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Salicylic Acid and Glycyrrhizic Acid Ammonium Salt in Colloidal and Nano Forms for Management of Potato Brown Rot Infection Caused by Ralstonia solanacearum

Eman O. Hassan [1*], Tahsin Shoala [2], Shereen A. Mohamed [3], Omnia A. M. Badr [3] and Ibrahim A. I. EL-Fiki [1]

[1]Department of Plant Pathology, Faculty of Agriculture, Benha University, Benha 13511, Egypt.
[2]Environmental Biotechnology Department, College of Biotechnology, Misr University for Science and Technology, Giza 12563, Egypt.
[3]Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University, Benha 13511, Egypt.

*** Corresponding author**
Eman O. Hassan

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Abstract

Potato (*Solanum tuberosum* L.) is a low-cost high-energy food possessing increasing demand around the world. Potato bacterial infections are serious biotic constraints to potato production, particularly in tropical and subtropical climates. This study aimed to manage *Ralstonia solanacearum* the causal agent of potato brown rot using salicylic acid (SA) and glycyrrhizic acid ammonium salt (GAS) in the colloidal and nano forms. SA and GAS were used in colloidal and nano form in three concentrations: 0.15, 0.30, and 1.25 mM to reduce the growth of the pathogen. Potato crop production and percentage of brown rot severity in Cara and Spunta cultivars were calculated. The gene expression profiles of Glucan endo-1,3-β-D-glucosidase, Polygalacturonase, Indole-3-acetic acid-amido synthetase GH3.3, and Cytoplasmic heat shock protein were assessed in Cara and Spunta cultivars under different treatments. The inhibition zone of *Ralstonia solanacearum* growth reached the highest record of 2.85 mm in response to 1.25 mM of GAS-NPs in comparison to other treatments. Interestingly, the treated Spunta cultivar with 1.25 mM recorded of 0% severity in comparison to 78.8% in the control. The Cara cultivar crop production increased to 4.62 kg in response to 1.25 mM SA-NPs compared to 3.72 kg, 3.9 kgs, and 1.82 kgs in the treated plants with 1.25 mM SA, negative and or positive control, respectively. GAS-NP treatments showed the lowest expression profile of plant defense genes and maintained the negative control expression profile. SA and GAS-NPs enhanced potato plant resistance and crop production in response to the phytopathogenic bacterial brown rot disease.

Keywords: potato, Ralstonia solanacearum, glycyrrhizic acid ammonium salt, salicylic acid, nanoparticles, gene expression.

Introduction

The potato (*Solanum tuberosum* L.) is an important solanaceous crop commonly cultivated in Egypt for local consumption and international export. It is susceptible to infection by actinomycetes, bacteria, fungi, nematode diseases, and physiological disorders (Walter et al., 2001). One of the most significant biotic barriers to potato production is pathogenic bacteria, particularly in tropical, subtropical, and warm regions around the world. About six major bacterial diseases infect potatoes worldwide causing considerable damage and tuber reduction, bacterial wilt, and hind leg are the most serious diseases while ring rot, conjunctivitis, and scab maintain minor effects in potatoes (Charkowski et al., 2020). In addition, potato blight is the most restrictive and limiting factor in exporting tubers to foreign markets.

Potato brown rot, popular as bacterial wilt, is a vascular disease caused by R. solanacearum. This gram-negative proteobacterium is one of the most dangerous bacterial phytopathogens in the world, capable of invading over two hundred plant species, such as potatoes, tomatoes, peanuts, peppers, eggplant, and bananas (Coll and Valls, 2013; Peeters et al., 2013). Although R. solanacearum is widespread in subtropical and tropical areas, phylotype IIB-1 strains such as UY031 are temperature tolerant and have caused significant epidemics in temperate areas (Ciampi et al., 1980; Janse et al., 2004; Champoiseau et al., 2009).

Salicylic acid (SA) is a multitasking signaling molecule capable of providing both systemic and local defense systems against a wide variety of plant diseases. SA was first recognized as a key plant growth regulator of systemic acquired resistance (SAR), a derived defense elicited by a virulent pathogen that encompasses the entire plant and protects it against a variety of diseases (Durrant and Dong, 2004). The plant hormone SA has received more attention due to its effect on numerous plant developmental stages besides its critical role in the resistance of various biotic and abiotic stressors (Vlot et al., 2009). Glycyrrhizic acid (GA), a triterpenoid saponin glycoside, is the primary water-soluble constituent of licorice root, while 18-glycyrrhetinic acid is the major metabolite of glycyrrhizic acid. Across plant metabolic pathways, glucuronidase converts glycyrrhizin into two pentacyclic stereoisomers, the triterpenoids, 18α- and 18β-glycyrrhetinic acid, separately (Maatooq et al., 2010). Glycyrrhizic acid has attracted more interest from the biological perspective due to its antibacterial abilities. Glycyrrhizic acid and its derivatives have many metabolic functions, including antioxidant (Kalaiarasi and Pugalendi, 2011), antiviral (Zhao et al., 2014), and antimicrobial properties (Huang et al., 2016).

In plants, glucan-1,3-β-glucosidase generates diverse gene families when multiple copies of glucan-1,3-β-glucosidase genes were discovered in a separate plant species (Doxey et al., 2007). Glucan-1,3-β-glucosidases are enzymes that can cleave the β-glycosidic linkages of glucans. They can be categorized as exo or endo. Exo-hydrolases catalyze the hydrolysis of β-glucan chains by chelating glucose substances from the non-reducing end and producing glucose as the sole hydrolysis product. Endo-hydrolases are enzymes that cleave bonds at various locations along the polysaccharide chain, producing small oligosaccharides (Pitson et al., 1993). The enzyme glucan-1,3-β-glucosidase is needed to hinder the development of pathogenic fungi and reduce damage from perturbations in fruit. This enzyme can affect the cell walls of some microbes that contain glucans (Confortin et al., 2019).

The plant cell wall serves as a crucial barrier against pathogenic organisms. Most of it is made up of cuticular wax, cutin, glycans, cellulose, pectic substances, and cell wall structural proteins (Agrios, 2005). Several microorganisms produce a wide range of cell wall-degrading enzymes (CWDEs) to break down plant cell wall components. cutinases, pectinases, cellulases, glucanases, and proteases are indications of CWDEs (Agrios,

2005). Enzymes from the glycoside-hydrolases (GH) family can break polysaccharide glycosidic linkages (Kubicek et al., 2014). Polygalacturonases are pectin-degrading enzymes that have both endo-polygalacturonases (EndoPGs) and exo-polygalacturonases (Exo-PGs). Endo-PGs digest the polysaccharide at random, producing oligogalacturonides, whilst the Exo-PGs solubilize the polymer from the non-reducing end, producing a single galacturonic acid (Abbott and Boraston, 2007; Schacht et al., 2011). EndoPGs and Exo-PGs are both representatives of the GH28 family (Schacht et al., 2011).

The hormone's bioactive pattern is free of IAA, which is inactivated by amino acid conjugation. As a result, indole-3-acetic acid (IAA), and amido synthetases (GH3) conjugate IAA to amino acids, which is an important phase in auxin homeostasis (Peat et al., 2012). When alanine or leucine is covalently linked to IAA, an inactivated but smoothly digested storage state is formed. On the other hand, IAA conjugation with aspartate or glutamate leads to hormone degradation. IAA-Trp conjugation has been shown to have anti-auxin activity in plant growth as a key operator during storage, degradation, and inhibition of auxin signaling (Westfall et al., 2013).

Indole-3-acetic acid (IAA) is necessary for maintaining the meristematic cell state of the cambium zone. The highest IAA levels were detected in the cambium zone of the stems (Nieminen et al., 2008). By mitigating the effects of intrinsic GA, IAA supplementation increased tuber progression in potatoes (Naskar et al., 2009). Potato auxin levels were high before tuber induction and decreased during tuber development.

The heat shock protein (HSP) family is one of the most widespread and genetically systematic groups of pathogens' molecular chaperons. The primary responsibility of HSPs is chaperone function, which prevents proteins from being irretrievably denatured, misfolded, or aggregated in response to a variety of exogenous abiotic environmental stresses (Bosl and Walter, 2006; Goloubinoff and De Los Rios, 2007). Wang et al. (2004) classified the HSP family into five groups based on their molecular weight: HSP100s, HSP90s, HSP70s, HSP60s, and tiny HSPs (sHSPs; 12-40 kDa). The Classification of HSPs is the most complex and difficult classification of HSPs in plants and plays a crucial role in plant stress tolerance due to their abundance and diversification.

The study aimed to control the causative agent of bacterial brown rot caused by *Rasltonia solanacearm* using salicylic and glycyrrhizic acid in addition to ammonium salt in colloidal and nano forms. Gene expression profiles of Glucan endo-1,3-β-D-glucosidase, Polygalacturonase, Indole-3-acetic acid-amido synthetase GH3.3, and Cytoplasmic heat shock protein were assessed as molecular defense genes in Cara and Spunta cultivars.

Material and Methods

Bacterial culture

The *R. solanacearum* Smith isolate was provided by the Bacterial Department, Institute of Plant Pathology, Agricultural Research Center, Giza, Egypt. Pathogenicity tests and Koch's postulates were successfully performed on potato seedlings (cv. Spunta) in the laboratory to confirm its pathogenicity, and re-isolated pure cultures of R. solanacearum were maintained on nutritional agar slants at 4OC.

Immunofluorescence Antibody Staining (IFAS)

To verify the established R. solanacearum identity, an isolate was grown on a KBA medium and the characteristics of the R. solanacearum colonies were serologically assessed using IFAS. Using typical bacterial colonies, a suspension was prepared to contain 106 cells per ml from the culture and the reference. A measured standard volume (20 L for 6 mm window diameter) was pipetted onto five consecutive windows of a 10-window test slide. The slides were air dried at room temperature (25-30°C) before being lightly heat fixed with a flame. All slide wells received 25 µl of antiserum

(polyclonal against R. solanacearum) in four dilutions (1:800, 1:1600, 1:3200, and 1:6400). Slides were incubated for 30 minutes at room temperature in a wet chamber, washed with Tween's buffer and then incubated for 30 minutes with 25 µL of Nordic SW/AR anti-rabbit fluorescein isothiocyanate conjugated at a 100-fold dilution. The slides were washed again with 0.01 M PB, and Tween buffer and excess moisture was carefully removed with filter paper. A drop of 0.1 M phosphate-buffered glycerol (pH 7.6) was added to each independent well and the slides were covered with long glass coverslips. A microscope (tube factor 1.25), an epifluorescence light source, and appropriate filters containing fluorescein isothiocyanate (FITC), a 100X oil immersion lens, and a 10X eyepiece were used to examine the slides. The presence or absence of normal bacterial cells with typical morphology was observed in at least 20 microscope spots per window (Janse, 1988).

Chemical synthesis of nanomaterials

Sigma-Aldrich supplied glycyrrhizic acid ammonium salt and salicylic acid was used. 10 mg of each chemical were dissolved in 10 ml of 100% ethanol and sonicated at room temperature (20–25°C) for one hour at a power of 50 kHz (XUBA3 Analogue Ul-ta-sonic Bath, Grant Company, Saint Joseph, MO, US).

Transmission Electron Microscopy (TEM)

Electron microscopy was used to describe the shape of a glycyrrhizic acid ammonium salt and salicylic acid nanomaterials. A drop of the nanoparticle solution was sonicated before adhering to carbon-coated copper (CCG) grids, which were then fully dehydrated at ambient temperature. Electron micrographs were taken at Al-Azhar University Regional Center for Mycology and Biotechnology (RCMB) using a JEOL GEM-1010 transmission electron microscope set at 70 kV.

Effect of different treatments on the growth of *R. solanacearum in vitro*

Salicylic and glycyrrhizic acid ammonium salts were used in colloidal and nano forms at three concentrations: 0.15, 0.30, and 1.25 mM for each of the bacterial infections studied. About 0.1 ml of bacterial suspension (1 x 10^{-8} dilution, 24 hr. old culture) was spread onto nutrient agar medium. Saturated filter paper disks (5 mm) of each treatment were placed on top of the inoculated plates. Discs with no treatment (sterile water only) were used as controls. Plates were incubated at 25-28°C for 48 hours with four disks/plate and three replicates for each treatment according to Loo et al., (1945). The consequences of the tested materials were determined as an inhibition zone surrounding the discs.

Control of potato bacterial wilt disease under field conditions

Field trials were conducted at the Faculty of Agriculture, Benha University, Egypt. Cara and Spunta potato varieties were obtained from the Horticultural Research Institute, Vegetable Sector in Dokki, Giza. These strains were chosen for their unique morphology and popularity in Egypt. The selected field has a history of high R. solanacearum infestation. The land (sandy loamy soil) was prepared for potato cultivation as usual. The land was divided into plots of 42 m^2 (6 x 7 m) and on the 27th of October, apparently healthy (free of any biotic infestation) small holey tubers of potato varieties Cara and Spunta were planted in rows of 25-30 cm cultivated. Three replicate split plots were used to arrange experimental plots. All agricultural practices, i.e., planting, irrigation, fertilization, weed, and pest control, etc., were carried out according to the Ministry of Agriculture, Egypt. Two-week-old healthy trifoliate seedlings of potato, Cara, and Spunta cultivars were inoculated with 100 ml bacterial suspension (10^7-10^8 CFU/ml) using the Janse-Stammpierce technique (1988). Similarly, sterile water control was performed. Treatments with salicylic acid and glycyrrhizic acid ammonium salt in colloidal and nano forms were started 48 hours after inoculation with three doses.

Disease and total yield evaluations

At harvesting time, the disease severity index % was analyzed and computed using a disease index scale ranging from 0 (no wilting symptoms) to 5 (all leaves wilted-dead plant) using the technique given by (Winstead, 1952) and Cooke, (2006). When 75% of the plants had attained senescence, harvesting began. Each treatment's total potato yield (kg/plant) was reported.

Primer Design

To ensure maximum specificity and efficiency during qPCR amplification under a standard set of reaction conditions, Allele ID 7.7 software was used to design qRT-PCR primers. ITS as an internal housekeeping gene was used as a reference gene in this study (Table 1).

Table1: Glucan endo-1,3-β-D-glucosidase, polygalacturonase, indole-3-acetic acid-amido synthetase GH3.3 and cytoplasmic small heat shock protein class I designed primers for qPCR.

Gene name	Accession	Forward	Reverse	TM
Glucan endo-1,3-β-D-glucosidase	NM-001288281.1	ATTTTGTCCCAGTCCGACCC	GGCCAGCATTTGGTTGGAAG	60
Polygalacturonase	XM-015306186.1	TGAAAGCCAATTCGAAACAAGCA	ATCAGAAGCCGTTCCACGAG	60
Indole-3-acetic acid-amido synthetase GH3.3	CP055235.1	ACCACGTCACTTTCCACGTA	CACGGCAATGCTTCGTTCAA	60
Cytoplasmic small heat shock protein class I	CP046693.1	TGGGCATCACAAGAGTGAAA	CACACATGCTAAACAAAATTCCACA	60

Differential expression analysis using quantitative real-time PCR

Total RNA was extracted from potato leaf tissues from treated and control groups using the RNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. The RNA obtained was treated with DNase at 37°C for 1 h to remove any DNA residues. RNA concentration and purity were measured with a Nano-Drop 2000C spectrophotometer (Thermo Scientific, USA). The reverse transcription reaction was performed using the High-Capacity cDNA Reverse Transcriptase Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then cDNA samples were stored at 20°C until use.

Except for NTC and cDNA controls, each PCR reaction contained 2.5 µL of cDNA, 12.5 µL of SYBR Green Mix (Cat. #204143), 0.3 M of each forward and Reverse primer (Table 2), 1 µL RNase inhibitor, and the final volume was completed to 25 µL by adding Nucleases-free water. A two-stage cycling protocol was performed utilizing the AriaMx Real-Time PCR System (Agilent Technologies, USA) under the following settings: 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 60 seconds. Results were normalized to ITS as an internal housekeeping gene. Relative gene expression ratios (RQ) between the treated and control groups were calculated using the formula: $RQ = 2^{-\Delta\Delta CT}$.

Statistical analysis

The presented data were laid out in triplicates and were statistically analyzed for the least significant difference (L.S.D.) according to Gomez and Gomez (1984).

Results

Immunofluorescence Antibody Staining (IFAS)

Figure (1) shows that the isolated cells had a short rod shape and were similarly stained in fluorescent light green by immunofluorescent antibody staining according to the method of IFAS. The identification results indicated that the isolate belonged to *R. solanacearum* race 3 biovar 2.

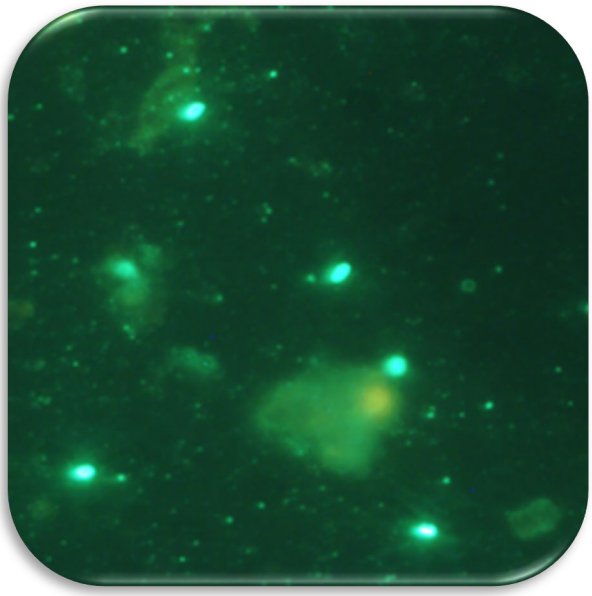

Figure 1. Under an immunofluorescent microscope, the typical morphological shapes of potato bacterial brown rot *R. solanacearum*.

Transmission Electron Microscopy (TEM)

The TEM characterization of salicylic acid nanoparticles applied in this study ranged from 5.48-20 nm. The results showed that the largest size for SA-NPs is 48.3 nm and the smallest size for SA-NPs is 17 .3 nm (Fig. 2)

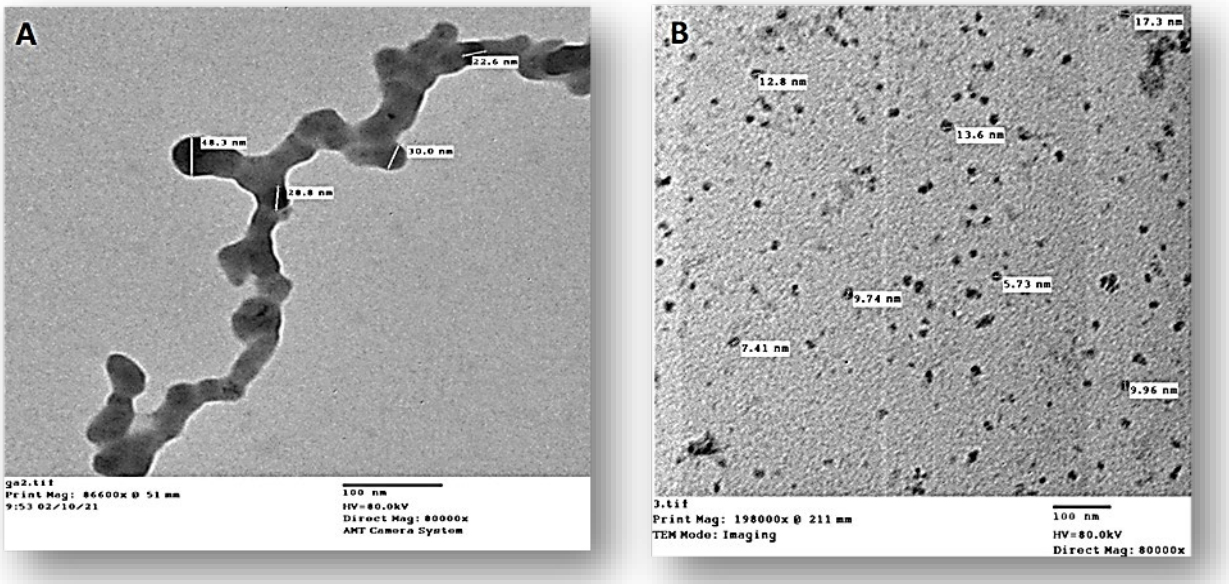

Figure 2. Transmission electron microscopy (TEM) image of prepared glycyrrhizic acid ammonium salt nanoparticles (A) and, salicylic acid nanoparticles (B).

Effect of different treatments on the growth of *R. solanacearum in vitro*

The results **of Fig. 3** showed that the zone of inhibition of *R. solanacearum* growth reached the highest record of 28.5 mm in response to 1.25 mM GAS-NPs compared to other treatments. Meanwhile, both 0.30 mM GAS-NPs and 1.25 mM SA-NPs suppressed the bacterial growth zone to 21.25 mm. Interestingly, 1.25 mM GAS inhibited the bacterial growth zone to 21.5 mm and 18.5 mm with 1.25 mM SA treatment. The lowest zone of inhibition for bacterial growth was 8.0 mm in response to treatment with 0.15 mM SA.

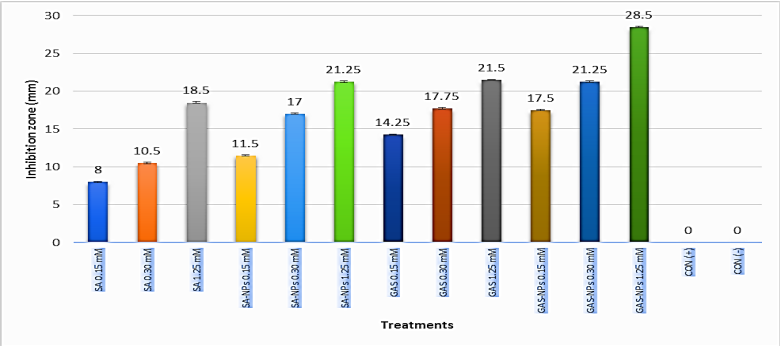

Figure 3. The inhibitory effect of salicylic acid (SA) and, glycyrrhizic acid ammonium salt (GAS) in the colloidal and nanoparticle (NP) forms at concentrations of 0.15, 0.30 and 1.25 mM against *R. solanacearum in vitro*.

Control of potato bacterial wilt disease under field conditions

The results presented in **Fig. 4** showed that the severity of brown rot in the treated Cara cultivar under 1.25 mM GAS-NPs treatment decreased to 4.0% compared with 68.6% in the untreated plants. Interestingly, no brown rot infection was investigated on the Spunta cultivar under the same treatment.

Effect of tested treatments on potato crop yield

The produced tubers of the cultivar Cara increased to 4.62 kg in response to 1.25 mM SA-NPs which differed from 3.72, 3.90, and 1.82 kg tubers in the treated plants with 1.25 mM SA, negative or positive control, respectively. In addition, tuber production of the Cara cultivar recorded 3.72 and 2.70 kg of tubers in response to 1.25 mM GAS-NPs and GA, respectively **(Fig. 5)**. The Spunta cultivar treated with 1.25 mM GAS-NPs increased tuber production to 4.90 kg compared to 3.88, 3.52, and 1.30 kg tubers in response to 1.25 mM GAS, negative control, and positive control, respectively. Meanwhile, Spunta cultivars treated with 1.25 mM by SA-NPS, and SA produced 3.63 and 2.68 kg tubers, respectively.

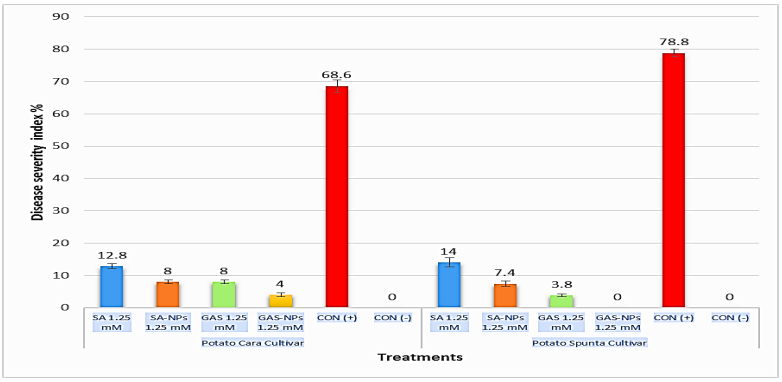

Figure 4. Effect of salicylic acid (SA) and glycyrrhizic acid ammonium salt (GAS) in the colloidal and nanoparticle (NP) forms at concentrations of 0.15, 0.30 and 1.25 mM on potato (Cara and Spunta cvs.) bacterial brown rot disease.

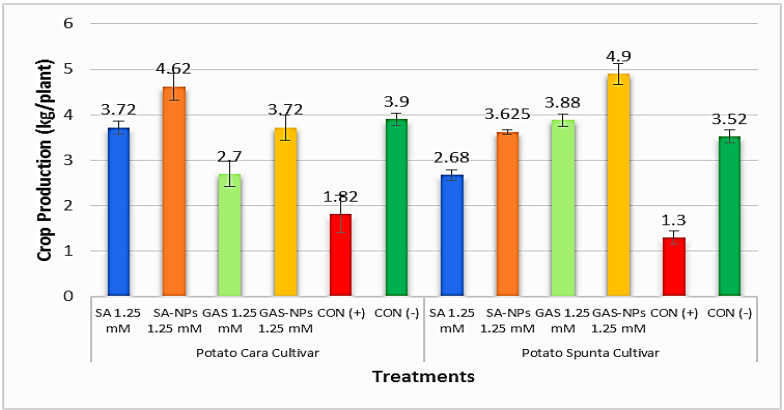

Figure 5. Effect of salicylic acid (SA) and glycyrrhizic acid ammonium salt (GAS) in the colloidal and nanoparticle (NP) forms at concentrations of 0.15, 0.30 and 1.25 mM on potato (Cara and Spunta cvs.) crop production.

Expression analysis of plant defense response genes against *R. solanacearum*

The data presented in **Fig. 6** illustrates relative gene expression profiles of glucan endo-1,3-β-D-glucosidase as a pathogen response (PR) gene, indole-3-acetic acid amido synthetase GH3.3 as auxin metabolism regulatory gene, cytoplasmic small heat shock protein as a cell death control gene, and polygalacturonase as a regulatory gene for pectin degradation. Pathogenic infection of *R. solanacearum* resulted in an up-regulation of all target genes in both varieties compared to the negative control group. All treated groups showed down-regulation compared to the positive control group, demonstrating the improving effect of all treatments. The glycyrrhizic acid nano-treatments in the two varieties showed the lowest expression profile of plant defense genes (glucan endo-1,3-β-D-glucosidase, indole-3-acetic acid amido synthetase, cytoplasmic small heat shock protein) in comparison with the other treatments. Remarkably, glycyrrhizic acid nano-treatments restored the gene expression profile of negative control groups. Cultivar Cara showed slightly up-regulated profiles compared to positive controls of Spunta cultivar and this may reflect stronger resistance in cultivar Cara than cultivar Spunta. Interestingly, polygalacturonase transcript levels showed a different pattern in the two cultivars, as transcript levels were down-regulated in cultivar Spunta, and expression profiles were up-regulated in cultivar Cara compared to the positive control.

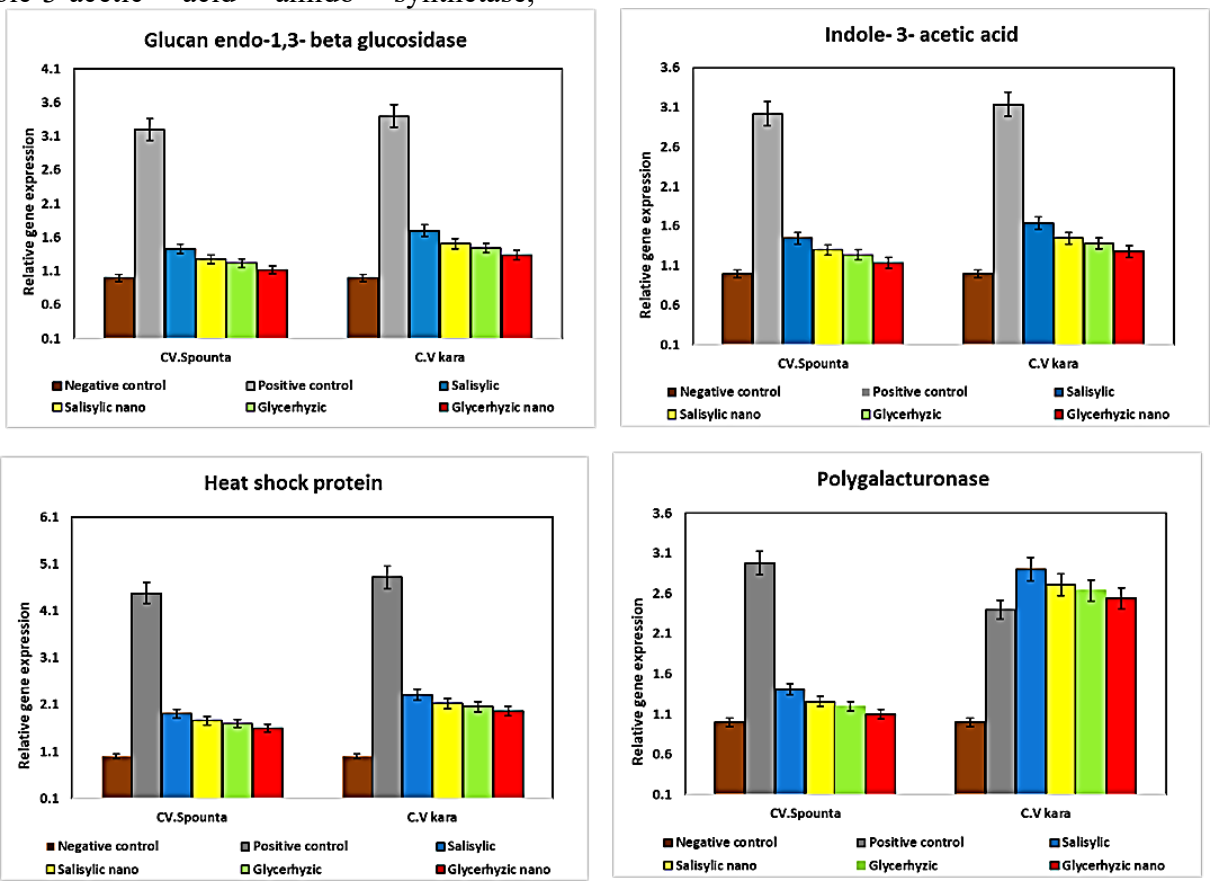

Figure 6. Relative gene expression of glucan endo-1,3-β-D-glucosidase, polygalacturonase, indole-3-acetic acid-amido synthetase GH3.3 and cytoplasmic small heat shock protein class I genes in potato cultivars Cara and Spunta in response to salicylic acid and glycerhizic acid ammonium salt in the colliodal and nano forms after 48 hours post-inoculation with *R. solanacearum*

Discussion

Bacterial brown rot disease caused by *R. solanacearum* is a broad-host-range, soil-borne disease that cannot be effectively controlled with a single management approach. In order to minimize tuber yield and decline infection by the disease, integrated disease management (IDM) strategies comprising various elements should be practiced.

The priming of plant tissues by inducers, allowing rapid activation of effective defense systems in response to phytopathogens, is a common aspect of induced resistance to the disease. Elicitors are non-toxic compounds that can trigger powerful plant defense mechanisms. After elicitor priming and subsequent inoculation of the plant with phytopathogens, we found an improvement in the disease resistance of the potato to the bacterial brown rot pathogen *R. solanacearum*. Treatment with the triggers salicylic acid and glycyrrhizic acid ammonium salt in colloidal and nano forms could increase the number of antimicrobial phenols in potato plants. The production of phenolic compounds at the host cell wall is a common host response, and the cross-linking of these phenylpropanoid esters results in the formation of lignin-like polymers that provide excellent defenses against pathogen attack. Lignin, phenolic compounds, suberin, and callose are examples of cell wall reinforcement. Lignin suppresses the secretion of virulence effectors and thus contributes to disease resistance by blocking pathogen entry into host cells **(Park and Ikeda, 2008; Luna *et al.*, 2011)**. Salicylic acid and glycyrrhizic acid ammonium salts in colloidal and nano forms were found to promote robust and rapid lignin deposition in potato root cell walls, which could be used as a target against the brown rot pathogen *R. solanacearum*.

Foliar spraying with SA before inoculation with *Fusarium oxysporum* has shown to increase resistance in Arabidopsis, as reflected in reduced leaf necrosis and plant death **(Edgar *et al.*, 2006)**. The defense response of *Solanum tuberosum* to salicylic acid and glycyrrhizic acid ammonium salt was supported by lignin deposition that was preceded by defense enzyme stimulation. The report by **Baysal *et al.*, (2005)** evidenced the effectiveness of the pretreatment of tomatoes with the elicitor DL-aminobutyric acid against *Xanthomonas vesicatoria* and *Clavibacter michiganensis* ssp. **(Cohen, 2002).**

The glucan endo-1,3-β-D-glucosidase enzyme was reported to break down β-1,3-glucans in bacterial cell walls to protect the host plant **(Zuluaga *et al.*, 2015)**. As a result, its transcript levels were highly expressed during infection (positive control) and significantly suppressed in response to the ameliorating effect of various therapies. The up-regulated GH3.3 gene encodes an enzyme that converts amino acids into indole-3-acetic acid (IAA). This gene along with GH3.5, GH3.6, and the gene encoding the growth-promoting peptide phytosulfokine (PSK) has been identified as positively regulated in auxin-mediated adventitious root initiation **(Amano *et al.*, 2007; Gutierrez *et al.*, 2015)**. **Zuluaga *et al.* (2015)** reported the up-regulation of small heat shock protein-encoding genes in response to *R. solanacearum* infection. They also mentioned the up-regulation of four genes in the susceptible accession and down-regulation of the same genes in resistant accessions, these results agree with our obtained data. Polygalacturonase was one of these genes, showing up-regulation in resistant accessions and down-regulation of expression in susceptible accessions. On the other hand, polygalacturonase is a crucial class of pectin-modifying enzymes that show different expression patterns and biological functions during different developmental and cell dynamic processes **(Yang *et al.*, 2018)**.

Abbreviation

SA= salicylic acid
GAS= glycyrrhizic acid ammonium salt
mM= millimole
GAS-NPs= glycyrrhizic acid ammonium salt nanoparticles
SA-NPs= salicylic acid nanoparticles
cv.= Cultivar

OC= Degree Celsius
v/v= Volume: volume
DLS= Dynamic laser light-scattering technique
PDI= Zeta potential and polydispersity index
hrs= Hours
mm= millimeter
i.e., = For example
%= Percentage
m2= Meter square
cm= Centimeter
CFU= Cell forming unit
ml= Milliliter
qPCR= Quantitative PCR
RNA= Ribonucleic acid
µL= Microliter
cDNA= Complementary DNA
rpm= Round/minuet
nm= Nanometer

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Authors' contributions

Conceptualization, Eman O. Hassan and Ibrahim A. El-Fiki; Data curation, Eman O. Hassan; and Ibrahim A. El-Fiki; Formal analysis, Eman O. Hassan, Ibrahim A. El-Fiki and Tahsin Shoala; Methodology, Eman O. Hassan, Omnia badr, Shereen A. Mohamed, Tahsin Shoala and Ibrahim A. El-Fiki; Resources, Eman O. Hassan, Ibrahim A. El-Fiki and Tahsin Shoala; Supervision, Eman O. Hassan and Ibrahim A. El-Fiki; Validation, Ibrahim A. El-Fiki, Omnia badr and Shereen A. Mohamed; Visualization, Eman O. Hassan, Ibrahim A. El-Fiki, Omnia badr, Shereen A. Mohamed and Tahsin Shoala; Writing – original draft, Eman O. Hassan and Ibrahim A. El-Fiki; Writing – review & editing, Eman O. Hassan, Omnia badr, Shereen A. Mohamed, Tahsin Shoala and Ibrahim A. El-Fiki

Competing interests

The authors declare that they have no competing interests.

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