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Technical Note

Conservation of post-harvest leaves of green onion (*Allium fistulosum* L.) with the use of salicylic acid solution

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Abstract

The green onion (Allium fistulosum) is a plant belonging to the family of the Aliaceae, which is used as condiment and seasoning. Its postharvest durability is very short, mainly due to its high metabolic rate and high water content, coupled with poor techniques of management and transport to the consumer. The salicylic acid is an inducer of resistance which acts antagonistically to the ethylene biochemical route, delaying the senescence of plants. The study aimed to evaluate the effect of salicylic acid on post-harvest conservation of green onions leaves. It were used five treatments in a completely randomized design and the treatments consisted of four concentrations of salicylic acid (1µM, 2µM; 8µM and 4mm) and the control (distilled water), in four replicates of ten leaves. After the harvest, selection and standardization of the samples, the bases of the green onions leaves were immersed in solutions with the different treatments for thirty minutes. The leaves were kept in a BOD incubator for seven days at a temperature of 10°C. The variables used for evaluation of the experiment were: weight loss, chlorophyll a, b and total, coloration, rottenness, protein content and activity of the phenylalanine ammonia-lyase enzyme. The obtained results showed that the mass loss was significant, with linear descending behavior with the increase of concentrations, up to five days after treatment application, as well as the decay of leaves, which obtained a quadratic behavior, on the seventh day, therefore demonstrating potential form of salicylic acid in the improvement of post-harvest conservation of green onions.

Keywords: horticulture; vegetable storage; phenylalanine ammonia-lyase.

Conservação pós-colheita de folhas de cebolinha-verde (*Allium fistulosum* L.) com o uso de ácido salicílico em solução

Resumo

A cebolinha-verde (*Allium fistulosum*) é uma planta pertencente à família das Aliáceas, a qual é utilizada como condimento e tempero. Sua durabilidade na pós-colheita é muito curta, devido principalmente a sua alta taxa metabólica e alto teor de água, aliado a técnicas precárias de manejo e transporte até o consumidor. O ácido salicílico é um indutor de resistência que atua de forma antagônica a rota bioquímica do etileno, retardando a senescência dos vegetais. O trabalho teve como objetivo principal avaliar do efeito do ácido salicílico na conservação em pós-colheita de folhas de cebolinha. Foram utilizados cinco tratamentos em delineamento inteiramente ao acaso, sendo que os tratamentos consistiram de quatro concentrações de ácido salicílico (1µM; 2µM; 4µM e 8µM) e a testemunha (água destilada), em quatro repetições de dez folhas. Após a colheita, seleção e padronização das amostras, as bases das folhas de cebolinha foram imersas nas soluções com os diferentes tratamentos, durante trinta minutos. As folhas foram mantidas em incubadora B.O.D., durante sete dias, à temperatura de 10ºC. As variáveis utilizadas para avaliação do experimento foram: perda de massa, clorofila a, b e total, coloração, podridões, teor de proteínas e atividade da enzima fenilalanina amônialiase. Os resultados obtidos mostraram que a perda de massa foi significativa, com comportamento linear descendente com o aumento das concentrações, até cinco dias após a aplicação dos tratamentos, assim como as podridões das folhas, que obteve um comportamento quadrático, no sétimo dia, demonstrando dessa forma potencial do ácido salicílico na melhoria da conservação pós-colheita de cebolinhas.

Palavras-chave: horticultura; armazenamento vegetal; fenilalanina amônialiase.

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Conservación post- cosecha de las hojas de cebolleta verde (*Allium fistulosum* L.) utilizando ácido salicílico en solución

Resumen

La cebolleta verde (*Allium fistulosum*) es una planta perteneciente a la familia de Aliáceas, que se utiliza como especia y condimento. Su durabilidad poscosecha es muy corto, debido principalmente a su alta tasa metabólica y el alto contenido de agua, junto con técnicas precarias de manejo y transporte hasta el consumidor. El ácido salicílico es un inductor de resistencia que actúa de forma antagonista a la vía bioquímica del etileno, retrasando la senescencia de las plantas. El trabajo tuvo como objetivo evaluar el efecto del ácido salicílico en la conservación postcosecha de hojas de cebolleta. Fueron utilizados cinco tratamientos en un diseño completamente al azar, los tratamientos consistieron en cuatro concentraciones de ácido salicílico (1µM; 2µM; 4µM e 8µM) y el control (agua destilada), con cuatro repeticiones de diez hojas. Después de la cosecha, selección y normalización de las muestras, se sumergieron las bases de las hojas de cebolleta en soluciones con diferentes tratamientos durante treinta minutos. Las hojas se mantuvieron en una incubadora B.O.D. durante siete días a una temperatura de 10°C. Las variables utilizadas para la evaluación del experimento fueron: pérdida de masa, clorofila a, b y total, colores, podredumbre, proteína y la actividad de la enzima fenilalanina amônialiase. Los resultados mostraron que la pérdida de masa fue significativa, con comportamiento lineal decreciente, hasta cinco días después de la aplicación de los tratamientos, así como la podredumbre de las hojas, que obtuvo un comportamiento cuadrático, en el séptimo día, lo que demuestra el potencial del ácido salicílico en mejorar el almacenamiento postcosecha de cebolletas.

Palabras clave: horticultura; almacenamiento de vegetales; fenilalanina amônialiase.

Introduction

The green onion (*Allium fistulosum* L.) belongs to the family of the Aliaceae, and is used as condiment and seasoning, it is originally from Siberia or the East, being cultivated in annual or biennial form. It has morphological characteristics similar to the onion, however, it does not form bulbs, but a thickening in the stem bases (CAMARGO, 1992).

The green onion is a highly perishable vegetable, due to its moisture content, high metabolic rate and deficient postharvest management and transport of the same (GONZÁLES et al., 2012).

Few studies are described in the literature about postharvest conservation of green onion. CANTWELL et al. (2001) report that postharvest treatments with water at 52.5 ^{o}C, or combined with chlorine at concentrations of 50 to 400 mg L^{-1} of NaOCl were more effective in the maintenance of the visual aspect and in the inhibition of initial microbial infections than the treatments with lower temperatures, with 20 ^{o}C and the same concentrations of chlorine. GONZÁLES et al. (2012) state that the used of vacuum packaging at the temperature of 4 °C increases the durability of the green onion leaves when compared to the storage without vacuum packaging and at ambient temperature. Using electrolytic water as treatment of green onion leaves for 3 minutes, HSU et al. (2013) observed a great potential for maintenance of visual quality of the vegetal material and decrease of microorganisms which promote decay.

The use of nutritive solutions or of resistance inducers can also be an alternative as method to increase the plants resistance to diseases and to prolong the postharvest durability of the vegetal materials (FINGER et al., 2004; DANNER et al., 2008; DALLAGNOL et al., 2006).

Among the resistance inducers which have been tested in vegetal materials, mainly in fruits and flowers, is the salicylic acid (REGLINSKI et al., 1997; POOLE et al., 1998; YAO and TIAN, 2005). The salicylic acid is a regulator of vegetal growth involved in the systematic resistance to pathogens (TAIZ and ZEIGER, 2004), due to its accumulation in the vegetal tissue (PASCHOLATI, 2011), in the biosynthesis and ethylene action (RASKIN, 1992). This compound can be considered an antagonist to ethylene, the main phytoregulator of fruits maturation (CIA et al., 2007), which can be also interesting for the use in leafy vegetal materials, such as the green onion, which is consumed *in natura*.

However, it does not exist in the literature studies which report the use of resistance inducers, as well as the salicylic acid in the postharvest conservation of green onions leaves.

The present study had as objective the assessment of different concentration of salicylic acid in solution, to increase of the postharvest durability of green onion leaves (*Allium fistulosum*).

Material and Methods

The present experiment was conducted in the Laboratory of Plant Health of the Universidade Tecnológica Federal do Paraná – Campus Dois Vizinhos during the year of 2013.

The green onion leaves (*Allium fistulosum*), cultivar "Todo Ano" were obtained from an organic vegetable garden in the municipality of Dois Vizinhos – PR. In the laboratory they were selected, eliminating leaves with mechanical damage or visual symptoms of pathogens, standardized, with an average size of 30 cm of height and 1 to 2 cm of diameter, then were arranged in experimental units (packets) which consisted of ten leaves each.

The used experimental design was completely randomized, with five treatments and four repetitions, being that the treatments consisted of immersion of the leaves during 30 minutes in four different concentrations of salicylic acid (1μM; 2μM; 4μM and 8μM) and the control (distilled water).

The leaves received the treatments through diffusion of sap, with only the base of the vegetal material immerse in the solution. Next, the leaves were placed in properly sterilized plastic packages and stored in a BOD incubator at 10 °C for seven days.

Among the used variables for evaluation of the experiment, there is the fresh mass loss, which was obtained by the difference of the mass in the samples at the experiment installation day and the value found in the weighing at the fifth and seventh days after the treatments application.

The contents of chlorophyll a, b and total (SPAD reading) were assessed at the fifth and seventh days after the treatment application, with the assist of a chlorophilometer. The readings were done in two points of each side of the leaf midrib, in the adaxial side.

The evaluation of the rottenness severity (leaf stains characteristic of pathogens action) was done at the seventh day after the treatment application, and was considered the presence, absence and percentage of damages, considering the damaged leaf, which presented typical characteristics of pathogens action. The confirmation was given by the visualization through a stereoscope microscope and realization of layers to identify the structure of the same

The green coloration of the leaves was assessed at the seventh day after the treatment application, through a scale with indexes from 1 to 4, where the index 1 indicates a green pigmented surface equivalent to 0 to 25% of the epidermis total area, the index 2 is 25 to 50%, the index 3 is 50 to 75% and the index 4 of 75 to 100% of the epidermis total area.

The biochemical analysis was done from the healthy samples of the foliar tissue. For dosage of total proteins, the samples were macerated in a mortar with 10 mL of phosphate buffer 0.2 M (pH 7.5). Next, the material was centrifuged (14.000g for 10 min at 4° C) and the supernatant was collected. For quantification of the total content of proteins of the samples, it was used the BRADFORD (1976) test. The total proteins reading were done in a spectrophotometer at 630 nm, using bovine serum albumin as standard.

The determination of the phenylalanine ammonia-lyase activity was through colorimetric quantification of the trans-cinnamic acid, liberated from the phenylalanine substrate, according to the methodology described by KUHN (2007).

The obtained results were submitted to statistical procedures, through the analysis of variance and complementary tests, with assist of the software ASSISTAT 7.5 BETA.

Results and Discussions

The obtained results in this experiment show that the application of salicylic acid provided a reduction in the mass loss, in the assessment done at the fifth day after the application of the treatments. Such mass loss of the green onion leaves behaved in a decreasing linear form, according to the increase of the concentration of salicylic acid (Figure 1).

The fresh mass loss is mainly caused by the loss of water in the leaves, through the processes of transpiration and respiration. The green onion, due to its thin epidermis and its high content of moisture, has high susceptibility to water loss, in function of the transpiration in face of low air relative humidity and high temperatures, resulting in dehydration of the same.

The effect of the salicylic acid in the reduction of fresh mass loss possibly is related to the decrease of the metabolic activity, such as respiration and production of ethylene. The inhibition of ethylene synthesis through the application of salicylic acid (SA) was proved by LESLIE and ROMANI (1996), who suggested that the AS acts in the inhibition of the 1-amino-cyclopropane carboxylic acid (ACC), which is the immediate precursor in the synthesis

Figure 1

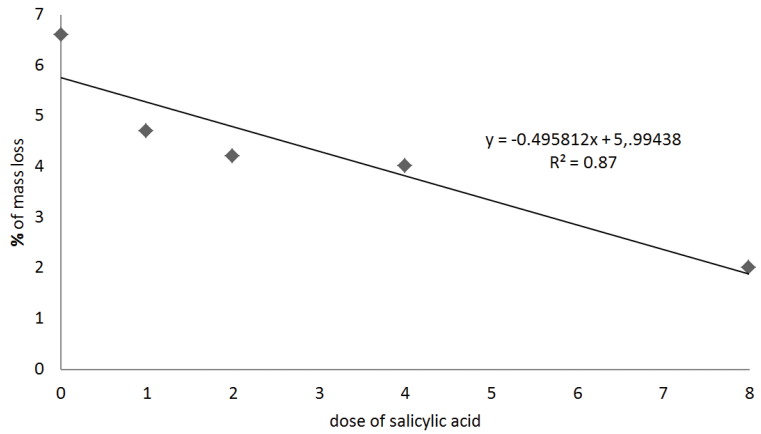

Figure 1. Percentage of mass loss of the Green onion leaves treated with different concentrations of salicylic acid. Dois Vizinhos, 2013.

of ethylene. The ethylene is produced in all superior plants from the methionine, being derivate of carbons 3 and 4 of this amino acid.

A promising result was also observed in the reduction of rottenness of the green onion leaves, at the seventh day after the treatments application, such parameter behaved in a quadratic form (Figure 2), being that the point of inflexion, i. e., where the percentage of rot was lesser, is the concentration of 1.97% of salicylic acid.

According to CIA et al. (2007), derivate compounds of benzoic acid, such as the salicylic acid can promote the resistance induction against pathogens. The exogenous application of salicylic acid in different plant species induced the expression of genes of PR-proteins, not only in the application local, but also in systemic form, suggesting that the salicylic acid acts as indicator in the systemic acquired resistance (SAR), which is a form of induced resistance that increases the plants endurance against subsequent infections and attacks of phytopathogens (KESSMANN et al., 1994).

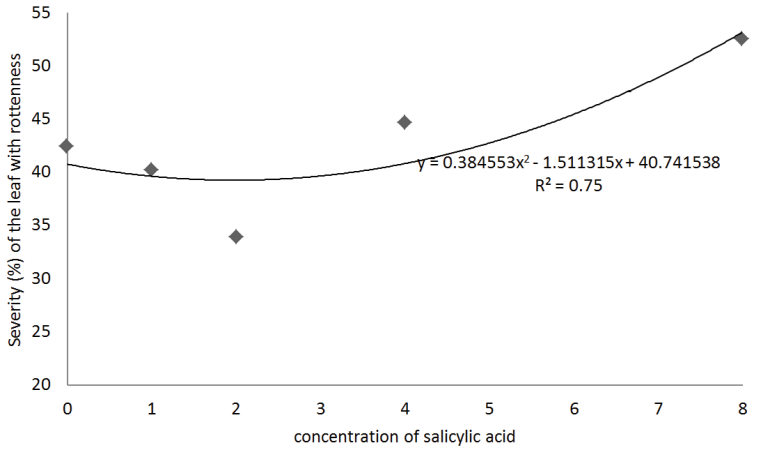

Figure 2. Severity of rottenness of the green onion leaves treated with different concentrations of sacylic acid. Dois Vizinhos, 2013.

Several routes can be activated in the resistance induction, such as the one from the phenylpropanoids (shikimic acid), with activation of the phenylalanine ammonia lyase enzyme (PAL), for formation of secondary metabolites for vegetal defense (DANNER et al., 2008; MAZARO et al., 2009), or through the route of the salicylates, with activation of pathogenicity-related proteins (PRPs), such as the chitinase and glucanase (CAMARGO, 2011; DI PIERO and GARDA, 2008).

In this study was not observed significant effect for activity of the FAL in response to the treatments application. This suggests that the rottenness reduction observed in this study can be related to other routes of vegetal defense, and not preferably the one of the phenylpropanoid.

There is also the possibility that the FAL activation have occurred in a different moment of the evaluation, i. e., soon after the treatments application and in the moment of the biochemical assessments, at the seventh day, it could already be in similar levels to the control. The metabolites contents of plants defense, in response to elicitors can vary according to the time of application of the same. DI PIERO and GARDA (2008) report that the glucanases activity in common bean was greater at the fourth day after the plants treatment with chitosan in comparison with the second and sixth day after the inducer application.

Thus, we suggest further studies that include the assessment of the activity of the FAL enzyme during the experiment, as well as insertion of enzymatic activity analysis, for the peroxidase, chitinase and glucanase enzymes.

The data shows that did not occur effect of the treatments on the proteins content, content of chlorophyll a, b and total as well as on the leaves coloration. Such data suggest that the treatments do not act significantly on the proteins metabolism, neither on the chlorophyll degradation in the assessed periods.

The use of resistance inducers in plants can alter the proteins and chlorophylls metabolism. As for the protein contents, it can decrease through application of inducers (KUHM, 2007) or increase by application of the same (VIECELLI et al., 2009), or remain unaltered, as occurred in this study. Such processes, as the activation, maintenance or suppression are dependent of many factors, being among them the specificity of the vegetal species and the used inducer.

As for the non activation of the chlorophyll molecules and green onion coloration, such parameters are directly related, i. e., with the reduction of the levels of chlorophyll would occur a loss of green coloration to yellowing of the leaves, being that this did not occur in the study. One of the hypotheses of the non alteration of the chlorophyll contents is that the leaves were detached from the plant and submitted to dark condition (BOD), and therefore ceased to be the main source of energy production for synthesis of the compounds of plant defense, considering that the chlorophyll molecules a and b constitute the two systems of pigments responsible for the absorption and transference of radiant energy.

In bean plants infected with *Uromyces appendiculatus* occurred an increment in the chlorophylls content, but when were infected with *Phaeoisariopsis griseola*, it was observed reduction in the contents of chlorophyll a and b in the moderately susceptible cultivar, whereas there was no significant difference in the highly susceptible cultivar, when compared to the control (STANGARLIN and PASCHOLATI, 2000).

In this sense, in the same way as the proteins, the alteration in the chlorophyll contents is very dependant of the species, inducer and mainly environment conditions, and in the case of this experiment, in the conditions proposed in the study were not especially relevant to metabolic alteration of the same.

Conclusions

The application of salicylic acid presents potential for postharvest conservation of green onion leaves, demonstrating action in the decrease of mass loss and on the rottenness reduction, however, are necessary more studies verifying if there were activated, compounds of defense as well as which was the activated metabolic route.

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