH. After 10 min, the absorbance at 510 nm was measured. Total flavonoid concentration was determined from a standard curve of vitexin. Results are expressed as mg vitexin equivalents per g FW.

Measurement of NO Accumulation and Nitric Oxide Synthase (NOS) Activity. Frozen samples were ground to a fine powder in a mortar precooled with liquid nitrogen. The powder was mixed with three volumes of extraction buffer [50 mM HEPES-KOH buffer (pH 7.6), 0.5 M sorbitol, 5 mM EDTA, 5 mM ethyleneglycol bis (2-aminoethyl ether) tetraacetic acid, 10 μM flavin adenine dinucleotide (FAD), 10 mM dithiothreitol, and 10 μM (*p*-amidinophenyl) methylsulfonyl fluoride]. After centrifugation at 14,000*g* for 30 min, supernatants were collected and used for assays.[28]

NO levels were measured on the basis of the spectrophotometric method of Murphy and Noack.[29] Crude enzyme mixture (1 mL) was first incubated with 100 U catalase and 100 U superoxide dismutase for 5 min to remove endogenous reactive oxygen species (ROS). Then, 0.5 mL of 10 mM oxyhemoglobin was added and the mixture incubated at room temperature for 3 min. All measurements were performed at 37 °C, and the absorbance was measured at 550 nm. All measured values were subtracted from the blank values, where the extracts were replaced in the reaction mixture by the same volume of extraction buffer. Results are expressed as μ mol per g FW.

Crude enzyme mixtures were prepared in advance and stored at −80 °C until analysis. Frozen samples (1 mL) were homogenized in 5 mL of homogenization buffer (50 mM triethanolamine hydrochloride, pH 7.5, containing 0.5 mM EDTA, 1 mM leupeptin, 1 mM pepstatin, 7 mM glutathione, and 0.2 mM phenylmethyl sulfonyl fluoride). After centrifugation at 9000*g* for 30 min at 4 °C, the supernatant was collected and recentrifuged at 100,000*g* for 50 min at 4 °C. The supernatant was used as the cytosolic fraction. The pellet was resuspended in 5 mL of homogenization buffer and used as the microsomal fraction. NOS activity was analyzed using the hemoglobin assay.[29]

Effect of L-Arginine on Mycelia Growth of *Botrytis cinerea* in Vitro. The effects of L-arginine on mycelia growth were assayed by

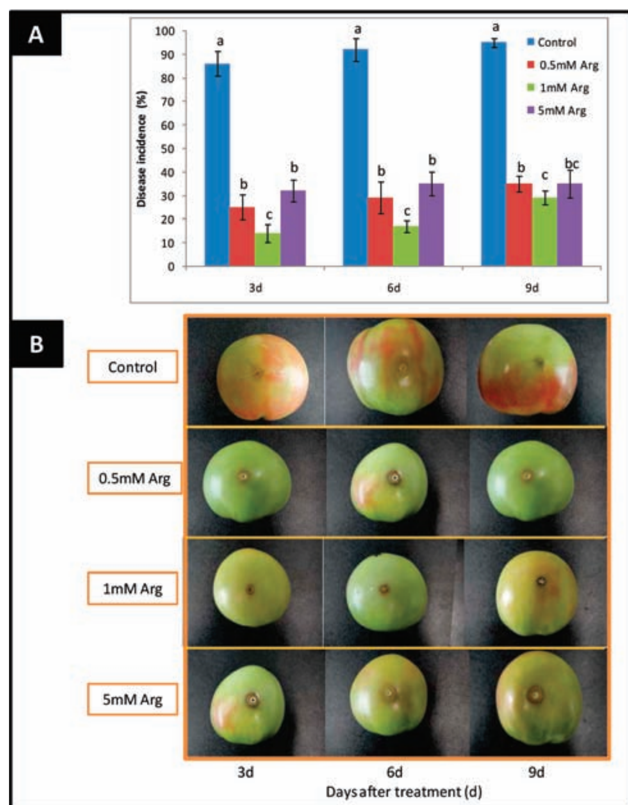

Figure 1. Disease incidence (A) and lesion are (B) in response to different L-arginine concentrations after inoculation with *B. cinerea*. Each column represents the mean of three replicates (each of 15 fruit). Bars represent standard deviations of the means. Values followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$.

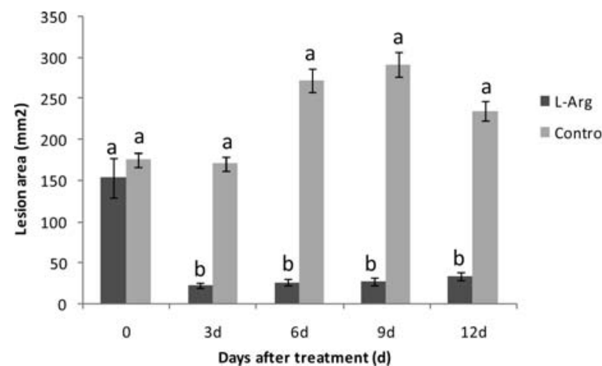

Figure 2. Effect of 1 mM L-arginine on lesion size in No.4 Zhongshu tomatoes inoculated with *B. cinerea*. Each column represents the mean of three replicates. Bars represent standard deviations of the means. Values followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$.

the method of Yao[7] with some modification. *Botrytis cinerea* were cultured on potato dextrose agar (PDA) plates for 7−10 days at 25 °C. L-Arginine concentration was 1 mM in the molten PDA-agar. After the agar had solidified, 7 mm disks of *B. cinerea* were placed in the center of each Petri dish (diameter: 90 mm). Dishes were incubated at 25 °C. Colony diameter was determined 2, 3, and 4 days after treatments. Each treatment was replicated three times, and the experiment was repeated twice. Effects of L-arginine on the mycelial growth of *B. cinerea* were calculated according to the following formula: inhibitory rate on *B. cinerea* (%) = (colony diameter of the control − colony diameter of treatment)/(colony diameter of the control − 7 mm) × 100.

Statistical Analysis and Experiment Replicates. Data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, United States). Significant effects were determined using Duncan's multiple comparison tests, and differences at the 5% level were considered significant. All experiments were conducted in a completely randomized design.

RESULTS

Effects of Different Concentrations of L-Arginine on Disease Incidence and Lesion Area in Tomatoes. Treatment of L-arginine significantly reduced disease incidence and lesion area in tomato fruits inoculated with *B. cinerea* in three different concentrations. The disease incidence in treated fruit appears to have 29%, 16%, 37% of control fruit in 0.5, 1, and 5 mM L-arginine, respectively, at day 3, and 31%, 18%, 38%, respectively, at day 6 (Figure 1A). At 12 days after treatment, the effect of L-arginine on disease incidence was less evident (data is not shown).

Meanwhile, the lesion area in L-arginine-treated fruit was lower than that in control fruit (Figure 1B), and the effect of 1 mM L-arginine treatment on inhibitory lesion expansion was the most evident ($P < 0.05$). Therefore, this concentration was used for further analyses.

Persistence of L-Arginine on Lesion Area in Tomatoes. Compared with controls, fruits treated with L-arginine showed reduced disease incidence after inoculation with *B. cinerea* until 12 days after treatment. The lesion sizes in L-arginine-treated fruit were lower than that of the control, continuing to 12 days after treatment ($P < 0.05$). The inhibition ratios were 86%, 90%, 90%, and 85% in fruits inoculated 3, 6, 9, and 12 days, respectively, after treatments (Figure 2).

Effects of L-Arginine on the Activities of Defense Enzymes. An increase in PAL activity has been used as a marker of the plant's reaction to environmental stress.[30] Preharvest treatment with L-arginine increased PAL activity in fruits in comparison with that of the control (Figure 3A). However, increased PAL activity induced by L-arginine was significant only for 9 days ($P > 0.05$).

Polyphenol oxidase (PPO), which catalyzes the O_2-dependent oxidation of phenolic compounds to form quinines, has been proposed as a component of elaborate plant defense mechanisms. PPO activity is associated with induced defense responses in plants.[31] PPO activity increased rapidly in response to preharvest treatment of L-arginine and reached 0.53U/g FW at 3 days after treatment. After this time, PPO activity decreased gradually in the later stages (Figure 3B).

Chitinases (CHI) and β-1,3-glucanase (GLU), which belong to the PR-2 and PR-3 families,[32] catalyze the hydrolysis of chitin and β-1,3-glucan, respectively. Both polymers are major components of fungal cell walls.[33] Preharvest L-arginine treatment significantly enhanced the activity of CHI at 3 days after treatment, when in L-arginine-treated fruit, it was 1.9-fold higher than that of the control. Then the value of induced CHI activity decreased rapidly (Figure 3C). GLU activities decreased in different treatment stages. Although L-arginine application did not alter the tendency, the activities of GLU were promoted all stages, especially around 3, 6, and 9 days after treatment, when the GLU activities

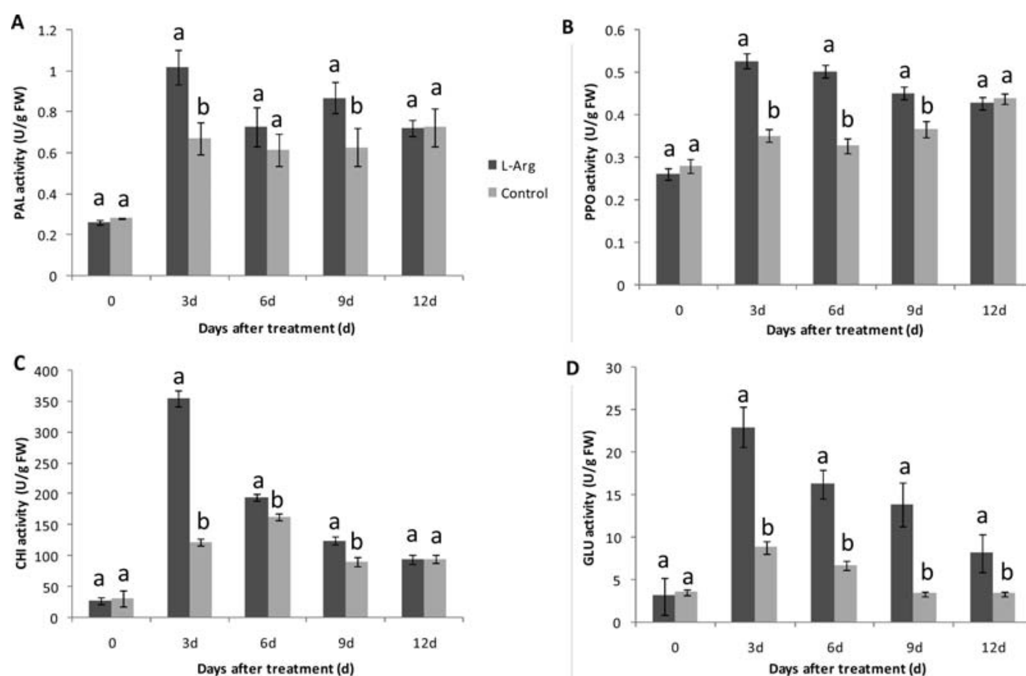

Figure 3. Effect of 1 mM L-arginine preharvest treatment on the activities of defensive enzyme: (A) phenylalanine ammonia-lyase; (B) polyphenol oxidase; (C) Chitinase; and (D) β-1,3-glucanase. Each point represents the mean of three replicates. Bars represent standard deviations of the means. Values followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$.

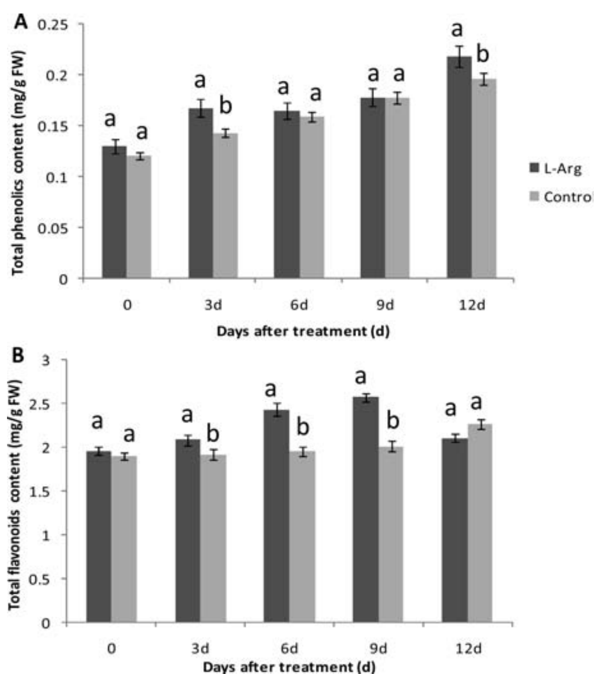

Figure 4. Effect of 1 mM L-arginine preharvest treatment on the content of total flavonoids (A) and total phenols (B). Each point represents the mean of three replicates. Bars represent standard deviations of the means. Values followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$.

were 1.6, 1.4 and 3.1-fold higher with L-arginine treated fruit than that in the control (Figure 3D).

Effects of L-Arginine on Accumulation of Phenolic Compounds. The effects of the L-arginine treatment on total flavonoids and total phenolics contents in fruits are shown in Figure 4. In both treatments, total phenolics content increased throughout the course of the experiment. In L-arginine-treated fruit, total phenolics content showed the most significant difference, and the content level reached peaked 1.17 times higher than that in control fruit at 3 days after treatment. Total flavonoid content was evidently induced in L-arginine treated fruit and showed a similar trend with total phenolic content.

Effects of L-Arginine on NO Concentration and NOS Activity. L-Arginine-treated fruit contained higher NO concentration than control fruit (Figure 5A). NO content in treated fruit increased up to 3 days after treatment and reached 6.71 μmol/g FW ($p < 0.05$). After 3 days, NO production in arginine-treated fruit decreased to a level slightly higher than that of fruit. Compared with that in control fruit, NOS activity increased rapidly in L-arginine-treated fruit (Figure 5B). In L-arginine-treated fruit, NOS activity peaked at 3 days after treatment and reached a level of 2.81 U/g FW. In contrast, NOS activity in control fruit fluctuated in a narrow range.

Effect of L-Arginine on *B. cinerea* in Vitro. L-Arginine had little inhibitory effect on the mycelial growth of *B. cinerea* compared with that of the control, and there was no significant difference between L-arginine treatment and the control. The growth of mycelia by L-arginine treatment did not show obvious changes.

DISCUSSION

Plants employ a complex array of physical and defense mechanisms to resist or evade biotic attacks. In addition to the constitutive defense mechanisms such as trichomes, thick secondary wall, or toxic compounds, plants are also equipped with inducible defense mechanisms,[34,35] among which NO is an essential signal for the development of resistance to the invading

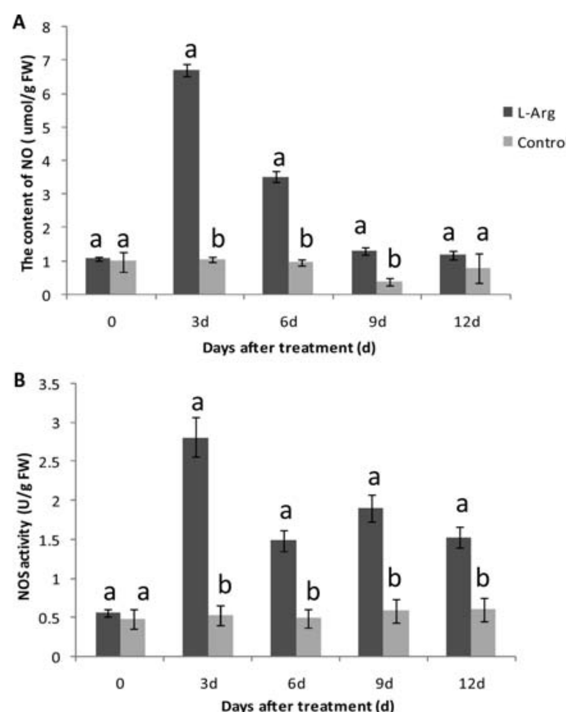

Figure 5. Effect of 1 mM L-arginine preharvest treatment on NO concentration (A) and NOS activity (B). Each point represents the mean of three replicates. Bars represent standard deviations of the means. Values followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$.

pathogen. After pathogen infection, a key feature of the plant resistance response is the generation of an NO burst.[12] In animals, NO is synthesized by nitric oxide synthase (NOS), which catalyzes L-arginine into L-citrulline.[36] In plants, the NO produced during the plant−pathogen interaction originates from NADPH and an enzyme with O_2-dependent NOS-like activity, which converts L-arginine into L-citrulline.[37] Given the important physiological function of L-arginine, it was essential to do further research on L-arginine in order to get more evidence which could prove the importance of NO during disease resistance in tomato fruits.

Resistance of plants to pathogens is based on both constitutive defense mechanisms such as pre-existing antimicrobial compounds and inducible defense mechanisms. Preharvest treatment was undertaken to control the initial infection in the field and to obtain fruit free of infection.[38] The present study showed that all three concentrations of preharvest treatments of L-arginine were effective in controlling the infection of *B. cinerea* in tomato fruits (Figure.1), in which preharvest treatment of 1 mM with L-arginine could significantly reduce the disease incidence of tomato fruits stored at 25 °C. The results of this study also indicated that preharvest treatment with L-arginine was not only concentration effective but also time effective. The protection of tomato fruits from an invasion of *B. cinerea* induced by L-arginine was affected by concentration and time (Figure 2). To get the best research results taking into account the operational and field application costs at the same time, 1 mM L-arginine was chosen for the rest of the experiments.

The disease resistance of tomato fruit was largely due to activation of a highly coordinated biochemical and structural defense system that helps ward off the spread of pathogens.[39,40] Previous researchers have suggested that Chitinase, β-1,3-glucanase, PAL, and PPO are related to induced resistance in plants. Chitinase and β-1,3-glucanase encoded by PR2 and PR3 are thought to be involved in plant defense mechanisms against fungal infection through hydrolyzing the polymers of fungal cell walls.[41,42] PAL is associated with the biosynthesis of secondary metabolites and is established as an enzyme downstream of NO action in these pathways.[43] An increase in PAL activity has been used as a marker of the plant's reaction to environmental stress.[30] PPO, which catalyzes the O_2-dependent oxidation of phenolic compounds to form quinines, has been proposed as a component of elaborate plant defense mechanisms. PPO activity is associated with induced defense responses in plants.[31] Our results indicate that preharvest treatment with L-arginine induces higher activities of Chitinase, β-1,3-glucanase, PAL, and PPO in tomato fruits than those in the controls during the whole storage period (Figure 3A−D). Synthesis of phenolic compounds results from signaling processes that are initiated very quickly after injury, pathogen attack, or elicitation.[44] Thus, an increase in total phenolic compounds can be a marker for the defense response in plants. L-Arginine preharvest treatment resulted in marked increases in total phenolic and total flavonoid content and throughout the experimental period (Figure 4). Together, these data confirmed that preharvest L-arginine treatment induced disease resistance in tomatoes.

Although resistance was enhanced by L-arginine pretreatment, the mechanisms by which L-arginine induces disease resistance remain unclear. NO is a major signaling molecule in plants. It plays crucial roles in the regulation of both defense responses and in inducing resistance to fungal pathogens. Exogenous application of NO promotes the activities of the defensive enzyme,[13,45] triggers accumulation of secondary metabolites,[46] and activates the expression of a series of resistance-related genes encoding phenylalanine ammonia-lyase (PAL) and pathogenesis-related proteins. The specific release of endogenous NO induced by L-arginine may be important for resistance. In our study, endogenous NO production increased after L-arginine treatment (Figure 5A), and NO content was positively correlated with PAL, PPO, CHI, and GLU activities after treatment. The Pearson coefficients were 0.71 ($P < 0.05$) and 0.94 ($P < 0.01$); 0.97 ($P < 0.01$) and 0.87 ($P < 0.05$), respectively. These results suggest that there is a close relationship between the change in NO content and the activities of defense enzymes. Meanwhile, the content of endogenous NO and disease incidence showed a significantly negative correlation with pearson coefficients of −0.88 ($P < 0.05$). The effects of L-arginine are not restricted to enzymes associated with disease resistance. Increases in NOS activity were observed in fruits in response to L-arginine preharvest treatment (Figure 5B). There is evidence that NOS activity is closely related to NO formation. The Pearson coefficient between endogenous NO content and NOS activity was 0.77 ($P < 0.05$). This suggests that the L-arginine−NOS−NO pathway is involved in preharvest L-arginine-induced disease resistance.

The results of our experiments suggest that preharvest treatment increased the activities of NOS and the content of endogenous NO and enhanced the defense responses in tomato fruits. However, we have to speculate whether L-arginine showed direct fungitoxicity on *B. cinerea* and inhibited mycelia growth or spore germination of the fungus in vitro. In an antibacterial test in vitro, we found that L-arginine treatment did not significantly inhibit the growth of the mycelium. The effect of L-arginine may be due to some physiological and biochemical reactions, related

to other antibacterial substances in induced disease resistance in tomato fruits and not because of its own toxic effect. This inference should be useful in the integrated control of postharvest diseases of tomatoes.

Preharvest treatment is a new trend of disease control in harvested fruit since the process is involved with the metabolism and modulation of plants. For example, nitrate and nitrite were produced in the L-arginine metabolic pathway and are important safety indicators in fruit and vegetable products. In this experiment, we had detected the contents of nitrate and nitrite, which were under the minimum detection limit (data are not shown). Whether preharvest treatment affects produce quality during the process of disease resistance induction needs further studies. In conclusion, the work presented here showed that the application of L-arginine in preharvest treatment, for inducing the defense resistance system against postharvest diseases in tomato fruits, may be a useful and promising measure for controlling postharvest decays on a commercial scale.

AUTHOR INFORMATION

Corresponding Author

*Phone: +86-10-62738456. Fax: +86-10-62736474. E-mail: pingshen@cau.edu.cn.

Notes

SafetyThis work was safe without explosive tendencies, special precautionary handling, and toxicity.

Funding Sources

This work was supported by the National Basic Research Program of China (No. 2011CB100604), the National Natural Science Foundation of China (No. 31071623 and 30671471), and the Commonweal Industry Scientific Researcher Project from the Ministry of Agriculture (No. 200803033).

ACKNOWLEDGMENT

We thank research scientist Aijun Zhang from USDA-ARS, USA, for his help with revising the grammar.

ABBREVIATIONS USED

CHI, Chitinase; FW, fresh weight; GLU, β-1,3-glucanase; NO, nitric oxide; NOS, nitric oxide synthase; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase.

REFERENCES

(1) Eckert, J. W.; Ogawa, J. M. The chemical control of postharvest diseases: deciduous fruit, berries, vegetables and root/tuber crops. *Annu. Rev. Phytopathol.* **1988**, *26*, 433–469.

(2) Mercier, J.; Smilanick, J. L. Control of green mold and sour rot of stored lemon by biofumigation with Muscodor albus. *Biol. Control* **2005**, *32*, 401–407.

(3) Meng, X.; Li, B.; Liu, J.; Tian, S. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. *Food Chem.* **2008**, *106*, 501–508.

(4) Capanoglu, E. The potantial of priming in food production. *Trends Food Sci. Technol.* **2010**, *21*, 399–407.

(5) Joyce, D. C.; Johnson, G. I. Prospects for exploitation of natural disease resistance in harvested horticultural crops. *Postharvest News Inform.* **1999**, *10*, 45–48.

(6) Wang, F. D.; Feng, G. H.; Chen, K. S. Defense responses of harvested tomato fruit to burdock fructooligosaccharide a novel potential elicitor. *Postharvest Biol. Technol.* **2009**, *52*, 110–116.

(7) Yao, H. J.; Tian, S. P. Effects of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biol. Technol.* **2005**, *35*, 253–262.

(8) Bhaskara Reddy, M. V.; Belkacemi, K.; Corcuff, R.; Castaigne, F.; Arul, J. Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit. *Postharvest Biol. Technol.* **2000**, *20*, 39–51.

(9) Delledonne, M.; Xia, Y. J.; Dixon, R. A.; Lamb, C. Nitric oxide functions as a signal in plant disease resistance. *Nature* **1998**, *394*, 585–588.

(10) Clark, D.; Durner, J.; Navarre, D. A.; Klessig, D. F. Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Mol. Plant-Microbe Interact.* **2000**, *13*, 1380–1384.

(11) Zhang, C.; Czymmek, K. J.; Shapiro, A. D. Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signaling governing progression of the Arabidopsis hypersensitive response. *Mol. Plant-Microbe Interact.* **2003**, *16*, 962–972.

(12) Talbot, N. Emerging Themes in Plant—Pathogen Interaction. In *Plant—Pathogen Interactions, Annual Plant Reviews*; Talbot, N., Ed.; CRC Press: Boca Raton, FL, 2004; Vol. 11, pp 1—26.

(13) Durner, J.; Wendehenne, D.; Klessig, D. F. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10328–10333.

(14) Polverari, A.; Molesini, B.; Pezzotti, M.; Buonaurio, R.; Marte, M.; Delledonne, M. Nitric oxide-mediated transcriptional changes in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* **2003**, *16*, 1094–1105.

(15) Huang, X.; von, R. U.; Durner., J. Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. *Planta* **2002**, *215*, 914–923.

(16) Kakkar, R. K.; Bhadur, S.; Rai, V. K.; Kumar, S. Amelioration of NaCl stress by arginine in rice seedlings: changes in endogenous polyamines. *Biol. Plant* **2000**, *43*, 419–422.

(17) Chen, H.; McCaig, B. C.; Melotto, M.; He, S. Y.; Howe, G. A. Regulation of plant arginase by wounding, jasmonate, and the phytotoxin coronatine. *J. Biol. Chem.* **2004**, *279*, 45998–46007.

(18) Modolo, L. V.; Augusto, O.; Almeida, I. M. G.; Pinto-Maglio, C. A. F.; Oliveira, H. C.; Seligman, K.; Salgado, I. Decreased arginine and nitrite levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impair nitric oxide synthesis and the hypersensitive response to *Pseudomonas syringae*. *Plant Sci.* **2006**, *171*, 34–40.

(19) Stevens, M.; Rick, C. M. Genetics and breeding. In: Atherton, J. G., Rudich, G. (Ed.), *The Tomato Crop: A Scientific Basis for Improvement*. Londun, NY, 1986, pp 75—77.

(20) Koukol, J.; Conn, E. E. The metabolism of aromatic compounds in higher plants. IV. Purification and properties of the phenylalanine deaminase of *Hordeum vulgare*. *J. Biol. Chem.* **1961**, *236*, 2692–2698.

(21) Liu, J.; Tian, S. P.; Meng, X. H.; Xu, Y. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biol. Technol.* **2007**, *44*, 300–306.

(22) Boller, T.; Gehri, A.; Mauch, F.; Vogeli, U. Chitinase in bean leaves: induction by ethylene, purification, properties and possible function. *Planta* **1983**, *157*, 22–31.

(23) Siefert, F.; Langebartels, C.; Boller, T.; Grossmann, K. Are ethylene and 1-aminocyclopropane-1-carboxylic acid involved in the induction of Chitinase and β-1,3-glucanase activity in sunflower cell-suspension cultures? *Planta* **1994**, *192*, 224–232.

(24) Hinton, D. M.; Pressey, R. Glucanases in fruit and vegetables. *J. Am. Soc. Hortic. Sci.* **1980**, *105*, 499–502.

(25) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phophomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(26) McCue, P.; Zheng, Z.; Pinkham, J. L.; Shetty, K. A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. *Proc. Biochem.* **2000**, *35*, 603–613.

(27) Lamaison, J. L.; Carnat, A. Teneursen principaux flavonoides des fleurs et des feuilles de Crataegus monogyna Jacq et de Crataegus laevigata (Poiret) DC. en fonction de la végétation. *Plantes Médit. Phytoth.* **1991**, *28*, 12–16.

(28) Hevel, J. M.; Marletta, M. A. Nitric oxide synthase assays. *Methods Enzymol.* **1994**, *233*, 250–258.

(29) Murphy, M. E.; Noack, E. Nitric oxide assay using haemoglobin method. *Methods Enzymol.* **1994**, *233*, 241–250.

(30) Pociecha, E.; Płażek, A.; Janowiak, F.; Zwierzykowski, Z. ABA level, proline and phenolic concentration, and PAL activity induced during cold acclimation in androgenic Festulolium forms with contrasting resistance to frost and pink snow mould (*Microdochium nivale*). *Physiol. Mol. Plant P.* **2008**, *73*, 126–132.

(31) Ramiro, D. A.; Guerreiro-Filho, O.; Mazzafera, P. Phenol contents, oxidase activities, and the resistance of coffee to the leaf miner *Leucoptera coffeella*. *J. Chem. Ecol.* **2006**, *32*, 1977–1988.

(32) van Loon, L. C.; van Strien, E. A. The families of pathogenesis-related proteins, their activities and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* **1999**, *55*, 85–97.

(33) Webster, J. *Introduction to Fungi*; Cambridge University Press: New York, 1986; pp 57−64.

(34) Paiva, N. L. An introduction to the biosynthesis of chemicals used in plant-microbe communication. *J. Plant Growth Regul.* **2000**, *19*, 131–143.

(35) Walling, L. L. The myriad plant responses to herbivores. *J. Plant Growth Regul.* **2000**, *19*, 195–216.

(36) Palmer, R. M. J.; Hickery, M. S.; Charles, I. G.; Moncada, S.; Bayliss, M. T. Induction of nitric-oxide synthase in human chondrocytes. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 398–405.

(37) Luzia, V. M.; Ohara, A.; Irene, M. G. A.; Pinto-Maglio, C. A. F.; Oliveira, H. C.; Seligman, K.; Salgado, I. Decreased arginine and nitrite levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impair nitric oxide synthesis and the hypersensitive response to *Pseudomonas syringae*. *Plant Sci.* **2006**, *171*, 34–40.

(38) Aharoni, Y.; Barkai-Golan, R. Pre-harvest fungicide sprays and polyvinyl wraps to control Botrytis rot and prolong the post-harvest storage life of strawberries. *J. Hortic. Sci.* **1987**, *62*, 177–181.

(39) Lawton, K.; Friedrich, L.; Hunt, M.; Weymann, K.; Kessmann, H.; Staub, T.; Ryals, J. Benzothiadiazole induces disease *resistance in Arabidopsis* by activation of the systemic acquired resistance signal transduction pahway. *Plant J.* **1996**, *10*, 71–82.

(40) Schroder, M.; Hahlbrock, K.; Kombrink, E. Temporal and spatial patterns of β-1,3-glucanase and Chitinase induction in potato leaves infected by *Phytophthora infestans*. *Plant J* **1992**, *2*, 161–172.

(41) Collinge, B.; Kragh, K. M.; Mikkelsen, J. D.; Nielsen, K. K.; Rasmussen, U.; Vad, K. Plant chitinases. *Plant J.* **1993**, *3*, 31–40.

(42) Schlumbaum, A.; Mauch, F.; Vogeli, U.; Boller, T. Plant chitinases are potent inhibitors of fungal growth. *Nature* **1986**, *324*, 365–367.

(43) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell.* **1995**, *7*, 1085–1097.

(44) Graham, T. L.; Graham, M. Y. Signaling in soybean phenylpropanoid responses, Dissection of primary secondary and conditioning effect of light wounding and elicitor treatments. *Plant Physiol.* **1996**, *110*, 23–33.

(45) Hao, G. P.; Du, X. H.; Zhao, F. X.; Shi, R. J.; Wang, J. M. Role of nitric oxide in UV-B-induced activation of PAL and stimulation of flavonoid biosynthesis in *Ginkgo biloba* callus. *Plant Cell, Tissue Organ Cult.* **2009**, *97*, 175–185.

(46) Xu, M. J. Nitric oxide: A potential key point of the signaling network leading to plant secondary metabolite biosynthesis. *Prog. Nat. Sci.* **2007**, *17*, 1397–1404.