

# Changes in the Production of Salicylic and Jasmonic Acid in Potato Plants (*Solanum tuberosum*) as Response to Foliar Application of Biotic and Abiotic Inductors

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## Abstract

An alternative to the use of chemical fungicides is to enhance the defensive response of plants by appropriate stimulation, a phenomenon known as induction of resistance. The aim of this study was to determine the changes of endogen levels of salicylic acid (SA) and jasmonic acid (JA) in potato plants as response to foliar application of biotic and abiotic inductors. Treatments T1 = Best Ultra F (*Bacillus* spp.  $10^8$  cfu/mL and *Pseudomonas fluorescens*  $10^8$  cfu/mL) 0.5%, T2 = FullKover HF (microbial jasmonic acid 1500 ppm) 0.2%, T3 = T1 0.5% + T2 0.1%, T4 = Milor® (Chlorothalonil + Metalaxyl) 0.5% and T5 = control (water) were applied in potato plants. The application of biotic and abiotic inductors improved the SA and JA production in potato plants. The production of salicylic acid in potato plants was observed by application of *Bacillus* spp. and *Pseudomonas fluorescens* (T1) and fungicide Milor® (T4). The application of T1 Best Ultra F, T2 FullKover HF (microbial JA), T3 (T1 + T2) and T4 Milor® improved the JA production in potato plants.

## Keywords

Salicylic Acid, Jasmonic Acid, *Bacillus* spp., *Pseudomonas fluorescens*

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## 1. Introduction

Excessive use of pesticides to control plant diseases is an important problem in the agricultural fields, so it is a priority study for biological control, because the current production systems demand the crop protection by innovative and environmentally methods compatible with sustainable agriculture as an alternative to chemical application [1]. An option is to enhance the natural defensive response of plants through adequate stimulation, a phenomenon known as induced resistance [2], which provides an efficient disease control and increases crop yields [3].

Currently there are two ways to induce resistance, the acquired systemic resistance (ASR) and induced systemic resistance (ISR), which can be differentiated by the nature and regulatory paths of the inductor (also called elicitor) [4]. Salicylic acid is a plant hormone that acts as a marker and regulator of plant responses against pathogens and abiotic stress is the molecule involved in ASR pathways [5]. ASR is activated after infection by a pathogen and is characterized by a hypersensitive reaction, local and systemic increase of endogenous SA levels in the plant, and by the production of pathogenesis-related proteins (PR) [6]. Most of the PR proteins are hydrolases, as glucanases (PR-2), chitinase (PR-3), peroxidases (PR-9), defensins (PR-12), thionins (PR-13) and lipid transfer proteins (PR-14) [7]. In plants, the SA biosynthesis is from the shikimate-phenylpropanoids pathway; SA is synthesized from phenylalanine by action of the phenylalanine ammonia lyase enzyme [8]. It has been found that the exogenous application of SA in plants induces stress tolerance, increases the biological response against salinity and extreme temperatures, modifies the concentrations of antioxidants, nutrients and chlorophyll [9] and increases the protection against pathogen attack [10]; defense is related to the attack mode and form of obtaining nutrients by the pathogen (biotrophs or necrotrophs) [11]. According to studies in *Arabidopsis thaliana*, defense against biotrophic pathogens usually involves dependent signaling of SA, while the induced defense against herbivorous insects and necrotrophic pathogens depend of JA [12].

It has been reported that ISR (another type of resistance) is effective against viral, bacterial and fungal diseases, is dependent of JA signaling and occurs when the plant roots are colonized by some nonpathogenic rhizobacteria or herbivores insect damage [13]. ISR has many similarities with ASR, and provides resistance in uninfected plant parts against pathogens. In plants, JA is synthesized from linolenic acid released from lipid membranes and converted into JA. JA synthesis can be also activated by herbivores, (tissues and leaves damages), and injuries caused by mechanical damage. JA induces trichomes formation on leaves, which confer protection to the leaf, another essential role of JA in the immunity activation against pathogens that feed on dead tissues, such as some necrotrophic fungi or bacteria [12]. In plants, the resistance against pathogenic infection can be improved by biotic and abiotic treatments, also called inductors. The biotic inductors include: necrotrophs and rhizobacteria infection, such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agrobacterium*, non-pathogenic microorganisms, such as *Trichoderma* [14] [15]. The abiotic inductors include chemical products or molecules as responsible of disease resistance signaling [16].

The aim of this study was to determinate the changes of endogen levels of SA and JA in potato plants as a response to foliar application of biotic and abiotic inductors.

## 2. Materials and Methods

### 2.1. Potato Plants

Potato minituber var alpha were seeded in plastic packages containing a mixture of peat-most and forest land (1:1), the cultures were kept under greenhouse conditions at the Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila, México. When the plants reached 30 days old the treatments application was carried out.

### 2.2. Treatments and Sampling

The biological treatments evaluated were provided by the company Green Corp Biorganiks of México and consisted of: T1 = Best Ultra F (*Bacillus* spp.  $10^8$  cfu/mL and *Pseudomonas fluorescens*  $10^8$  cfu/mL) concentration 0.5%, T2 = FullKover HF (aqueous solution of microbial origin jasmonic acid 1500 ppm) 0.2% and T3 = mix T1 Best Ultra F 0.5% + T2 FullKover HF 0.1%; T4 = Milor<sup>®</sup> (Chlorothalonil + Metalaxyl) 0.5% and T5 = absolute control (water). Application was made by spraying on the foliage using a hand sprayer. Samples were collected at 0, 1, 3, 6, 12, 24 and 48 h after treatment application, time 0 was collected before application. The samples were lyophilized, macerated and stored at  $-20^{\circ}\text{C}$  until use.

### 2.3. Quantification of SA

To extract SA, 50 mg of macerated tissue were placed in Eppendorf tubes and 1 mL of extraction solution was added (10% methanol: 1% acetic acid: 89% distilled water), tubes were stirred by vortex during 15 sec and then sonic vibration was used. Tubes were centrifuged at 13,000 rpm by 10 min, the supernatant was recovered, filtered through 0.45  $\mu$ m nylon membrane and placed in a new tube. Quantification of SA was performed using an Agilent 1120 LC chromatography system with UV detector, separation was performed on a Agilent 4.6  $\times$  150 mm C18 5  $\mu$ m column at 30°C. Mobile phases were 50% phase A (94.9% water: 5% acetonitrile: 0.1% formic acid) and 50% phase B (5% water: 94.9% acetonitrile: 0.1% formic acid) [17], flow rate 0.6 mL/min by 12 min at 250 nm wavelength, sample injection 20  $\mu$ L. The hormone was determined on three independent samples from each sampling time and treatment. The concentration was calculated using a calibration curve with standard salicylic acid.

### 2.4. Quantification of JA

To extract JA, 100 mg of macerated tissue were placed in Eppendorf tubes and 450  $\mu$ L of extraction solution were added (95% methanol: 5% ethyl acetate), tubes were stirred by vortex during 15 sec and then sonic vibration was used. Tubes were centrifuged at 13,000 rpm by 10 min, the supernatant was recovered, filtered through 0.45  $\mu$ m nylon membrane and placed in glass vial. Solvent was evaporated in an oven at 50°C and the residue was resuspended in 1 mL of mobile phase (60% methanol: 40% water: 1% acetic acid) [18]. Quantification of JA was performed using a HPLC equipment with HP Ultraviolet detector HP7985A, mobile phase was 60% methanol: 40% water with 1% acetic acid. Flow rate was 0.85 mL/min using a Hypersil ODS column of 4.6 mm  $\times$  25 cm  $\times$  5  $\mu$ m, JA concentration was determined at 295 nm during 15 min. Samples were analyzed in duplicate and the JA concentration was calculated using a calibration curve with jasmonic acid standard.

The experiment was carried out only one time in a randomized experimental design, with three replicates per treatment in each time. Data were analyzed using ANOVA and with Tukey test, 0.5 significance.

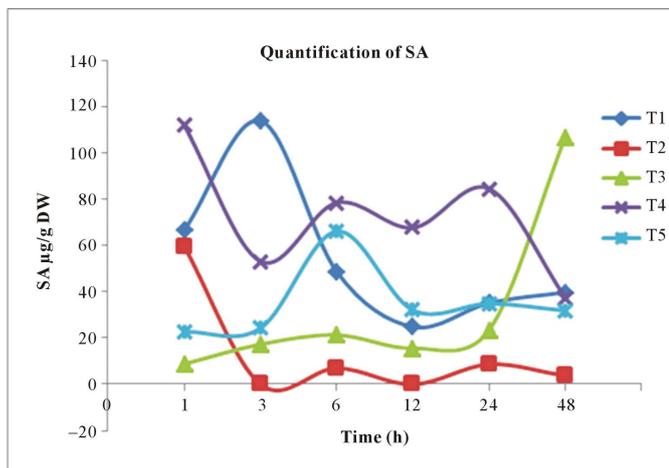
## 3. Results and Discussion

### 3.1. Quantification of SA

The results shown significant differences between treatments in each sampling time analyzed separately by ANOVA. SA concentration in potato plants it was between 3.68 and 114.02  $\mu$ g/g dry weight (DW) during the experiment. In the first hour T4 (Milor<sup>®</sup>) was statistically different from the control with 112.05  $\mu$ g/g DW (492% more than control), however, this value decreased from 3 h to reach statistically similar levels to the control. T1 (Best Ultra F) increased significantly the SA concentration at 3 h reaching 114.02  $\mu$ g/g DW (469% more than control), the results are shown in the **Figure 1**. T1, T4 and control did not show significant differences between them at 6 h, but this treatment did show significant differences compared to other treatments. There are no significant differences at 12, 24 and 48 h for the SA concentration in the treated plants compared with the control.

T4 (Milor<sup>®</sup>) is a mixture of fungicides, Metalaxyl with systemic action and Chlorothalonil with contact action, it is widely used to control diseases caused by oomycetes. In this study it was demonstrated the protectant effect and the potential as defense mechanism activator of Milor<sup>®</sup> by SA accumulation. It is known that ASR activation is given by abiotic elicitors, such as chemicals products that interact with signaling pathways associated with plants resistance stimulating their defenses, also have a direct negative effect on the pathogen. For example, the fungicide Probenazole induces ASR in *Arabidopsis* by signaling and accumulation of SA [19]; the herbicides Acifluorfen and Paraquat induce ASR against *Colletotrichum lagenarium* on cucumber plants [20].

As for T1 (Best Ultra F) containing spores of *Bacillus* spp. and *P. fluorescens*, a significant increase was observed in the production of SA 3 h after application, which coincides with the results reported by Segarra *et al.* [21] by *Trichoderma asperellum* spores inoculation in cucumber plants, activating the systemic resistance to *Pseudomonas syringae* pv. *lachrymans*, where the highest SA production was detected at the same time reported in this study. Best Ultra F containing *P. fluorescens*, a bacterium that has been reported as ISR activator, effective against different types of pathogens [22]. Saikia *et al.* [23] reported values up to 2000 ng/g fresh weight of SA in chickpea plants treated with different strains of *P. fluorescens*. This can be attributed to different mechanisms that have been suggested for the pathogens control with resistance induction in plants by *P. fluorescens* that include the production of HCN, ammonium, antibiotics, siderophores and volatile compounds. Siderophores,



**Figure 1.** SA production in potato leaves. T1 = Best Ultra F (*Bacillus* spp. and *P. fluorescens*), T2 = FullKover HF (JA 1500 ppm), T3 = T1 + T2, T4 = Milor<sup>®</sup> (chlorothalonil + metalaxyl) and T5 = control (water).

particularly SA, are involved in the resistance induction in plants [24], but when *P. fluorescens* is applied in plants is difficult to establish if the SA is produced by the plant or bacteria, because free SA generated locally or systemically is transferred to other tissues throughout the phloem [23]. Chen *et al.* [25] mention that SA produced by *P. fluorescens* cannot be correlated with the ISR activity, ISR is activated by rhizobacteria and does not require SA because is JA and ethylene dependent [26].

T2 (FullKover HF) containing JA of microbial origin did not induce significant increase of SA compared to control, this can be explained by the fact that JA is the ISR messenger. When ISR is activated, it is characterized by an accumulation of JA, therefore, exogenous application of JA in plants should increase the JA concentration, but not SA concentration, as was observed in this study.

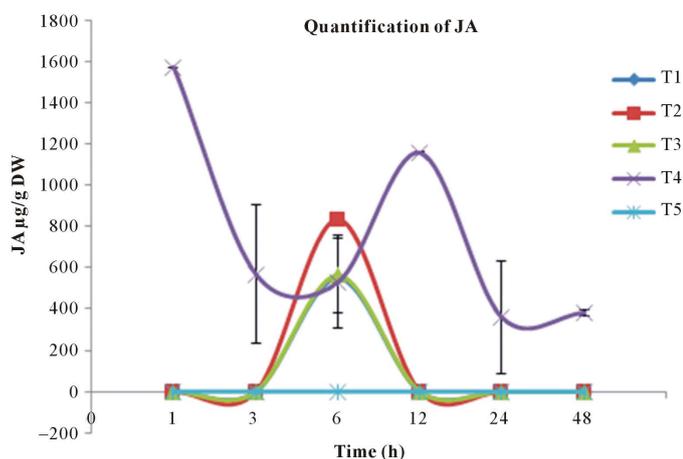
On the other hand, T3 formulated with *Bacillus* spp., *P. fluorescens* and JA (T1 + T2) induced a time delay for the JA production, this could be attributed to a possible antagonistic interaction of these compounds. It is known that both, SA and JA have a cross signaling, both pathways are mutually antagonistic (Kunkel and Brooks [27]). Similarly, Niki *et al.* [28] have reported that the SA application in tomato leaves blocks the JA biosynthesis and SA production is inhibited by JA in transgenic tobacco plants; thus, the SA and JA production is inhibited antagonistically by JA and SA, respectively.

### 3.2. Quantification of JA

No JA levels were detected in the control, only in treated plants. The maximum concentrations were obtained at 1 h (1572.18 µg/g DW) and 3 h (569.28 µg/g DW) with T4 (Milor<sup>®</sup>). All treatments, except the control had similar JA levels at 6 h, T1 (Best Ultra F) reached 550.02, T2 (FullKover HF) 833.32, T3 (T1 + T2) 562.48 and T4 (Milor<sup>®</sup>) 529.62 µg/g DW. T4 (Milor<sup>®</sup>) was the only that kept the JA production at 12, 24 and 48 h (Figure 2).

JA is the ISR messenger, in plants the level of this hormone depends on the tissue, cell type, development stage and response to different environmental stimuli [29], JA induces the expression of certain genes when is exogenously applied and is accumulated in vegetal tissues after traumatic injury or treatment with inducers, such as T2 FullKover HF (microbial JA solution). Similarly, the JA production using T1 Best Ultra F (*Bacillus* spp., and *P. fluorescens*) and T3 (T1 + T2) is attributed to both treatments containing *Bacillus*. It has been demonstrated that *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides* and *B. sphaericus* are able to elicit and activate ISR increasing the biochemical compounds levels related with this defense [15] [30]. Furthermore it has been reported that some fluorescent *Pseudomonas* species can induce systemic resistance in plants [14].

T4 Milor<sup>®</sup> induced in the plant JA production at all times (Figure 2), its ability to induce the SA (above mentioned) and JA production in plants, it is probably because the abiotic inducers, such as chemical products, act in one or several points of the pathways involved in disease resistance [16]. The effect of the presence of both



**Figure 2.** JA production in potato leaves. T1 = Best Ultra F (*Bacillus* spp. and *P. fluorescens*), T2 = FullKover HF (JA 1500 ppm), T3 = T1 + T2, T4 = Milor® (chlorothalonil + metalaxyl) and T5 = control (water).

signals by the production of SA and JA, can be explained by a possible crosstalk that is still not entirely clear and that generates a synergistic interaction between these hormones, this synergism depends on the combination and concentrations used [31].

Although the experiment was performed once, the results show the potential use of *Bacillus* spp., *P. fluorescens* and JA of microbial origin from formulated products to induce resistance in plants as a strategy in the management of diseases and reducing levels of environmental pollution caused by the use of conventional pesticides.

#### 4. Conclusion

The application of biotic and abiotic inducers improved the SA and JA production in potato plants. The application of T1 Best Ultra F (*Bacillus* spp. and *Pseudomonas fluorescens*) and T4 Milor® improved the SA production in potato plants. The application of T1 Best Ultra F, T2 FullKover HF (microbial JA), T3 (T1 + T2) and T4 Milor® improved the JA production in potato plants.

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