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The Effect of Salicylic Acid on the Reduction of *Rizoctonia solani* Damage in the Tubers of Marfona Potato Cultivar

[1]M.R. Hadi and [2]G.R. Balali

[1]Department of Biology, Sciences and Research Branch of Fars, Islamic Azad University, Iran
[2]Potato Biotechnology Research Department, University of Isfahan, Iran

Abstract: This study was carried out to evaluate the effects of salicylic acid in Marfona potato cultivar (*Solanum tuberosum* L.) for reducing the pathogenesis of *Rizoctonia solani* in potato tubers under greenhouse conditions. The pots sterilized soils had been infected by *R. solani* (21B1 strain) two weeks before transplanting the potato plantlets. The seedlings were treated with 0 (control), 0.1, 0.2, 0.5, 1, 2 and 4 mM of salicylic acid every week. The results showed that treatment with 0.2 mM salicylic acid resulted in 73% reduction in the infection symptoms on the potato tubers (sclerotia). Moreover, the intensity of infection symptoms were further reduced by an increase in the concentration of salicylic acid. The least infection was observed when 0.5 mM salicylic acid was applied. The results also showed that the number of potato tubers was increased by the application of 2 mM salicylic acid to plants that had been infected with fungi. Base of the results obtained, the application of 0.2 - 0.5 mM salicylic acid resulted in the reduction of tuber losses caused by *Rizoctonia* fungi.

Key words: Solanum tuberosum · Rizoctonia solani infection · Salicylic acid

INTRODUCTION

The Food and Agricultural Organization (FAO) of the United Nations has named the year 2008 as the international year of the potato (*Solanum tuberosum*). This signifies the importance of this crop which plays an important role in coping with ever increasing world food demand. Potato, after rice, wheat and corn is the most common food material consumed in the world. The history of the potato is almost as old as the history of the man itself. It dates back nearly to 8,000 years ago when the Indians inhabiting in the south of present day Peru domesticated the wild potatoes plants that used to grow around the Lake Titicaca. According to FAO report, Asian countries produced nearly 131 million tons of potatoes in 2006, followed by Europe with 126 million tons. China is the largest potato producer and India as the second largest. In the same year the total amount of potato produced was 314 million tons. Potato fields are treated against numerous pathogens including fungi that each year cause great losses in potato crops. Plants have both constitutive and induced mechanisms that aid them to defend against diseases. Following a localized infection, many plants have been shown to manifest a systemic reaction that leads them to a wide resistance spectra against various pathogens, including fungi, bacteria and viruses. Because the entire plant manifests the resistance response, this form of induced resistance is called systemic acquired resistance. The onset of systemic acquired resistance has been shown to be accompanied by the accumulation of salicylic acid, a wide variety of mRNA species and their encoded protein products [1]. Salicylic acid which has recently been considered as one of the plant hormones [2] is involved in reducing the damage caused by various pathogens such as bacteria, fungi and viruses. It is also considered as the most important factor in systemic acquired resistance against the above pathogens [3]. The levels of salicylic acid increase in plants undergoing systemic acquired resistance. Different investigations have also shown that the application of salicylic acid causes the induction of resistance in plants such as Arabidopsis and tobacco against viruses and fungi [4]. Furthermore, application of salicylic acid induces the same set of genes and spectrum of resistance specificities as found in biologically-induced systemic acquired resistance [1]. Salicylic acid causestheinduction of a range of defense genes [5], the most important of which is the expression of diseaserelated proteins which have shown special anti-bacterial and anti-fungal characteristics about which little knowledge with regard to the role of salicylic acid in resistance induction is available. These observations led

Corresponding Author: M.R. Hadi, Department of Biology, Sciences and Research Branch of Fars, Islamic Azad University, Iran

to the hypothesis that salicylic acid acts as a signal that triggers systemic acquired resistance. This hypothesis was validated by experiments in which tobacco plants were engineered to express a bacterial salicylate hydroxylase gene, whose product degrades salicylic acid. This study evaluates the effect of salicylic acid in reducing lesions on potato tubers caused by *Rizotonia solani* fungus.

MATERIALS AND METHODS

Growth Conditions: The experiment was conducted during 2007-2008 under greenhouse conditions at Potato Research Center, University of Isfahan, Iran. Disease free Marfana potato cultivar Marfana was obtained from Agricultural Biotechnology Research Institute of Iran (ABRII). Tubers were propagated by nodal cuttings made under aseptic conditions and were kept in growth chamber at 24 °C ±3 with light intensity of 55.6 μmol m^{-2} s^{-1} and 16h/8h light/dark period. The 4 week-old plantlets grown from each single nodal cutting were planted in 3.8 liter clay pots (one seedling per pot) [6] filled with Methylbromide fumigated soil (two parts sandy loam, one part decayed compost) on November 2007. The light intensity was 83.3 μmol m^{-2} s^{-1} with 13h/11h dark/light period. After being hardened for four weeks under plastic sheets, the plantlets were grown in the greenhouse at 23 ± 2°C/16 ± 2°C (day/night temperatures) under natural light. Plants were irrigated as required to maintain adequate moisture levels. The pots were fertilized monthly with a 250-ppm solution of 20:20:20 (N:P:K) starting the 2nd week of planting.

Fungal Inoculation: Pure fungal (*Rizoctonia solani*), strain $21B_1$ culture was obtained from the Department of Biology, University of Isfahan. Two weeks before transplanting the potato seedlings in the pots, the soils, except control, were inoculated with the fungus inoculums at four areas (2 x 2 cm^2).

Salicylic Acid Treatment: The leaves were sprayed with deionized water (control) and solutions of 0.1, 0.2, 0.5, 1, 2 and 4 mM salicylic acid. Ten ml of these solutions were also added to the soils in the pots weekly. Treatments were arranged as randomized complete blocks with 30 pots (replications) for each treatment.

Parameters Measured: Both the total number and total weight of potato tubers per pot were determined 120 days after planting. The number of infected spots on every potato tuber was determined. The scales of infected spot (presence of sclerotia) on the potato tubers were selected from 1 to 5 as follows: scale 1: (1-5), scale 2: (5-10), scale 3: (10-15), scale 4: (15-20) and scale 5: (20-25) sclerotia spots.

Statistical Analysis: Analysis of variance (ANOVA) was performed separately to investigate the effects of salicylic acid on reducing fungal damage and also on the number and weight of tubers per pot in a randomized complete design.

RESULTS

Effects of Salicylic Acid on the Weight and Number of Potato Tubers: Salicylic acid was tested for its effects on weight and on the number of potato tubers at 0 (control), 0.1, 0.2, 0.5, 1, 2 and 4 mM in plants that were infected with fungi (*Rizoctonia solani*). Deionized water was used as control. Results are shown in Figs. 1 and 2. Except at 2 mM, all other salicylic acid treatments did not affect the weight of potato tubers as compared to control significantly (Fig. 1).

Similarly, there were no inhibitory effects of salicylic acid on the number of potato tubers (Fig. 2). In contrast, salicylic acid at 2 mM, increased the number of potato tubers compared to the control significantly. Also, our results showed that salicylic acid treatment at 4 mM did not affect on potato tubers as compared to control (Fig. 3). However, the effects of salicylic acid at 4 mM on number of the potato tubers in plants that were infected with fungi (Fig. 1) were greater than on plants that were not infected (Fig. 3). At 4 mM concentration, salicylic acid did not have any inhibitory effects on growth, weight and on the number of potato tubers in both infected and uninfected.

Effect of Salicylic Acid on Reduce Disease: The results showed that different treatments of salicylic acid were effective in reducing the pathogenic effects of *Rizoctonia* fungus on the potato tubers. Salicylic acid at 0.2 mM, reduced fungal infection significantly (Fig. 4). In fact, salicylic acid was effective in reducing both the intensity (extent) and the number of infected spots (presence of sclerotia on the potato tubers). Also, salicylic acid at 0.5 m M, decreased the number of infected spots by about 72%. Furthermore, no significant difference of the effects of all concentrations of salicylic acid used on reducing the pathogenic effects of Rizoctonia solani was observed.

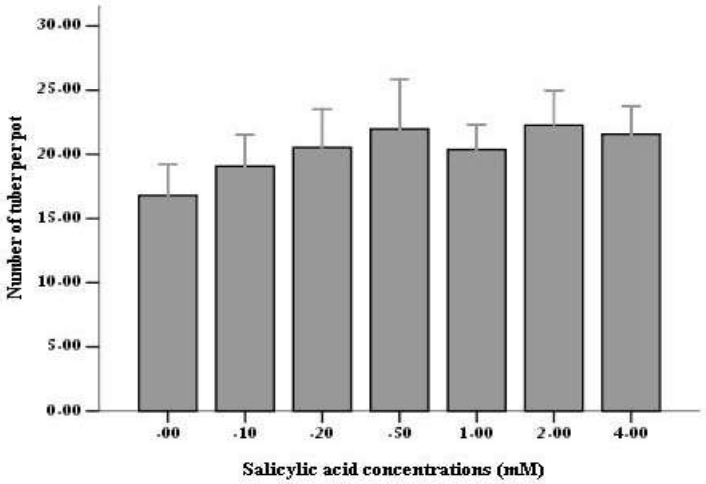

Fig. 1: The effects of salicylic acid on the number of tuber per pot in plants that were infected with fungi (*Rizoctonia solani*). Vertical bars indicate ± standard error

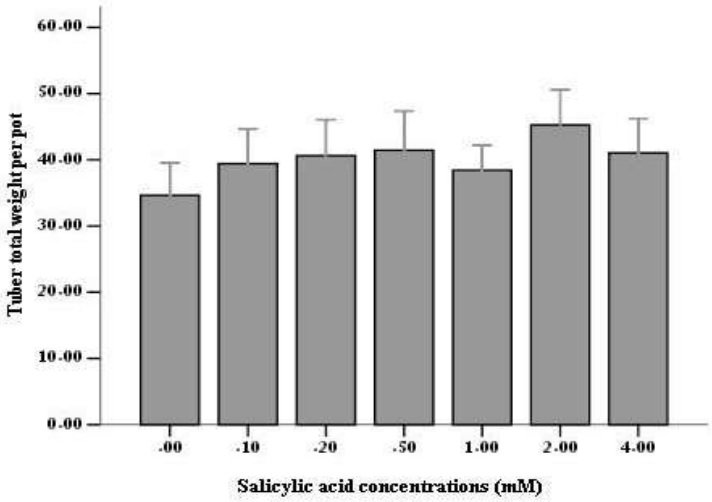

Fig. 2: The effects of salicylic acid on tuber total weight per pot in plants that were infected with fungi (*Rizoctonia solani*). Vertical bars indicate ± standard error

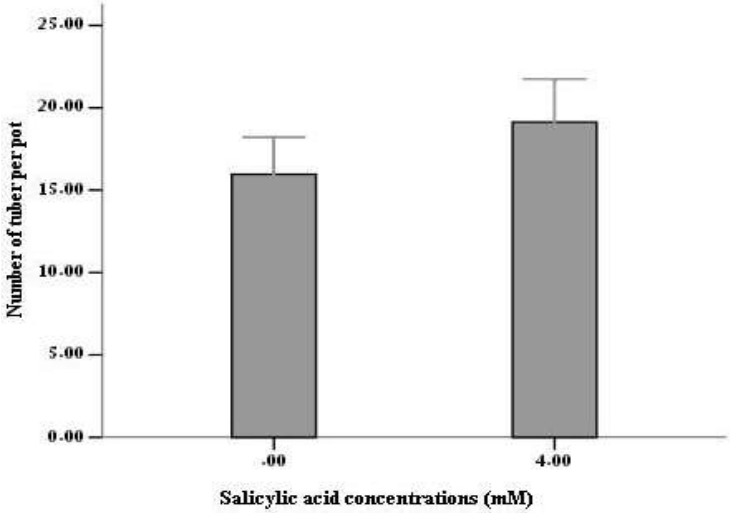

Fig. 3: The effects of salicylic acid on the number of tuber per pot in plants that were not infected with fungi. Vertical bars indicate ± standard error

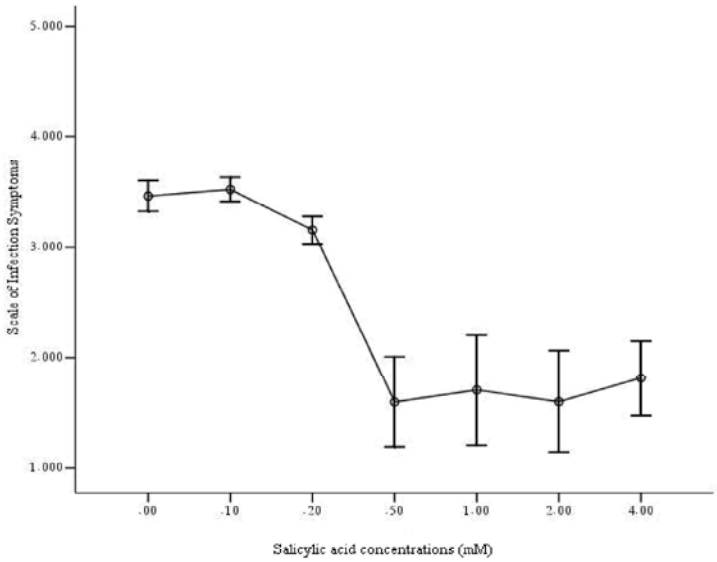

Fig. 4: The effect of salicylic acid on the reduction of fungal damage on the potato tubers in plants that were infected with fungi (*Rizoctonia solani*). Scale of infection symptoms (presence of sclerotia) on the potato tubers was selected from 1 to 5. Scales of 1, 2, 3, 4 and 5 represent number of 1 to 5, 5 to 10, 10 to 15, 15 to 20 and 20 to 25 sclerotia, respectively. Each point on the curve is mean number of infection symptoms (sclerotia) on the potato tubers (about 367 samples). Vertical bars indicate ± standard error.

DISCUSSION

Salicylic acid plays a critical role in the classical systemic acquired resistance in induced cucumber and tobacco plants [7]. Most previous studies on the role of salicylic acid have been focused on the interactions between plants and virulent or avirulent pathogens [8]. Exogenously applied salicylic acid on tobacco plants significantly reduced blue mold disease both in greenhouse assays and in the micro-titer plate assays [9]. However, little work has been done with regard to the ameliorative effects of salicylic acid on plants treated with fungi. Our results suggest that salicylic acid treatments against potato black scurf disease may be associated with the activation of some novel defense pathways. Investigation on the interplay between plants and bio-trophic fungal pathogens show that the relative roles of both plant and pathogen in these interactions are complex and poorly understood. One of the most important ways of fungal attack is either the use of resistant cultivars or improving the resistance of the cultivars through application of different methods including the use of chemical substances such as salicylic acid and its derivatives in the form of spray on plants [10]. Plants not expressing the gene salicylic acid are unable to accumulate this acid and more importantly were unable to mount a systemic acquired resistance response, demonstrating that salicylic acid accumulation was required for induction of systemic acquired resistance [11]. However, when examined carefully, these plants were also shown to permit growth of normally incompatible races of both fungal and bacterial pathogens, indicating that salicylic acid also plays a role in mediating genetically- determined resistance. Together, these results indicate that a common salicylic acid -dependent pathway exists that controls both systemic acquired resistance and gene-for-gene resistance [12]. Based on our results, it seems that application of 0.2 - 0.5 mM salicylic acid solution as spray on the leaves of potato plants can reduce losses caused by *Rizoctonia* fungus (sclerotia symptoms) on the potato tubers. It is possible that salicylic acid when applied on the leaves it is degraded to catechol by salicylate hydroxlase enzyme causing a decrease in the amount of plants salicylic acid. As a result salicylic acid will play a minor role in salicylic acid -dependent pathway that controls systemic acquired resistance against fungal pathogen. We believe that the artificial application of salicylic acid as spray on potato plants leaves will result in an increase in the amount of plants salicylic acid content which plays a major role in the salicylic acid - dependent pathway that controls systemic acquired resistance against fungal pathogen.

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