EFFECTS OF POTASSIUM PHOSPHITE ON BIOCHEMICAL CONTENTS AND ENZYMATIC ACTIVITIES OF CHINESE POTATOES INOCULATED BY *PHYTOPHTHORA INFESTANS*

MOHAMMADI, M. A.[1,2] – ZHANG, Z.[1] – XI, Y.[1] – HAN, H.[1] – LAN, F.[1] – ZHANG, B.[1] – WANG-PRUSKI, G.[1,3*]

[1]*Joint FAFU-Dalhousie Lab, College of Horticulture, Fujian Agriculture and Forestry University Fuzhou, 350002, China*

[2]*Department of Horticulture, College of Agriculture, Alberoni University Kapisa, 0204, Afghanistan*

[3] *Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture Dalhousie University, Truro, Canada*

*Corresponding author
e-mail: gefu.wang-pruski@dal.ca; phone: +90-2-893-6247

(Received 30th Jan 2019; accepted 28th Feb 2019)

Abstract. Potato late blight caused by *Phytophthora infestans* dominates the entire world where potatoes and other Solanaceae crops are grown. In this study, the effects of potassium phosphite (KPhi) based fungicide on two potato varieties infected by two strains of the pathogen were studied. Tubers coming from foliar spray of potassium phosphite wounded and/or inoculated with pathogens were sampled at 0, 6, 12, 24, and 48 hours. Phytoalexins, phenols, β-1, 3-glucanase (PR-2), chitinase (PR-3), peroxidase (POD), polyphenol oxidase (PPO), superoxidase dismutase (SOD) and catalase (CAT), were analyzed. Results demonstrated that plants applied with KPhi produced tubers with enhanced resistance to the pathogen than their untreated plants. Moreover, tuber slices from KPhi applied plants following infection showed a significant increase in the contents of phytoalexins and phenols. PR-3 activities were induced by KPhi and wounding with the highest level at 48 hours. The activities of PR-2 were not significantly induced by KPhi or wounding, but its content was significantly increased by pathogen infection with the highest in untreated tubers after 48 hours. The KPhi treated tubers produced more enzymatic activities significantly after wounding and pathogen infection than those that were not treated. Our findings suggested that KPhi stimulates a quick and vigorous response in tubers against the pathogen infection via activation of defense responses, such as defense biochemical compounds, pathogenesis-related enzymes and antioxidant enzyme activities.
Keywords: *potassium phosphite, potato late blight, antioxidants enzymes, pathogenesis-related (PR) enzymes, Phytophthora infestans*

Abbreviation: KPhi - Potassium Phosphite, PR-2 - β-1, 3-glucanase, PR-3 - chitinase, POD - peroxidase, PPO - polyphenol oxidase, SOD - superoxidase dismutase, CAT - catalase, BABA - ß-aminobutyric acid, ROS - Reactive Oxygen Species, SAR - systemic acquired resistance, FW - fresh weight，GAE – gallic acid equivalent

Introduction

Solanaceae is an important plant family which has a diverse group of plants, ranging from wild species to several economically important cultivated crops. Potatoes (*Solanum tuberosum* L.) belongs to this family and it is the first non-grain food crop worldwide and third highly consumed food crop in the world, after wheat and rice (Norton and Swinton, 2018). Global potato production in 2017 was revealed as 376.8 million metric tons with

major production from developing countries. Nowadays, China is leading potato producers throughout the world accounting for 99.1 million tons, followed by India and the Russian Federation (Norton and Swinton, 2018).

The causative agent of potato late blight is oomycete *Phytophthora infestans* (Mont.) De Bary. It is one of the most devastating plant diseases throughout the world. This disease causes leaf death, which leads to significant yield reductions. The pathogen can also infect tubers which cause storage reduction as well as reduced seed quality. Pathogenic infections trigger plant defense responses by changing their biochemical contents. Biochemicals can limit the multiplication of pathogens, making the host environment unsuitable for growth of pathogens or directly by targeting and eliminating the attack of the microorganism (Hammerschmidt, 1999).

Activating the plant's immune system by different chemical and symptomatic factors could be an alternative way to increase plant resistance to biotic stresses (Lim et al., 2013). It has been reported that various chemical and biological compounds can trigger plant defense reactions without a real attack of pathogens. These compounds are known as resistance inducers or plant enhancers (Silva et al., 2011). Phosphite ($H_2PO_3^{2-}$, Phi), an alkaline salt of phosphorous acid (H_3PO_3) and phosphonate [$HPO(OH)_2$], is commonly known for controlling plant diseases by enhancing plant defense responses (McDonald et al., 2001). Phi has direct effects on inhibiting oxidative phosphorylation in oomycete metabolism (Lobato et al., 2008), and indirect effects that stimulate host protection responses eventually inhibit the pathogen growth (Daniel and Guest, 2005). It plays an important role as a fungicide, fertilizer or biostimulator or can work with at least one of these properties in various research systems (Thao and Yamakawa, 2009). Also, Wang-Pruski et al. (2010) and Borza et al. (2017) also report a comprehensive protective effect of Phi, which increased when applied in combination with a chlorothalonil protective herbicide. Further, Borza et al. (2014) studied foliage and postharvest treatments of Phi and its uptake and translocation in leaves and tubers. Machinandiarena et al. (2012), Wiesel et al. (2014) showed that Phi triggers disease resistance through increased hydrogen peroxide (H_2O_2) production, gene expression of pathogenesis-related (PR) protein PR1, glucanase (PR 2) and phenylalanine ammonia lyase, and increased soluble protein accumulation in *Arabidopsis*, potato and tomato plants (Chandrasekaran et al., 2017; Silva et al., 2011).

Potassium phosphite (KH_2PO_3) has been applied on plants to induce resistance to various oomycete pathogens, such as *Phytophthora* species (Kim et al., 2010) and *Pseudoperonospora* species (Silva et al., 2011). KPhi has been studied to inhibit normal metabolism of oomycetes, limiting their growth and stimulating defense mechanisms of plants, as well as boosting the synthesis and transportation of secondary metabolites (Kuć, 2001). KPhi can entourage reposition of defense molecules (Dalio et al., 2014; Ramezani et al., 2017), such as phytoalexin and phenols that are defensive chemicals for resistance to diseases and to overcome pathogenic attacks. The accumulations of phytoalexins and phenols were recorded in potato tubers after plants were treated with ß-aminobutyric acid (BABA) (Olivieri et al., 2009). KPhi can induce plant resistance by initiating hypersensitive reactions, resulting in programmed cell death of infected cells, increasing activities of phenylpropanoid biosynthetic enzymes, resulting in increased acquired systemic immunity against pathogens (Eshraghi et al., 2011). PR proteins are vital factors for the interaction of plants with pathogens and many groups of PR proteins can be a part of

first defense responses against pathogens (Kadota et al., 2014). Two PR proteins, chitinase and ß-1, 3-glucanase, were investigated in potato tubers in this study. Chitinase hydrolyzes chitin in cell wall of fungi (Dehestani et al., 2010), while the ß-1, 3-glucanase plays key role in plant defense responses to pathogen infections by catalyzing the cell wall cleavage of many pathogens (Adams, 2004). It has been shown that ß-1, 3-glucanase activity is correlated with systemic acquired resistance (SAR) that protects plants from various biotic stresses. Cellular defense function against oxidative stress can also be measured by increased POD, PPO, SOD, and CAT (Debnath et al., 2018b; Majer et al., 2014). Also, Lobato et al. (2011) studied that, after KPhi-treated potato leaves were mechanically wounded and/or infected by different pathogens, phytoalexins, antioxidant enzyme activities, and pathogenesis-related protein production were increased. In this study, we analyzed the protection function of KPhi against two pathogenic strains of *Phytophthora infestans*, in stored tubers obtained from the plants foliar treated with KPhi during the growing season. We also measured the contents of phytoalexins and phenols, and the enzymatic activities of chitinase, ß-1, 3-glucanase, POD, PPO, SOD and CAT, in two tubers of two potato varieties that vary in their resistant levels to Late blight.

Materials and Methods

Experiment location

The plants were grown in the College of Horticulture greenhouse at Fujian Agricultural and Forestry University, Fuzhou city (latitude 260, 5 '16 "N, longitude 190, 14 '6" E, altitude 42.09 m), in China between February, and May 2017 from October 2017 to January 2018. During the growing seasons, the temperatures in the greenhouse varied between 15-24°C and natural daylight cycle were 10-14 hours.

Biological samples

Two Chinese potatoes (*Solanum tubrusum* L.) varieties Xingjia No. 2 (moderately resistant to late blight) and Zhongshu No. 3 (moderate susceptible to late blight) were used in this study. Xingjia No. 2 was provided by Institute of Agricultural and Forestry Sciences in Daxinganling region, Heilongjiang Province; Zhongshu No. 3 was provided by Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science. Pieces of seed potatoes (~ 50 g of weight with 2-3 eyes) were planted in 5-liter plastic pots containing a mixture of vermiculite, peat and perlite (1: 3: 1, v/v) in the greenhouse. Plants were watered by using a sprinkler irrigation system. The plants were grown between February 26, 2017 and May 25, 2017 and repeated once from October 15, 2017 to January 15, 2018. In each season, 50 plants were used for each variety. The 50 plants were divided into two groups of 25 each: Group 1 was sprayed with water; Group 2 was sprayed with 1% KPhi biweekly, total three times during the growing season. Plants from different treatment groups were randomized in the greenhouse. KPhi was first sprayed to the leaves 35 days after the emergence. The second spray was 15 days after the first spray and the third spray was 15 days after the second spray. The control plants applied with sterilized water. Two strains of *Phytophthora infestans* (Pi) (Mont.) ASO and 1-12-25, isolated from Yunnan province by personnel of College of Plant Protection at Fujian Agriculture and Forestry University, were used in this study. They were grown on Zhongshu No. 3 potato tuber

slices and kept at 18°C and 90% RH. After seven days, mycelium was collected in sterile distilled water and stimulated to release zoospores by incubation at 4°C for 6 hours. After filtration through 5 layers of cheesecloth, the sporangial suspension was observed under a microscope for quantification before use as an inoculum. The rate of sporangia was adjusted to 4 x 10^{-4} sporangia/mL using a hematocytometer (Lobato et al., 2011).

KPhi stock solution, preparation and treatment

To prepare the KPhi stock solution (1%), the phosphorous acid crystals (Sigma-Aldrich) was neutralized with potassium hydroxide (KOH) by slowly mixing of phosphorous acid and potassium hydroxide solution until the pH was adjusted to 6.3. KPhi stock solution was diluted to 1% (1 g/100 mL) and sprayed to plants at the value of 30 mL per plant (4.5 L/ha) using a hand sprayer. Greenhouse plants were treated by KPhi three times during the growing season. KPhi was first sprayed to the leaves 35 days after the emergence. The second spray was 15 days after the first spray and the third spray was 15 days after the second spray. The control plants applied with sterilized water.

Wounding procedure and sample preparation

Tuber wounding procedure was performed according to the mechanism described by Kim et al. (2010) with a small modification. Collected tubers were first peeled, and internal flesh tissues were cross-sectioned by a mandolin cutter to pieces of about 10 mm thickness. The pith of the tubers was removed and tuber pieces were placed on a rack with a wet paper towel in five litter plastic boxes with lids at 18°C in the dark up to 7 days. Water was added to the bottom of the boxes in order to keep the humidity. Periderm samples were collected at 0, 6, 12, 24, 48 hours and seven days after wounding. Samples were immediately frozen in liquid nitrogen and stored at -80°C until use.

Late blight resistance evaluation

Tubers from plants of Xingjia No. 2 and Zhongshu No. 3 treated with KPhi and control were stored at 8°C and 55% RH for three months. The tubers were then washed in distilled water, sterilized by soaking in 2% sodium hypochlorite for 5 min and rinsed with distilled water and then used for phytopathological tests. Tubers slice (5-6 cm in diameter, 10 mm thickness) were infected with 50 µL of sporangia suspension (4×10^{-4} sporangia/mL) and incubated at 18°C in darkness. The disease severity symptom was evaluated on the upper surface of potato slices seven days after infection according to the method described by Lobato et al. (2008). The disease severity was recorded with the scale from 1 to 10, where 1 = no lesions, 2 = a few circles, 3 = up to 5%, 4 = 5–10%, 5 = 10–25%, 6 = 25–50%, 7 = 50–75%, 8 = 75–85%, 9 = 85–95% and 10 = 95–100% of leaf area with late blight symptoms (Lobato et al., 2008). Two strains of *Phytophthora infestans* (Pi) (Mont.) ASO and 1-12-25 were used in this study, and a total of 25 tubers in each variety and each treatment were used in three replications. The negative control has KPhi treated tuber slices but not infected by *P. infestans*.

Extraction and determination of phytoalexin and phenol contents

Phytoalexins was extracted and measured according to the method described by Lobato et al. (2008) Tubers (1 g) were mixed in 10 mL chloroform/methanol/acetic acid (50:5:45

v/v/v) using a blinder. The homogenate was kept overnight at room temperature (15°C) and then filtered through cheesecloth. Chloroform and 0.2 M acetic acid were added in equal volumes to the filtered mix. The blends were shaken well and let stand to separate into two layers. The top chloroform layer containing phytoalexins was removed and evaporated in 60°C an electrothermal blast dryer (Shanghai Yiheng scientific instrument Co., Ltd.) until it is dry. The dried sample is dissolved into 1 mL of cyclohexane and 2 mL of sulfuric acid (H_2SO_4) were added to the solution. The mixture was stirred and centrifuged at 12,000 rpm for 30 min. Then the red color of the lower sulfuric acid layer was measured at 500 nm with a spectrophotometer (METASH UV5100H) to determine the concentration of phytoalexin as µg/g FW. Phenols were extracted according to the Folin-Ciocalteu colorimetric procedure with minor modifications (Škerget et al., 2005). Briefly, 0.5 g of the tuber sample was homogenized with 2.5 mL Folin-Ciocalteau 0.2 N (Solarbio Life Sciences) for 5 min and then 2.0 mL sodium carbonate (75 g/L) was added. The mixture was kept at room temperature for 2 hours before it was measured at 760 nm using a spectrophotometer (METASH UV5100H). The phenol concentration was represented as gallic acid equivalent (GAE) (mg) on fresh weight (FW) (mg GAE/g FW).

Measurement of antioxidant enzyme activities

The square cut (100 mg) in the central part of tuber disks was grounded in a mortar and pestle at 0, 6, 12, 24 and 48 hours after inoculation or wounding. The mixture was filtered through four layers of cheesecloth and centrifuged at 12,000 rpm for 15 min. The supernatant, which represented the soluble tuber extract, was immediately processed.

The activity of chitinase and ß-1, 3-glucanase, was measured spectrophotometrically using the [Commercial chitinase and ß-1, 3-glucanase Assay Kits (GA-1-Y and JDZM-2-G), Beijing Suolai Bao Technology Co., Ltd, China] following the procedures described by the manufacturer.

POD was measured using the method by Shannon et al. (1966). The assay mixture containing 2.5 mL of phosphate buffer, 0.2 mL of suitably diluted tuber extract, and 0.1 mL of o-dianisidine (50 mg of o-dianisidine was dissolved in 50 mL of methanol) was incubated at 28°C in a water bath for 2 min. The reaction commenced by adding 0.2 mL of H_2O_2 (0.6%) and the absorbance was recorded at 470 nm against reagent blank.

PPO it was measured in 1 ml reaction mixture consisting of 20 µl of raw extract, 35 mM sodium phosphate buffer pH 6 and 100 µl 0.2 M catechol. The reaction was initiated by the addition of catechol to the mixture and absorption 420 nm was measured in 1 minute. Total SOD activity was determined by it is the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in a spectrophotometer at 560 nm as previously described (Cakmak and Marschner, 1992). The assay of CAT activity was based on its ability to decompose H_2O_2, with the absorbance of the supernatant at 240 nm (Choo et al., 2004).

Statistical analysis

All the experiments were organized in a complete random block design (CRBD) with three biological replicates for each treatment and repeated at least three times. The surface area showing damage symptoms data were analyzed by the *t-test*, phytoalexin and phenol contents, as well as enzymatic activities, were analyzed for significant variances and differences using one way ANOVA. SPSS (version 22, SPSS, Chicago) was used for

means value analysis. Differences between means were detected, when using Tukey's test
(P < 0.05) and graphs generated by Microsoft Office Excel 2016.

Results

Effect of KPhi on late blight suppression

The effects of leaf treatments of KPhi in greenhouse conditions have caused reduced
sensitivity to *Phytophthora infestans* in the tubers. The comparison between inoculated
tuber pieces of Xingjia No. 2 and Zhongshu No. 3 showed a decreased diameter
development of pathogen growth from the plants treated with KPhi in both cultivars
(*Figure 1A*). Data in *Figure 1B* showed a significant effect of the KPhi treatment, which
caused reductions in lesion size caused by *P. infestans* in tubers of both cultivars. Also, it is
confirmed that Xingjia No. 2 was less susceptible to both strains of *P. infestans*, and
Zhongshu No. 3 showed higher susceptibility to *P. infestans* as seen in the control tuber
slices (*Figure 1, A and B*). As well, the two strains showed a different range of
aggressiveness, ASO is more aggressive that of 1-12-25 (*Figure 1, A and B*).
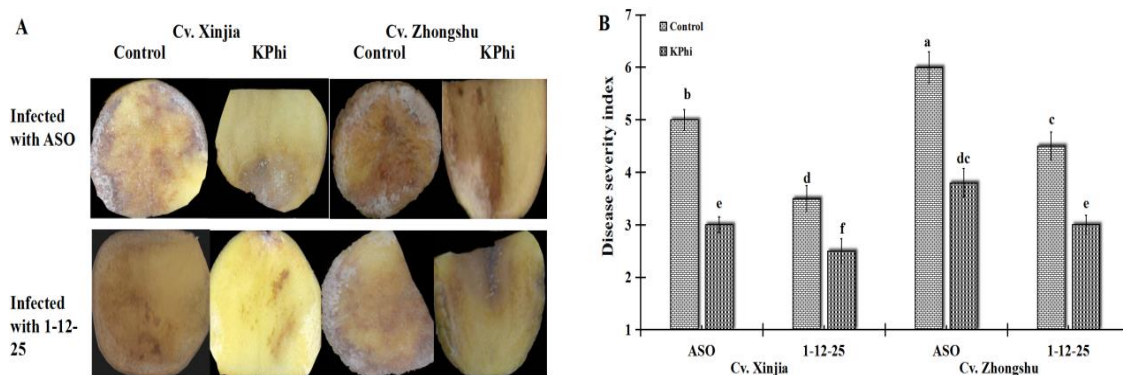
*Figure 1. Effect of foliar application of KPhi on tuber lesion diameter caused by two
Phythophthora infestans strains (A). Effect of foliar application of KPhi on disease severity in tuber
slices against Phytophthora infestans (B). Means that not sharing a common letter within a graph
are significantly different at p-value < 0.05 according to the Tukey's test. The experiments
included three biological replications and were performed two times*

Effect of KPhi on phytoalexin and phenol accumulations in tubers

The contents of phytoalexins and phenols were quantified in tuber pieces taken from
plants treated by KPhi or non-treated and infected by ASO isolate of *P. infestans* (*Figure 2*).
The highest amount of phytoalexins was accumulated a week after infection with
Phytophthora infestans, while the lowest was measured in non-treated tubers (*Figure 2, A
and B*). Phytoalexin contents were 3 fold increased in the treated inoculated tubers,
followed by un-treated tuber (CPi) (*Figure 2 A and B*). Wounding treatments (PhiW) also
showed a 1 fold increase than their controls (*Figure 2, A and B*). The lowest phytoalexin
detected in healthy tubers. As shown in *Figure 2*, 48 hours after infection Xinjia No. 2
tubers produced a higher accumulation of phytoalexin than Zhongshu No. 3. The phenol
contents were increased 3 fold in KPhi treated plants (PhiPi), followed by untreated plants

(CPi) 48 h after tubers inoculation (*Figure 2, C and D*). Wounding (CW) increased phenol contents in both Xingjia No. 2 and Zhongshu No. 3 (*Figure 2, C and D*). Pathogen infection (CPi) increased the phenol contents in both Xingjia No. 2 and Zhongshu No. 3 when compared with their controls. Phi treated samples (Phi) also had a significant increase in phenol contents when compared with the controls in both cultivars, but wounding after Phi treatments (PhiW) only increased phenol contents in Zhongshu No. 3 (*Figure 2, C and D*). The highest content of phenols was detected after inoculation of KPhi treated tubers in both cultivars; while the lowest were found in non-inoculated tubers of both cultivars (*Figure 2, C and D*).
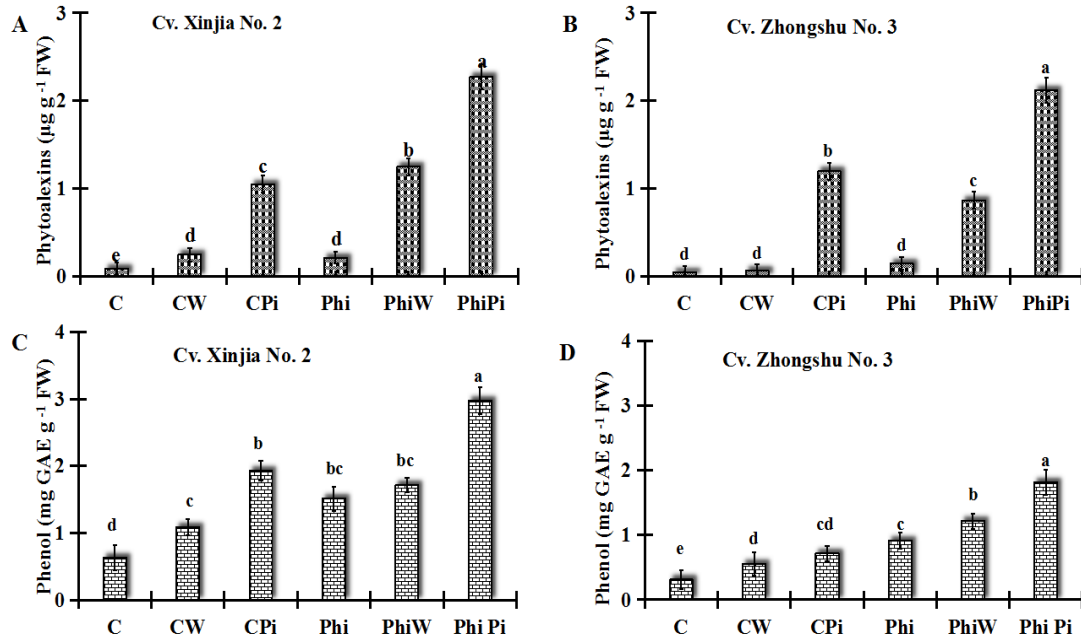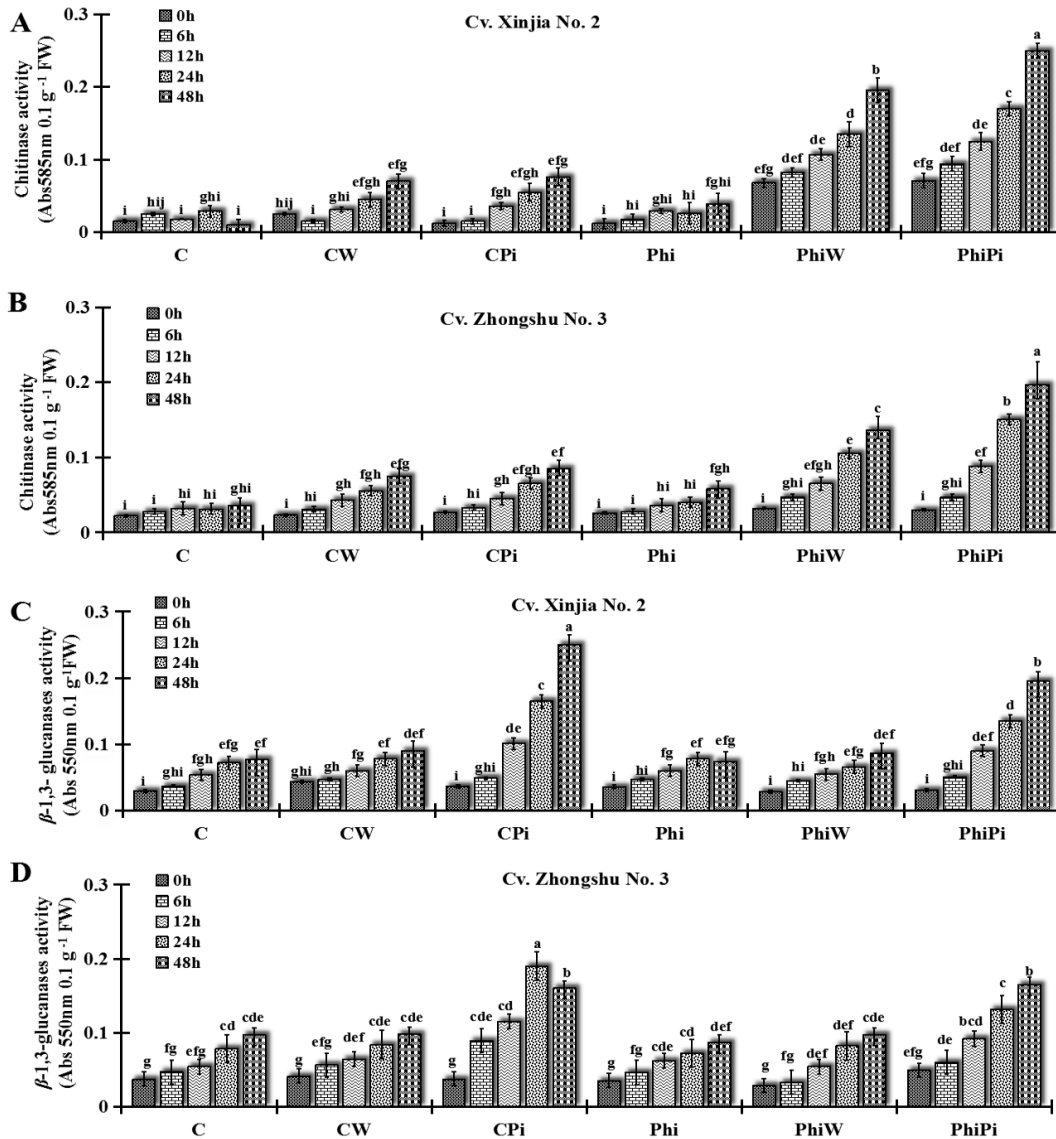

Figure 2. *Effects of KPhi application on phytoalexin contents of Cv. Xingjia No. 2 (A) and Zhongshu No. 3 (B), and phenol contents of Cv. Xingjia No. 2 (C) and Zhongshu No. 3 (D). Phytoalexin and phenol were quantified in tuber pieces after wounding (W), and inoculation by P. infestans (Pi) ASO isolate. Means not sharing a common letter within the same letters in the graph are significantly different at P < 0.05 according to Tukey's test. The experiments included three biological replications and were performed three times. C, control tubers; CW, control + wounded; CPi, control + inoculation with ASO isolate; Phi, tubers taken from KPhi applied plants; PhiW, tubers treated by KPhi + wounding; PhiPi, tubers from KPhi treated plants + inoculation with ASO isolate. FW, fresh weight*

Effect of KPhi on chitinase and ß-1, 3-glucanase activities in tubers

In this study, the effect of KPhi on chitinase and ß-1, 3-glucanase activities after wounding and inoculation with ASO isolate strain were determined at various time points. Tuber slices from KPhi-treated (Phi) and control plants (C), wounded (W) or inoculated with a pathogen (Pi) were measured at 0, 6, 12, 24 and 48 hours after the treatments (*Figure 3*). A gradual increase with time in chitinase activity after wounding and pathogen infection was observed in control potatoes of both cultivars. The level of chitinase activities in both cultivars without treatments was similar (*Figure 3, A and B*). Wounding

(CW) increased the chitinase activities in both cultivars, similarly to the Phi treatments (CPhi). The infection treatments (CPi) also showed a similar response as with wounding in both cultivars. Both wounding and pathogen infections in Phi treated tubers had significantly increased the chitinase activities in both cultivars (*Figure 3, A and B*).

Figure 3. Effects of KPhi application on chitinase activities in Xingjia No. 2 (A) and Zhongshu No. 3 (B) tubers, and β-1, 3-glucanase activities in Xingjia No. 2 (C) and Zhongshu No. 3 (D) tubers. Activities of chitinase and β-1, 3-glucanases were analyzed from tuber after wounding or inoculation with P. infestans for 0, 6, 12, 24, and 48 h, respectively. Means not sharing a common letter within the same letter in the graph are significantly different at P< 0.05 according to Tukey's test The experiments included three biological replications and were performed three times. C, control tubers; CW, control + wounded; CPi, control + inoculation with ASO isolate; Phi, tubers taken from KPhi applied plants; PhiW, tubers treated by KPhi + wounding; PhiPi, tubers from KPhi treated plants + inoculation with ASO isolate. FW, fresh weight

In wounded tubers taken from plants applied with KPhi, the rate of chitinase activity was increased 2 folds by 48 hours in KPhi treated tubers in Xinjia No. 2. In infected tubers taken from plants applied with KPhi, the rate of chitinase activity was increased 3 folds by 48 hours in Xinjia No. 2 tubers and then about 2 folds in Zhongshu No. 3. It is noted that the enzyme activities of wounded and inoculated tuber slices from Xingjia No. 2 after Phi treatments were higher when compared to that of Zhongshu No. 3 (*Figure 3, A and B*).

In contrast to chitinase, ß-1, 3-glucanase activities did not change significantly in potatoes tubers of KPhi treated plants when compared with that of the controls in both cultivars (*Figure 3, C and D*). Wounding treatment did not significantly alter the enzymatic activities in these samples either. The ß-1, 3-glucanase activity was increased sharply 48h after *P. infestans* infection in untreated tubers (CPi) more dramatic of cultivar Xingjia No. 2 (*Figure 3C*). An approximately two-fold increase in ß-1, 3-glucanase activity was recorded 24 hours after pathogen infection in Xingjia No. 2 cultivars when compared to control (*Figure 3C*). In Zhongshu No. 3, approximately 3 folds increase were noted at 24 hours after pathogen infection in untreated samples (CPi) and about 2 folds increase from the KPhi treatment plants and wounded tubers (PhiW) (*Figure 3D*) when compared to their corresponding controls. The ß-1, 3-glucanase activity increased significantly in potato tubers 24 hours after infection with a pathogen (CPi) (*Figure 3D*) followed by treated (KPhi) tubers and control after infection, compared to healthy tubers (*Figure 3, C and D*).

Effect of KPhi on POD and PPO in tubers

Inoculation by ASO late blight strain caused an increase in reactive oxygen species (ROS) response and up-regulation of antioxidant enzymes in the defense system to alleviate ROS-mediated damages. The POD and PPO activities were measured spectrophotometrically at various time points in tubers after wounding or inoculation by a pathogen (*Figure 4*). The highest POD activities values were recorded 48 hours after the pathogen inoculation, both in control (CPi) and KPhi treated plants (PhiPi). At 48 hours, the enzymatic activities increased up to 4 folds in Xingjia No. 2 and about 4 folds in Zhongshu No. 3 in the inoculated potatoes taken from tubers applied with KPhi compared with the tubers from the untreated plants (*Figure 4, A and B*). Meanwhile, the PPO activities showed similar increases, but the highest activities were detected at 12 hours after the inoculation (CPi) in Xingjia No. 2, and 24h after infection (CPi) in Zhongshu No. 3 (*Figure 4, C and D*). At these time points, the tubers obtained from the plants treated with KPhi presented increases in the PPO activities, approximately 4.8 times in Xingjia No. 2 and 4-times in Zhongshu No. 3 in comparison to the inoculated potatoes collected from untreated plants (*Figure 4, C and D*).

Effect KPhi on SOD and CAT response after inoculation by late blight

SOD and CAT activities were analyzed in a spectrophotometer at 0, 6, 12, 24 and 48 hours after pathogen inoculation with ASO isolate on KPhi and control tubers. As shown in *Figure* 5 (A and B), the lowest SOD activity was found at 0 hours while the highest value of SOD was found at 48h after inoculation (CPi) in Xingjia No. 2 (*Figure 5A*) and 24 hours after inoculation (CPi) in Zhongshu No. 3 (*Figure 5B*). The KPhi treated samples 48 hours after infection (PhiPi) had increased the SOD activity by about 3 folds in Xingjia No. 2, while it has increased by around about 2 folds in the Zhongshu No. 3 24hours after infection (*Figure 5, A and B*). Wounding did not significantly increase the SOD activities in

Phi treated samples. The CAT activity was found to be the lowest at 0 h in both cultivars and increased in the KPhi treated at 48 hours after inoculation (PhiPi) to about 4 folds in Xingjia No. 2 (*Figure 5C*) and only about 3 folds after inoculation in Zhongshu No. 3 cultivar (*Figure 5D*). Control samples at 24 hours had an increase of about 2 folds after inoculation (CPi), in contrast to the activities of controls inoculated by the late blight pathogen (*Figure 5, C and D*).
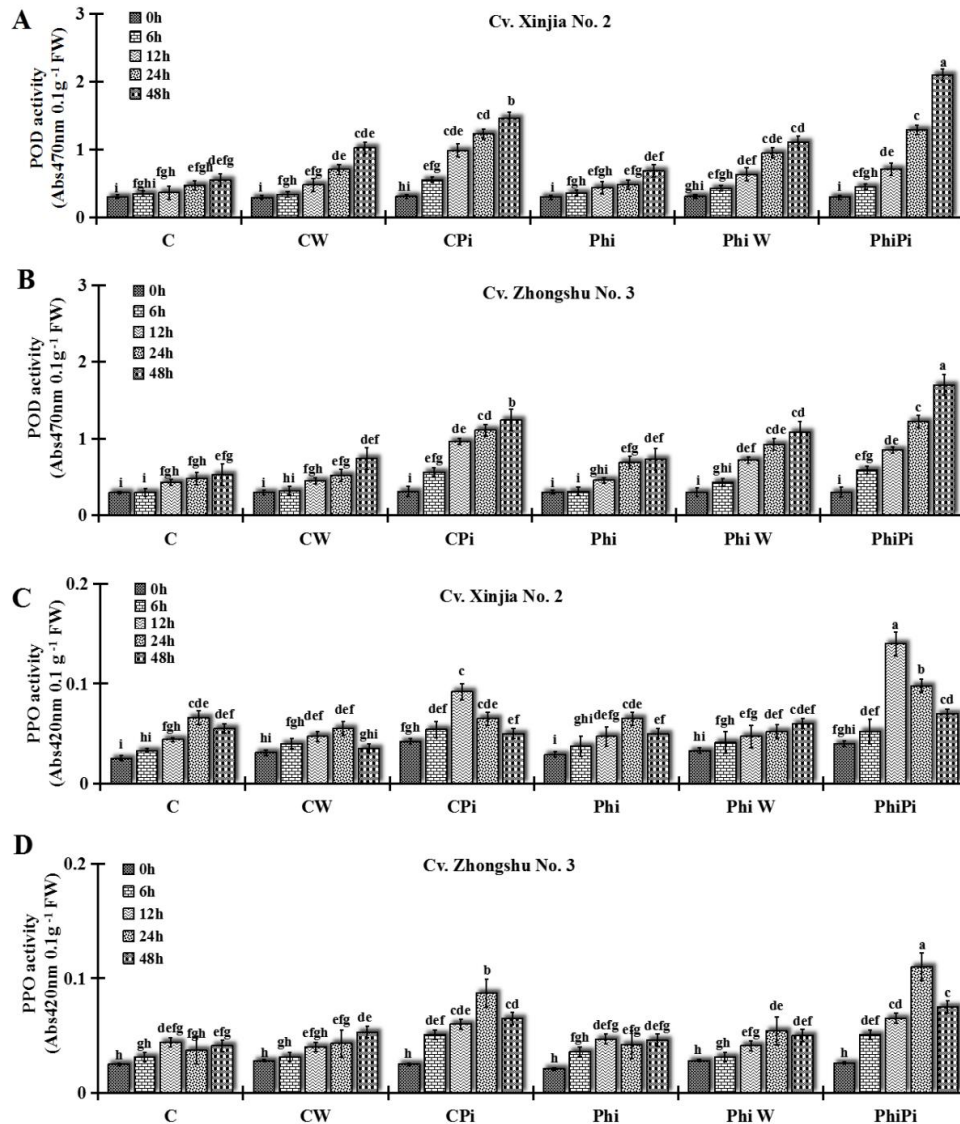
Figure 4. Effects of KPhi application on peroxidase (POD) activities in Xingjia No. 2 (A) and Zhongshu No. 3 (B) tubers, and polyphenol oxidase (PPO) activities in Xingjia No. 2 (C) and Zhongshu No. 3 (D) tubers. Measures not sharing a common letter within the same letter in the graph are significantly different at P < 0.05 according to Tukey's test. The experiments included three biological replications and were performed three times. C, control tubers; CW, control + wounded; CPi, control + inoculation with ASO isolate; Phi, tubers taken from KPhi applied plants; PhiW, tubers treated by KPhi + wounding; PhiPi, tubers from KPhi treated plants + inoculation with ASO isolate. FW, fresh weight
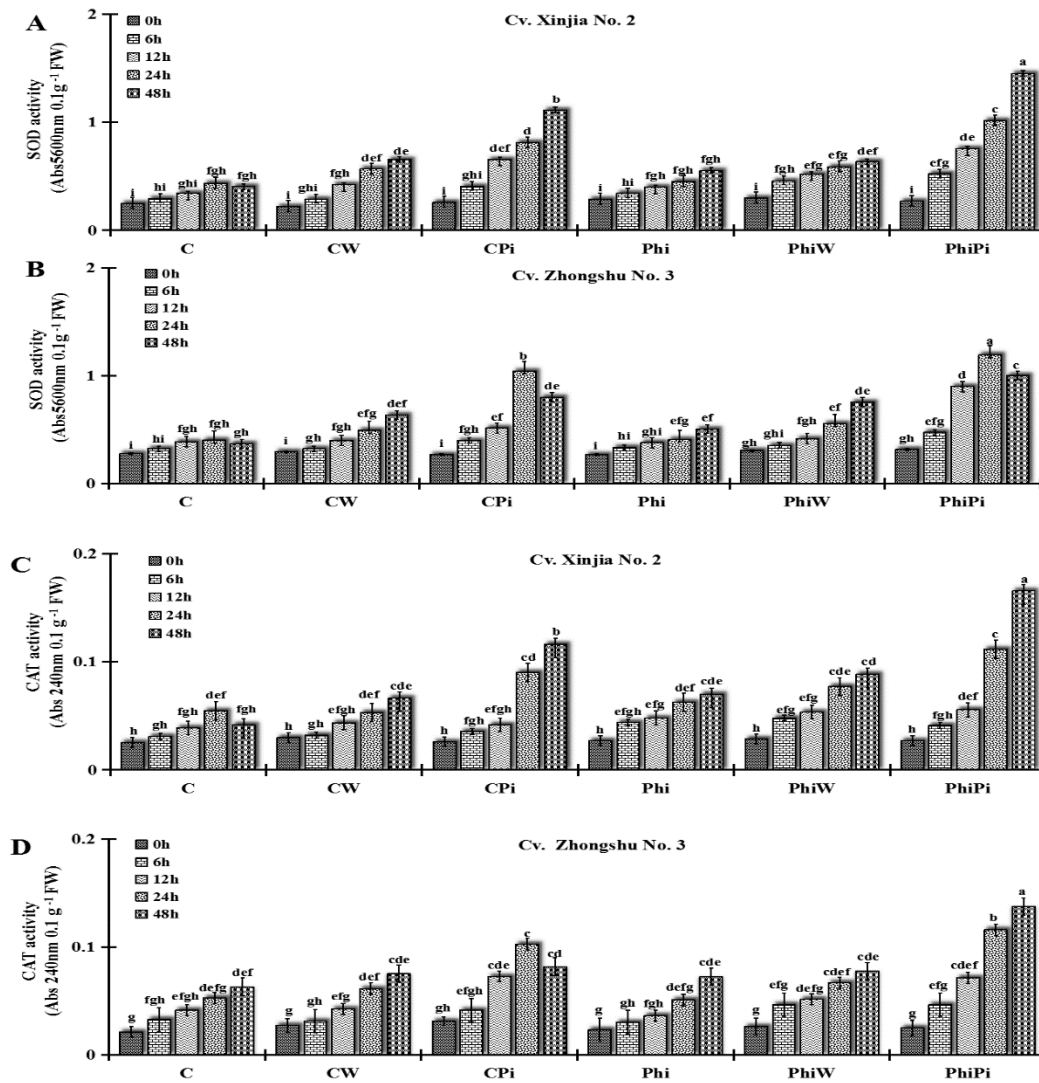
Figure 5. *Effects of KPhi application on superoxide dismutase (SOD) activity in Xingjia No. 2 (A) and Zhongshu No. 3 (B) tubers, and catalase (CAT) activity in Xingjia No. 2 (C) and Zhongshu No. 3 (D) tubers. Measures not sharing a common letter within the same letter in the graph are significantly different at P < 0.05 according to Tukey's test. The experiments included three biological replications and were performed three times. C, control tubers; CW, control + wounded; CPi, control + inoculation with ASO isolate; Phi, tubers taken from KPhi applied plants; PhiW, tubers treated by KPhi + wounding; PhiPi, tubers from KPhi treated plants + inoculation with ASO isolate. FW, fresh weight*

Discussion

In the present study, the action of KPhi on physio-biochemical contents and the enzyme activities of the potato plant in response to *Phytophthora. infestans* and wounding was studied, using two potato cultivars with different degrees of horizontal resistance against the two late blight isolate strains (*Figure 1*). It is demonstrated that the applications of KPhi on leaves results in the postharvest tubers with notably decreased disease symptoms after infection with two *P. infestans* pathogen strains. The significant protection was observed in

Mohammadi et al.: Effects of potassium phosphite on biochemical contents and enzymatic activities of Chinese potatoes inoculated by *Phythophthora infestans*

- 4510 -

moderately resistant Xinjia No. 2, after postharvest. These results were consistent with previous studies in potato. A study reported by Mayton et al. (2008) who analyzed various compounds of Phi on the protection against *P. infestans* in leaves and tubers of postharvest potato. Their experiments showed that *P. infestans* infection on leaves was well controlled by Phi as effective as a conventional fungicide against tuber late blight. Comparable to our results, Lobato et al. (2011) showed that the use of KPhi on the leaves resulted in a series of postharvest defense reactions at field conditions. Phytoalexin and phenol accumulations were related to defense reactions of the potatoes and our results showed great increase phytoalexins and phenols in potatoes tuber treated with KPhi compared to untreated slices from the control plants and wounded samples. This effect was relatively high in moderately resistant cultivar Xingjia No. 2 (*Figure 2, A and C*), and lower in the more susceptible cultivar. Zhongshu No. 3 (*Figure 2, B and D*). This means that KPhi has been able to activate a general defense response in the potato plants and the response is cultivar dependent. The involvement of phytoalexins and phenols in response to Phi were reported and various works had been reviewed by Gray et al. (2018). Plants produce enzymes, such as chitinases and ß-1, 3-glucanases (Silva et al., 2011) which can break down cell wall compounds of pathogens. These enzymes are essential determinants of plant resistance to fungi attack (Funnell and Phillips, 2004). Since these enzymes are PR proteins and their stimulations in the tubers after wounding or inoculation have been reported (Alexandersson et al., 2016; Lobato et al., 2017). Chitinases play essential roles in activating defense response in plants, alone or in combination with ß-1,3-glucanases (Mauch et al., 1988). Our results represented an increase in chitinase activity after infection by late blight pathogen. KPhi treated tubers at 48 hours after infection, clearly indicated the presence of the increased enzymatic activities (*Figure 3, A and B*). This result also confirms the efficacy of anti-fungal function of KPhi, as pointed out by Deliopoulos et al. (2010).

In the present study, ß-1, 3-glucanase was not significantly induced by KPhi after inoculation by the pathogen in Xingjia No. 2, and the highest enzymatic activity was found in untreated tubers 48 h (*Figure 3C*). Likewise, KPhi had a less effect on Zhongshu No. 3, with the highest activities of this enzyme found at 24 hours after inoculation in untreated tubers (*Figure 3D*). Compared to our results, cucumber plants inoculated with *Pseudoperonospora cubensis* showed a quick increase in chitinase and ß-1, 3-glucanase activities, which causes the degradation of the cell wall of fungi (Moazzameh et al., 2018). Greater induction of ß-1, 3-glucanase in the treated plants indicates that the activity of ß-1, 3-glucanase can weaken the fungi cell wall and prevent hypha colonization (Menu-Bouaouiche et al., 2003). Lim et al. (2013) used iTRAQ-based quantitative proteomics to identify significant changes in defense proteins including pathogenesis-related, stress-responsive and detoxification-related proteins in potato leaves after treatment with Phi. They identified that 93 (62 up-regulated and 31 down-regulated) differentially regulated proteins were identified in the leaf proteome of Phi-treated plants. To find out whether oxidation enzymes caused by stress can participate in the KPhi-induced defense mechanism, the activity of POD, PPO, SOD and CAT were measured in tubers after harvest. The increases in POD and PPO activities were highlighted that these enzymes could be a part of KPhi defense responses.

The antioxidant POD enzyme is necessary for inducing systemic resistance and could be used as a biomarker of induced resistance in plants (Kuć, 2001). The POD and PPO

activities is increased after wounding or inoculation with a pathogen in tubers taken from plants applied with KPhi (Lobato et al., 2011). In their studies, KPhi application on infected plants showed a direct relation between disease reduction and the enzyme activity. Similarly, in our result, the POD and PPO activities were increased significantly in contrast to the control plants 12 and 48 and hours after inoculation in both cultivars (*Figure 4*). Foundation PPO enzymes to wounding and enzymatic browning had been investigated in many plants (Demiİr and Kocaçalişkan, 2001). This enzyme plays a significant role in confirm of alkaloids under biotic and abiotic stress (Bilková et al., 2005). PPO activity in this study had increased at 12 hours in inoculated tubers collected from KPhi used plants in Xingjia No.2 (*Figure 4C*) and 24 hours after infection in Zhongshu No. 3 (*Figure 4D*). Similar to our results, Lobato et al. (2011) also showed that KPhi promoted POD and PPO activities after plants were infected by late blight.

It is known that SOD plays a substantial role in stress tolerance of plants and it is the first path of protection against damaging effects of high levels of ROS. Several environmental stresses usually lead to an increase in ROS production. As showed in *Figure 5 (A and B)*, the highest rate of SOD was found at 48 hours in Xingjia No. 2 (*Figure 5A*), followed by Zhongshu No. 3 (*Figure 5B*) in both varieties. Similar to our results, Mofidnakhaei et al. (2016) showed that potassium phosphite affected plant growth, and enzymatic activities of POD, SOD and CAT in cucumber plants challenged with *Pythium ultimum*.

Furthermore, CAT is necessary for ROS detoxification under stress situations by degrading H_2O_2 into H_2O and O_2 (Debnath et al., 2018a). In this study, CAT activity was increased in the plants treated with KPhi after inoculation by *P. infestans* in cv. Xingjia No. 2 (*Figure 5C*) and Zhongshu No. 3 (*Figure 5D*) in the potato tubers, respectively, compared to the wounded or control samples (*Figure 5C and 5D*). This is the first report demonstrating the responses of CAT and SOD to KPhi.

Conclusions

Plant defense pathways use some physiological and biochemical means to enhance resistance against pathogen invasions. KPhi induced the biochemical compounds and enzymes activity. Increased contents of phytoalexins and phenols were needed for plant defense processes. These metabolites will help plants to improve their resistance to pathogens.

Additionally, KPhi can increase the defense enzyme activities, inducing chitinase, ß-1, 3-glucanase activities and boosting production of antioxidants enzymes. The detailed analysis of cell wall composition and structure will elucidate and confirm our ideas of Phi involve in inducing resistance. KPhi treatment considerably mitigated POD, PPO, SOD and CAT activities which leads to late blight tolerance in potato plants. Finally, our data showed a partial characterization of some biochemical markers and certain phytopathological features involved in the response by KPhi in two cultivars with different degree of multigenic resistance to *Phytophthora infestans*. We believe this increased tolerance triggered by KPhi is belong to cell wall amplification and increased productions of biochemicals and a group of enzymatic antioxidants activities.

Furthermore, our data supported the application of KPhi as a priming inducer to enhance cell defense mechanisms. This suggests that the response to foliar KPhi treatment

could have a useful effect on physio-biochemical events and promoting tuber resistance against *Phytophthora infestans* if used in an IPM program. Overall, our data support the fact that KPhi improves resistance by improving cellular defense biochemical compounds, PR enzymes and key antioxidants enzymes activities.

REFERENCES

[1]   Adams, D. J. (2004): Fungal cell wall chitinases and glucanases. – Microbiology 150: 2029-2035.

[2]   Alexandersson, E., Mulugeta, T., Lankinen, Å., Liljeroth, E., Andreasson. E. (2016): Plant Resistance Inducers against Pathogens in Solanaceae Species – From Molecular Mechanisms to Field Application. – International journal of molecular sciences 17: 1673.

[3]   Bilková, A., Bezakova, L., Bilka, F., Psenak. M. (2005): An amine oxidase in seedlings of *Papaver somniferum* L. – Biologia Plantarum 49: 389-394.

[4]   Borza, T., Peters, R., Wu, Y., et al. (2017): Phosphite uptake and distribution in potato tubers following foliar and postharvest applications of phosphite-based fungicides for late blight control. – Annals of Applied Biology 170: 127-139.

[5]   Borza, T., Schofield, A., Sakthivel, G., et al. (2014): Ion chromatography analysis of phosphite uptake and translocation by potato plants: dose-dependent uptake and inhibition of *Phytophthora infestans* development. – Crop protection 56: 74-81.

[6]   Cakmak, I., Marschner. H. (1992): Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. – Plant Physiology 98: 1222-1227.

[7]   Chandrasekaran, M., Belachew, S. T., Yoon, E., Chun. S. C. (2017): Expression of β-1, 3-glucanase (GLU) and phenylalanine ammonia-lyase (PAL) genes and their enzymes in tomato plants induced after treatment with *Bacillus subtilis* CBR05 against *Xanthomonas campestris* pv. vesicatoria. – Journal of general plant pathology 83: 7-13.

[8]   Choo, K.-S., Snoeijs, P., Pedersén. M. (2004): Oxidative stress tolerance in the filamentous green algae *Cladophora glomerata* and *Enteromorpha ahlneriana*. – Journal of Experimental Marine Biology and Ecology 298: 111-123.

[9]   Dalio, R. J., Fleischmann, F., Humez, M., Osswald. W. (2014): Phosphite protects Fagus sylvatica seedlings towards *Phytophthora plurivora* via local toxicity, priming and facilitation of pathogen recognition. – PloS one 9: e87860.

[10] Daniel, R., Guest. D. (2005): Defence responses induced by potassium phosphonate in *Phytophthora palmivora*-challenged *Arabidopsis thaliana*. – Physiological and Molecular Plant Pathology 67: 194-201.

[11] Debnath, B., Hussain, M., Li, M., Lu, X., Sun, Y., Qiu, D. (2018a): Exogenous Melatonin Improves Fruit Quality Features, Health Promoting Antioxidant Compounds and Yield Traits in Tomato Fruits under Acid Rain Stress. – Molecules (Basel, Switzerland) 23.

[12] Debnath, B., Irshad, M., Mitra, S., et al. (2018b): Acid Rain Deposition Modulates Photosynthesis, Enzymatic and Non-enzymatic Antioxidant Activities in Tomato. – International Journal of Environmental Research: 1-12.

[13] Dehestani, A., Kazemitabar, K., Ahmadian, G., et al. (2010): Chitinolytic and antifungal activity of a *Bacillus pumilus* chitinase expressed in Arabidopsis. – Biotechnology letters 32: 539-546..

[14] Deliopoulos, T., Kettlewell, P. S., Hare. M. C. (2010): Fungal disease suppression by inorganic salts: a review. – Crop Protection 29: 1059-1075.

[15] Demiİr, Y., Kocaçalişkan. İ. (2001): Effects of NaCl and proline on polyphenol oxidase activity in bean seedlings. – Biologia Plantarum 44: 607-609.

[16] Eshraghi, L., Anderson, J., Aryamanesh, N., et al. (2011): Phosphite primed defense responses and enhanced expression of defense genes in *Arabidopsis thaliana* infected with *Phytophthora cinnamomi*. – Plant Pathology 60:1086-1095.

[17] Funnell, B. E., Phillips, G. J. (2004): Plasmid biology. – ASM Press Washington, DC,

[18] Gray, M. A., Hao, W., Förster, H., Adaskaveg, J. E. (2018): Baseline Sensitivities of New Fungicides and Their Toxicity to Selected Life Stages of Phytophthora Species from Citrus in California. – Plant Disease 102: 734-742.

[19] Hammerschmidt, R. (1999): PHYTOALEXINS: What Have We Learned After 60 Years? Annual – Review of Phytopathology 37: 285-306.

[20] Kadota, Y., Sklenar, J., Derbyshire, P., et al. (2014): Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. – Molecular cell 54: 43-55.

[21] Kim, H. K., Choi, Y. H., Verpoorte, R. (2010): NMR-based metabolomic analysis of plants. – Nature protocols 5: 536.

[22] Kuć, J. (2001): Concepts and direction of induced systemic resistance in plants and its application. – European Journal of Plant Pathology 107: 7-12.

[23] Lim, S., Borza, T., Peters, R. D., et al. (2013): Proteomics analysis suggests broad functional changes in potato leaves triggered by phosphites and a complex indirect mode of action against *Phytophthora infestans*. – Journal of Proteomics 93: 207-223.

[24] Lobato, M., Olivieri, F., Altamiranda, E. G., et al. (2008): Phosphite compounds reduce disease severity in potato seed tubers and foliage. – European Journal of Plant Pathology 122: 349-358.

[25] Lobato, M. C., Daleo, G. R., Andreu, A. B., Olivieri, F. P. (2017): Cell Wall Reinforcement in the Potato Tuber Periderm After Crop Treatment with Potassium Phosphite. – Potato Research: 1-11.

[26] Lobato, M. C., Machinandiarena, M. F., Tambascio, C., et al. (2011): Effect of foliar applications of phosphite on post-harvest potato tubers. – European Journal of Plant Pathology 130: 155-163.

[27] Machinandiarena, M. F., Lobato, M. C., Feldman, M. L., Daleo, G. R., Andreu, A. B. (2012): Potassium phosphite primes defense responses in potato against *Phytophthora infestans*. – Journal of Plant Physiology 169: 1417-1424.

[28] Majer, P., Czégény, G., Sándor, G., Dix, P. J., Hideg. É. (2014): Antioxidant defense in UV-irradiated tobacco leaves is centered on hydrogen-peroxide neutralization. – Plant physiology and biochemistry 82: 239-243.

[29] Mauch, F., Mauch-Mani, B., Boller. T. (1988): Antifungal hydrolases in pea tissue: II. Inhibition of fungal growth by combinations of chitinase and β-1, 3-glucanase. – Plant Physiology 88: 936-942.

[30] Mayton, H., Myers, K., Fry. W. (2008): Potato late blight in tubers—The role of foliar phosphonate applications in suppressing pre-harvest tuber infections. – Crop protection 27: 943-950.

[31] McDonald, A. E., Grant, B. R., Plaxton. W. C. (2001): Phosphite (phosphorous acid): its relevance in the environment and agriculture and influence on plant phosphate starvation response. – Journal of plant nutrition 24: 1505-1519.

[32] Menu-Bouaouiche, L., Vriet, C., Peumans, W. J., Barre, A., Van Damme, E. J., Rougé, P. (2003): A molecular basis for the endo-β1, 3-glucanase activity of the thaumatin-like proteins from edible fruits. – Biochimie 85: 123-131.

[33] Mofidnakhaei, M., Abdossi, V., Dehestani, A., Pirdashti, H., Babaeizad, V. (2016): Potassium phosphite affects growth, antioxidant enzymes activity and alleviates disease damage in cucumber plants inoculated with *Pythium ultimum*. – Archives of Phytopathology and Plant Protection 49: 207-221.

[34] Norton, G. W., Swinton, S. M. (2018): Precision agriculture: global prospects and environmental implications. – In Tomorrow's Agriculture: Incentives, Institutions, Infrastructure and Innovations-Proceedings of the Twenty-fouth International Conference of Agricultural Economists: Incentives, Institutions, Infrastructure and Innovations-Proceedings of the Twenty-fouth International Conference of Agricultural Economists. Routledge 269.

[35] Olivieri, F., Lobato, M., Altamiranda, E. G., et al. (2009): BABA effects on the behavior of potato cultivars infected by *Phytophthora infestans* and *Fusarium solani*. – European Journal of Plant Pathology 123: 47-56.

[36] Ramezani, M., Rahmani, F., Dehestani. A. (2017): The effect of potassium phosphite on PR genes expression and the phenylpropanoid pathway in cucumber (*Cucumis sativus*) plants inoculated with *Pseudoperonospora cubensis*. – Scientia Horticulturae 225: 366-372.

[37] Shannon, L. M., Kay, E., Lew, J. Y. (1966): Peroxidase isozymes from horseradish roots I. Isolation and physical properties. – Journal of Biological Chemistry 241: 2166-2172.

[38] Silva, O., Santos, H., Dalla Pria, M., May-De Mio, L., (2011): Potassium phosphite for control of downy mildew of soybean. – Crop Protection 30: 598-604..

[39] Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., Knez, Ž. (2005): Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. – Food Chemistry 89: 191-198.

[40] Sudipta, K., Kumara Swamy, M., Balasubramanya, S., Anuradha, M. (2014): Assessment of genetic fidelity, antioxidant enzyme activity and proline content of micropropagated and field grown plants of *Leptadenia reticulata*(wight & arn.)-an endangered medicinal plant. – Plant Cell Biotechnol Mol Biol 15: 127-135.

[41] Thao, H. T. B., Yamakawa, T. (2009): Phosphite (phosphorous acid): fungicide, fertilizer or bio-stimulator? – Soil Science and Plant Nutrition 55:228-234.

[42] Wang-Pruski, G., Coffin, R. H., Peters, R. D., et al. (2010): Phosphorous acid for late blight suppression in potato leaves. – Am. J. Plant Sci. Biotechnol 4.

[43] Wiesel, L., Newton, A. C., Elliott, I., et al. (2014): Molecular effects of resistance elicitors from biological origin and their potential for crop protection. – Frontiers in plant science 5: 655.