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# Preharvest multiple fungicide stroby sprays promote wound healing of harvested potato tubers by activating phenylpropanoid metabolism

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ABSTRACT

Wound healing is an inherent property of harvested potato tubers. However natural healing process usually needs 2-4 weeks, which increased water loss and pathogen invasion. Therefore, it is necessary to develop a technology to accelerate wound healing processing. Stroby is a biogenic fungicide with induced resistance and it has not been reported whether it can improve the wound ability of potato tubers. Potato plants (cv. Longshu 7) were repeatedly sprayed with 0.4 g $L^{-1}$ (w/v) Stroby during tuber development, and the effect of preharvest stroby spraying treatment on wound healing in harvested potato tubers was evaluated in this study. The results showed that Stroby sprays reduced weight loss and disease index of harvested tubers inoculated with *Fusarium sulphureum*. Stroby-treated potato tubers also showed accelerated accumulation and increased thickness of the suberin polyphenolic, suberin polyaliphatic and lignin at wound sites of tubers. As the major substrates of suberin synthesis, cinnamic, caffeic, ferulic and *p*-coumaric acids were accelerated, and the content of total phenolics, flavonoids and lignin were increased along with increased activity of phenylalanine ammonia-lyase (PLA) at wound sites of harvested tubers. The results suggest that preharvest multiple sprays with Stroby on potato plants could accelerate wound healing of harvested tubers via activated phenylpropanoid pathway.

## 1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth largest crop and ranks third in global consumption, representing an important staple vegetable in human diet and a mainstay of the global economy (Zhang et al., 2017). However, potato tubers are susceptible to mechanical damage at harvest and during postharvest handling. The wounds formed on the surface of tubers not only accelerates desiccation, but also opens a channel for the invasion of various pathogens, leading to increased decay and faster evaporation (Lulai, 2007). Interestingly, potato tubers have the ability to heal by forming wound healing structure on the surface of the wound, which effectively prevent evaporation and decay (Lulai and Neubauer, 2014). Natural healing of tubers takes a long time, which usually needs about 2-4 weeks under ambient conditions (12 – 25 °C, RH 60–70%) (Jiang et al., 2019). Incomplete wound healing of potato tubers could lead to a severe decay during storage. Therefore, it is necessary to develop the technology to accelerate wound healing of potato tubers.

Several exogenous chemicals effectively accelerated the wound healing of potato tubers. Abscisic acid (ABA) promoted the wound healing of tubers by eliciting phenylpropanoid metabolism and fatty acid metabolism (Kumar et al., 2010; Lulai et al., 2008). Salicylic acid and its analogue bezo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester improved the formation of healing structure, which was closely related to the induction of phenylpropanoid pathway and reactive oxygen metabolism at the wound sites of tubers (Li et al., 2018; Jiang et al., 2019).

Stroby, also known as kresoxim-methyl, is a new, broad-spectrum, high-efficiency, green fungicide. The main components of Stroby are derived from secondary metabolites, such as Strobilururus A produced by *Oudemansiella mucida* grown on larch (Kolosova et al., 2017). Stroby has been noted to reduce the fungal diseases caused by *Ascomycetes*,

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*Basidiomycetes*, *Deuteromycetes,* and *Oomycetes* (Wang et al., 2008). Stroby sprays decreaced the rice blast (Chen et al., 2015), early and late blight of potato plants (Chakraborty and Roy, 2012), and powdery mildew of cucumbers and grapes (Yu et al., 2008). Herms et al. (2002) found that Stroby promoted the gene expression of *PR-1* and increased the resistance of tobacco against viral and bacterial, indicating that that Stroby could induce resistance of tobacco. Our previous study indicated that Stroby multiple sprays in the field not only reduced powdery mildew and downy mildew of muskmelon plants, but also induced resistance against postharvest diseases of fruit (Ma et al., 2004). Although there are reports that Stroby application induced resistance in tobacco and muskmelons, the effects of preharvest Stroby sprays on wound healing of harvested potato tubers have not yet been examined.

The aims of this study were to evaluate the effects of Stroby multiple sprays in the field on wound healing of harvested potato tubers, to observe the accumulation of suberin and lignin at wound sites of tubers, and to determine key enzyme activity and metabolite contents of phenylpropanoid pathway.

## 2. Materials and Methods

### 2.1. Plant Material

Potato mini tubers (cv. Longshu 7) were provided by the Potato Institute of Gansu Academy of Agricultural Sciences, China. The tubers were directly planted in the open field of Huichuan Town, Weiyuan County, Gansu Province (104 °02.043 E, 35 °05.55 N, 2360 m altitude), China, with plant spacing of 25 – 30 cm and ridge distance of 50 – 55 cm. The field management was conducted according to the local potato cultivation standard. The tubers were harvested at the mature stage in early of October and were chosen based on the uniformity of shape and size, unblemished surface, without symptoms of disease. Picked tubers were packaged into mesh bags (25 kg $bag^{-1}$), and transported to the Postharvest Biology and Technology Laboratory of Gansu Agricultural University, China, within 24 h, and stored in cool room at 7 ± 2 °C for use.

### 2.2. Stroby sprays

Stroby solution of 0.4 g $L^{-1}$ (w/v) (≥ 50 %, BASF, Germany) was prepared with tap water, according to the manufacturer's instructions. The prepared Stroby solution was sprayed with a manual sprayer (SX-LK22C, Shixia Holdings Group, China) on plants for three times, at flowering stage (7 d after full blossom), tuber bulking stage (56 d after full blossom), and 1 week before harvest (80 d after full blossom), respectively. A total of 25 plants were sprayed with 1 L of Stroby solution. The test field was divided into six identical blocks, each containing 50 potato plants. According to the principle of completely randomized manner, 3 of the areas were selected to spray Stroby, the other 3 of areas were sprayed with tap water as the controls. The field spray experiments were conducted in 2017 and 2018.

### 2.3. Artificial wound and wound healing of tubers

Based on a modified method of Li et al. (2017), the tubers were washed with tap water and immersed in 1.5 % (v/v) sodium hypochlorite for 3 min to surface disinfection, and air-dried at room temperature. Afterwards, three artificial wounds (approximately length × 2 cm, width × 0.2 cm depth × 2 cm) per tuber were inflicted around the equator with a stainless-steel peeler (BR227, FOREVER, Japan). The wounded tubers were packed in polyethylene bag (40 cm × 25 cm and 0.01-mm thickness) with holes (1-mm-diameter holes distributed every 3 cm) and stored at ambient temperature (20 ± 2 °C, RH 75 % – 80 %) in dark for healing.

### 2.4. Determination of weight loss and disease index

The weight loss was measured based on the method of Bao et al. (2014). The potato tubers were weighed to analyze weight loss by calculating quotient of fresh weight decrease of tubers at each time to their corresponding initial weight. The weight loss was expressed as a percentage and three replicates per treatment were made 10 tubers.

The measurement of disease index was conducted according to the method of Jiang et al. (2019). *Fusarium sulphureum*, the major pathogen caused dry rot of potato tubers in China, was provided by the Institute of Plant Protection, Gansu Agricultural Sciences Academy, China, and was cultured on potato dextrose agar (PDA) and re-cultured at 28 °C once a week. A conidial suspension was obtained from 7 d old PDA cultures by flooding the surface of the culture with 10 mL of sterile distilled water containing 0.01 % (v /v) Tween-80. Subsequently, the conidial suspension was filtered through four layers of sterile cheesecloth and vortexed for 15 s, and the concentration of the suspension was adjusted to $1.0 \times 10^6$ spores $mL^{-1}$ using a hemocytometer prior to use.

On days 0, 3, 5, 7, 14, and 21 after healing, 20 μL of the prepared conidial suspension were evenly applied to the surface of the wound with an applicator then air-dried. The inoculated tubers were packed in polyethylene bags (40 cm × 25 cm and 0.02-mm thickness) with 1-mm-diameter holes distributed every 3 cm, stored in dark (20 ± 2 °C, RH 75 % – 80 %) for healing. The statistics of index was recorded after one week of inoculation. The situation of fungal growth on tuber surface were analyzed to define the disease index specified with 0 = normal (no fungi), 1 = trace (0 < visible fungal covering ≤ 1 /4), 2 = slight (1 /4 < visible fungal covering ≤ 1 /2), 3 = moderate (1 /2 < visible fungal covering ≤ 3 /4), 4 = severe (visible fungal 120 covering > 3 /4). Disease index was calculated according to the following equation:

$$\text{Disease index} = \frac{\Sigma(\text{The incidence level} \times \text{the number of wounds in each stage})}{\text{The total number of wounds} \times \text{The highest incidence level}} \times 100$$

Sixty tubers were used for each treatment and repeated three times at least.

### 2.5. Observation of suberin polyphenolic (SPP), suberin polyaliphatic (SPA) and lignin accumulation at the wound sites

The vertical wound surface of the tuber was cut into thin slices with thickness of 0.2 – 0.3 mm and width of about 1 cm using stainless steel blade (74-C, Gillette, China), washed with distilled water for three times.

The deposition of SPP was observed according to the method of Fugate et al. (2016). Slices were stained with 0.1 % berberine for 45 min. Subsequently, the stain was removed by washing with distilled water and 75 % alcohol for 2 – 3 times, and then washing again with 95 % alcohol for 1 – 2 times. Then, the tissue was stained with 0.25 % toluidine blue for 1 – 2 min, and washed with distilled water and 75 % alcohol.

The deposition of SPA was observed according to the method of Lulai and Corsini (1998). Slices were stained with 0.05 % tolouidine blue for 45 min. Subsequently, the stain was removed by washing with distilled water and 75 % alcohol for 2 – 3 times, and then washing again with 95 % alcohol for 1 – 2 times. Then, the tissue was stained with1 % neutral red III for 1 – 2 min, and washed with distilled water and 75 % alcohol. The fluorescence of SPP and SPA were observed by using a microscope (BX3, Olympus, Japan) with fluorescence excitation filter at 340 – 390 nm and emission filter at 420 nm.

The deposition of lignin was observed according to the method of Alba et al. (2010) with modifications. The vertical wound surface was hand-sliced (0.2 – 0.3-mm thickness) with a stainless-steel blade (74-C, Gillette, China). The prepared slices were immediately rinsed with distilled water to remove starch granules and then immersed in 1 % (w /v) phloroglucinol solution for 2 h on a glass slide with few drops of

concentrated hydrochloric acid. After 5 min, the images of red-stained deposited lignin were captured using a microscope (CX21FS1C, Olympus, Japan) under 10 × magnification.

The thickness of SPP, SPA, and lignified cell layers was measured by IS Capture 3.6 imaging analysis (TUCSEN, China).

### 2.6. Sampling

The method was performed according to Jiang et al. (2019) with some modification. Healing tissues (2 cm length × 2 cm width and 2 mm depth) were collected from the wounded site including suberized layers using a stainless-steel knife (B-51, Fengxing, China) after 0, 3, 5, 7, 14, and 21 d of healing. Each tuber was made three wounds and 30 tubers were sampled each treatment at each time point. The same group of samples at each time point were grouped together and grounded into powder with a grinding miller (IKA M20, Germany). Then the samples were weighed by 2 g as a sample and individually packed with an aluminum foil and stored at - 80 °C.

### 2.7. Determination of phenylalanine ammonia-lyase (PAL) activity and total phenolics, flavonoids and lignin contents

PAL activity was assayed according to the method of Wang et al. (2015) with some modifications. 2 g of frozen sample were put in an ice bath with 5 mL of boric acid-borax buffer (pH 8.8, containing 40 g $L^{-1}$ PVP, 2 mM EDTA, and 5 mM β-mercaptoethanol). Then, the extraction mixtures were sonicated at 4 °C for 10 min and centrifuged (3K30, Sigma, Germany) at 4 °C and 12,000 × *g* for 30 min, and the supernatant was crude enzyme. The reaction system included 0.5 mL of supernatant, 3 mL of 50 mM boric acid buffer (pH 8.8), and 0.5 mL of 20 mM L-phenylalanine. The absorbance determined at 290 nm after mixing the reaction system for 10 s was considered as the initial value ($OD_0$). Subsequently, the mixture was incubated at 37 °C for 1 h, and its absorbance at 290 nm was measured as termination value ($OD_1$). An increase of 0.01 in the absorbance per hour represented an enzyme activity unit (U). PAL activity was expressed as U $kg^{-1}$ by fresh weight.

The total phenolic and flavonoids content were determined depending on the method of Pirie and Mullins (1976). Frozen sample (2 g) were mixed with 5 mL of precooled 1 % HCL-methanol solution, ground in ice bath, and centrifuged at 12,000 × g and 4 °C for 30 min. Mixture was transferred to a centrifuge tube and placed in the dark at 4 °C for 20 min. Then the mixture was centrifuged at 12,000 × g and 4 °C for 30 min. The supernatant was collected for the determination of the flavonoids and total phenolics contents and the absorbance was measured at 280 and 325 nm, respectively. The total phenolic and flavonoid contents were expressed as $OD_{280}$ $kg^{-1}$ and $OD_{325}$ $kg^{-1}$ by fresh weight.

The lignin content was measured according to the method of Yin et al. (2010). Two grams of frozen sample were added in pre-cooled 5 mL of 95 % ethanol and centrifuged at 4 °C and 10,000 × g for 30 min, and the precipitate was rinsed with 95 % ethanol: hexane (1:2 v /v) for three times. Then, the washed precipitate was dried at 60 °C for 24 h, and dissolved in 1 mL of bromide: acetic acid (25 %:75 % v /v). The solution was incubated in a water bath at 70 °C for 30 min, and 1 mL of 2 M NaOH was added to stop the reaction. Finally, 2 mL of acetic acid and 0.1 mL of 7.5 M hydroxylamine hydrochloride were added to the mixture and centrifuged at 4 °C and 12,000 × *g* for 15 min. Then, 0.5 mL of the supernatant was top up to 5 mL with acetic acid and its absorbance was measured at 280 nm. The lignin contents were expressed as $OD_{280}$ $kg^{-1}$ by fresh weight.

### 2.8. Determination of phenolic acid contents in healing tissues

The content of four phenolic acids in the healing tissues was determined by the method of Ayaz et al. (2005). A total of 10.0 g frozen sample was ground in 70 % acetone (w /v = 1:3), then ultrasonically assisted extraction (40 kHz, 25 min) and filtered. The filtrate was dried with nitrogen and the aqueous phase was added 1 M NaOH to adjust pH to 7.0. The phenolic acids were extracted with 30 mL ethyl acetate and repeated 3 times. After separation of the ester phase by a separation funnel, the remaining aqueous phase was readjusted to pH 2.0 with 1 M HCL for hydrolysis and extracted with 25 mL ethyl acetate, repeat twice. Finally, the ester phase was mixed and concentrated under nitrogen-blow. The remains were dissolved in 3 mL methanol and filtered through a 0.45 μm organic membrane (Bojin Co., Ltd., China) before high-performance liquid chromatography (HPLC) analysis.

HPLC analysis was performed to an Agilent 1101 (Agilent Technologies Inc., America) fitted with an Eclipse Plus C18 column (150 mm × 4.6 mm, φ 5 μm). Solvent A: acetonitrile; solvent B: 1 % acetic acid in water. Gradient elution was performed according to the following: 0 – 5 min, 85 % B; 5 – 7 min, 80 % B; from then on, 70 % B. The flow rate was 1 mL $min^{-1}$ and the column temperature was set at 25 °C. 20 μL of the extract was injected. At the end of the elution, 100 % acetonitrile was used to wash the column for 10 min and equilibrate it to initial conditions. The spectral information of phenolic acid was detected at 323 nm with an UV-detector and identification of compounds was achieved by comparing their retention time with standard. The phenolic acid contents were expressed as mg $kg^{-1}$ by fresh weight.

### 2.9. Statistical analysis

The all determination is replicated 3 times at least. Results were presented as mean value ± standard errors. The statistical analysis was performed with SPSS version 19.0 (SPSS Inc., Chicagoc, IL, USA). Data differences were analyzed by ANOVA, followed by Duncan's multiple range tests at a 5 % level.

## 3. Results

### 3.1. Stroby sprays reduced weight loss and disease index of harvested tubers during healing

Weight loss and disease index are important indicators to evaluate the healing effect of tubers. Stroby sprays effectively reduced weight loss of harvested potato tubers during healing. Similarly, a significant difference was noted in the later stage of healing in both 2017 and 2018. The weight loss of treated tubers was 34.3 % and 48.9 % lower than those of the control at 21d of healing in 2017 and 2018, respectively (Fig. 1A, C). At the beginning of healing (0 d), the disease index of the treated tubers was significantly lower than that of the control in 2017, however, there was no obvious difference between treatment and control tubers in 2018 (Fig. 1B, D). Consistently, with the extension of healing time, the disease index gradually declined in both treated and control tubers in two years. Stroby significantly reduced disease index of inoculated tubers, which was 55 % and 40.6 % lower than the control at 5 d of healing in 2017 and 2018, respectively. The results of weight loss and disease index indicated that Stroby sprays promoted wound healing of harvested potato tubers.

### 3.2. Stroby sprays accelerated the accumulation of SPP, SPA, and lignin at the wound sites of harvested tubers

The accumulation rate and quantity of suberin and lignin at wound sites of tubers reflected the level of healing tissue formation. The same trend appeared in 2017 and 2018. The accumulation of SPP gradually increased at the wound sites of the treated and the control tubers during healing (Fig. 2A, Fig. 3A). The accumulation difference was observed between the treated and the control on the 3rd d of healing. The SPP accumulation rate and amount of treated tubers were significantly higher than that in the control. The thickness of the SPP cell layer in the treated tubers was 1.2 times and 92.6 % higher than that in the control at 3 d of healing in 2017 and 2018 (Fig. 2D, 3 D). The accumulation

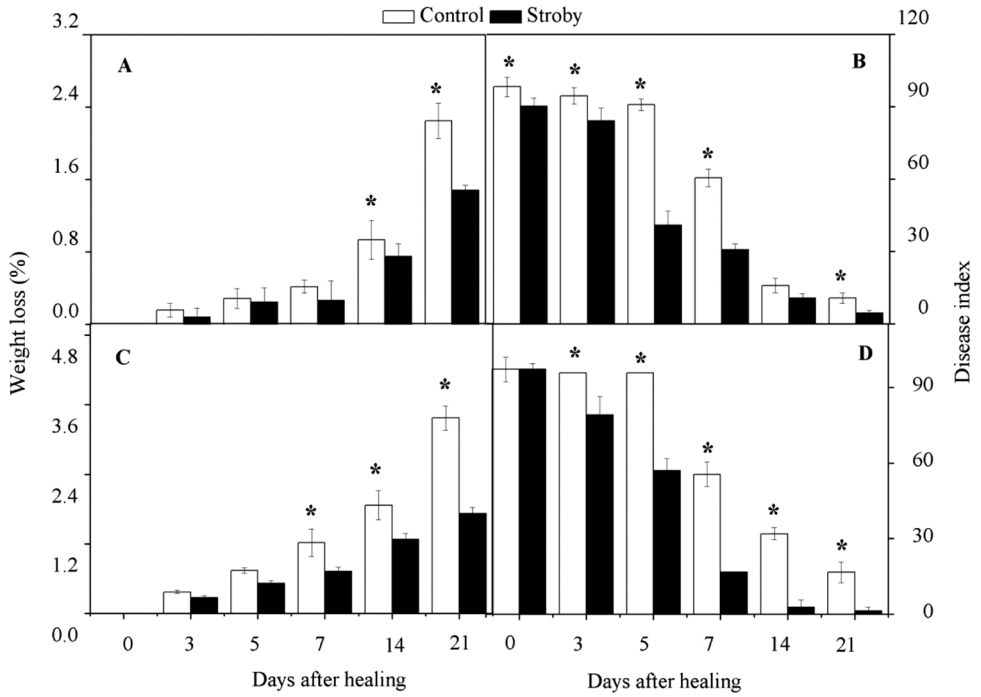


**Fig. 1.** Effect of preharvest multiple Stroby sprays on weight loss and disease index of harvested potato tubers during wound healing (A and C: weight loss in 2017 and 2018; B and D: disease index in 2017 and 2018). Bars indicate standard error (±SE). Asterisks denote significant differences ($P$ <0.05).

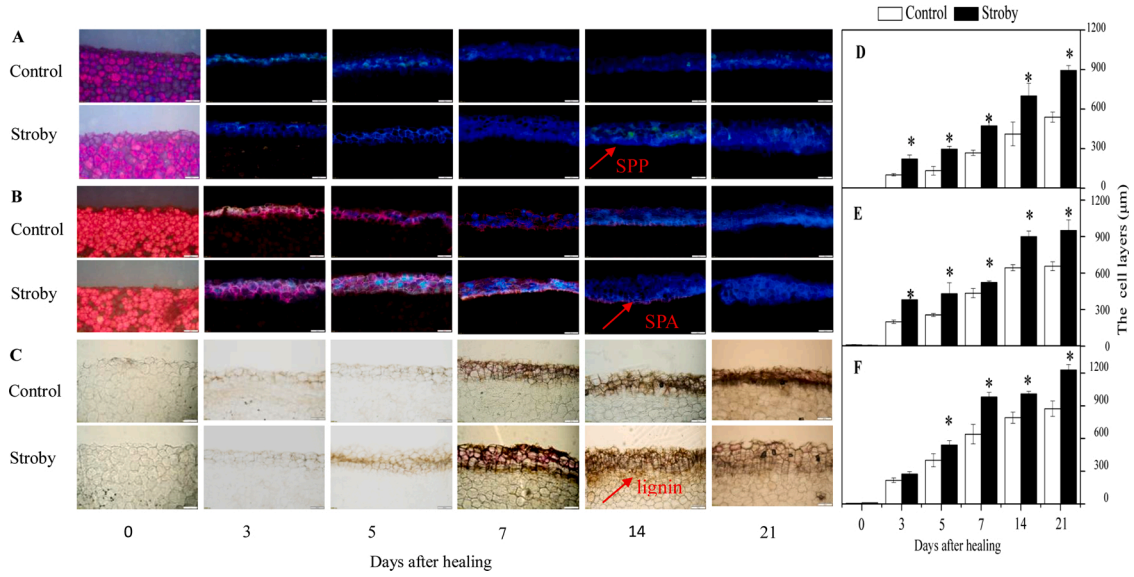


**Fig. 2.** Effect of preharvest multiple Stroby sprays on the accumulation of SPP (A), SPA (B) and lignin (C), and cell layers thickness of SPP (D), SPA (E) and lignin (F) at the wound sites of harvested potato tubers in 2017. Arrows denote SPP, SPA, and lignin, respectively. Scale bars = 200 μ. Bars indicate standard error (±SE). Asterisks denote significant differences ($P$ <0.05).

tendency of SPA showed a similar pattern to that of SPP (Fig. 2B, 3 B). The treated tubers accumulated more SPA at wound sites during healing. The thickness of the SPA cell layer in the treated tubers was 90.3% and 1.3-fold higher than the control on 3rd d of healing in 2017 and 2018 (Fig. 2E, 3 E). During healing, the lignin accumulation rate and amount of treated tubers were significantly higher than the control, and the significant difference accumulation was observed during the middle and late stages of healing in both 2017 and 2018 (Fig. 2C). The thickness of the lignin cell layer in the treated tubers was 35.2% and 54 % higher than the control on the 5th d of healing (Fig. 3F). The accumulation results of SPP, SPA and lignin indicated that Stroby sprays accelerated the deposition of suberin and lignin at wound sites of harvested potato tubers during healing.

### 3.3. *Stroby sprays activated phenylpropanoid metabolism at the wound sites of harvested tubers*

The phenylpropanoid metabolic activity of the wound tissue is a reflection of the tuber healing ability. At 0 d of healing, the PAL activity in the treated tubers was significantly higher than that in the control, which was 4.3 times higher than control (Fig. 4A). The treated tubers kept a higher PAL activity at wound sites during healing, which

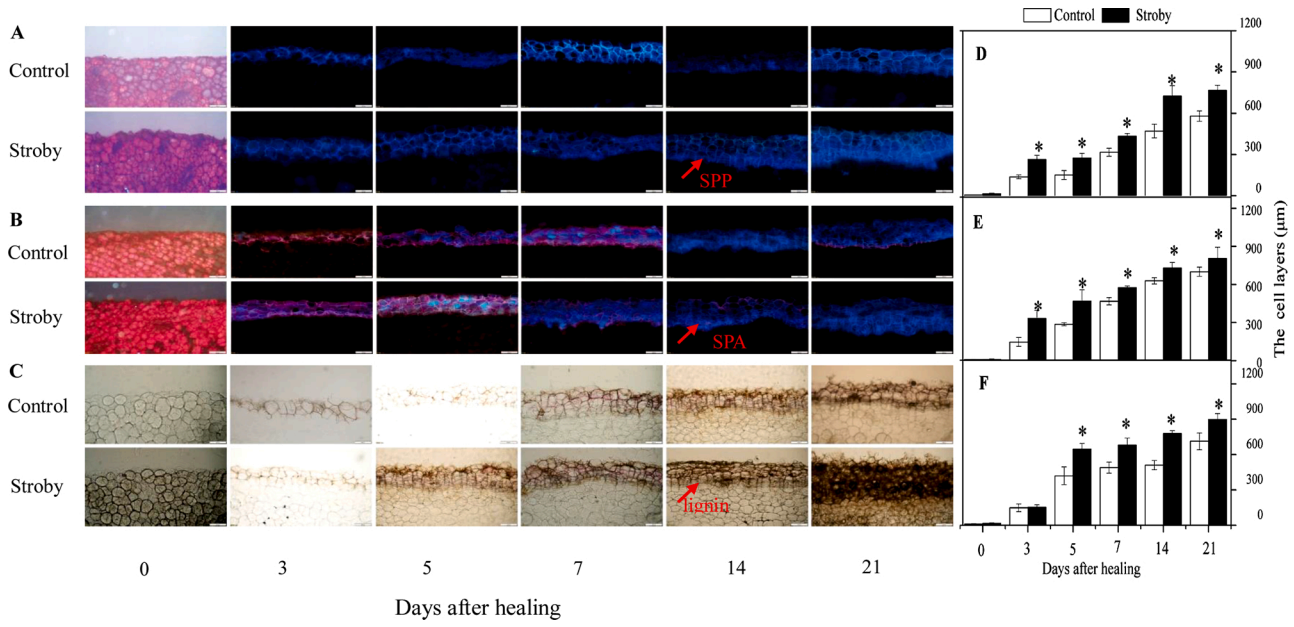


**Fig. 3.** Effect of preharvest multiple Stroby sprays on the accumulation of SPP (A), SPA (B) and lignin (C), and cell layers thickness of SPP (D), SPA (E) and lignin (F) at the wound sites of harvested potato tubers in 2018. Arrows denote SPP, SPA, and lignin, respectively. Scale bars = 200 μ. Bars indicate standard error (±SE). Asterisks denote significant differences ($P$ <0.05).

increased by 2.1 times when compared with the controls at 7 d of healing. Similarly, the total phenolic content of the treated tubers was significantly higher than the control at 0 d of healing, which was 18.8% higher than the control (Fig. 4B). During the healing, the total phenolic content of the treated tubers increased rapidly at early 3 d of healing, and then tended to be gentle. Treated tubers were significantly higher than the control during the healing, and it was 61.3 % and 39.4 % higher than the control at 3 and 5 d of healing, respectively. At 0 d of healing, the flavonoids and lignin contents between treatment and control was no obviously different. The content of flavonoids and lignin in the

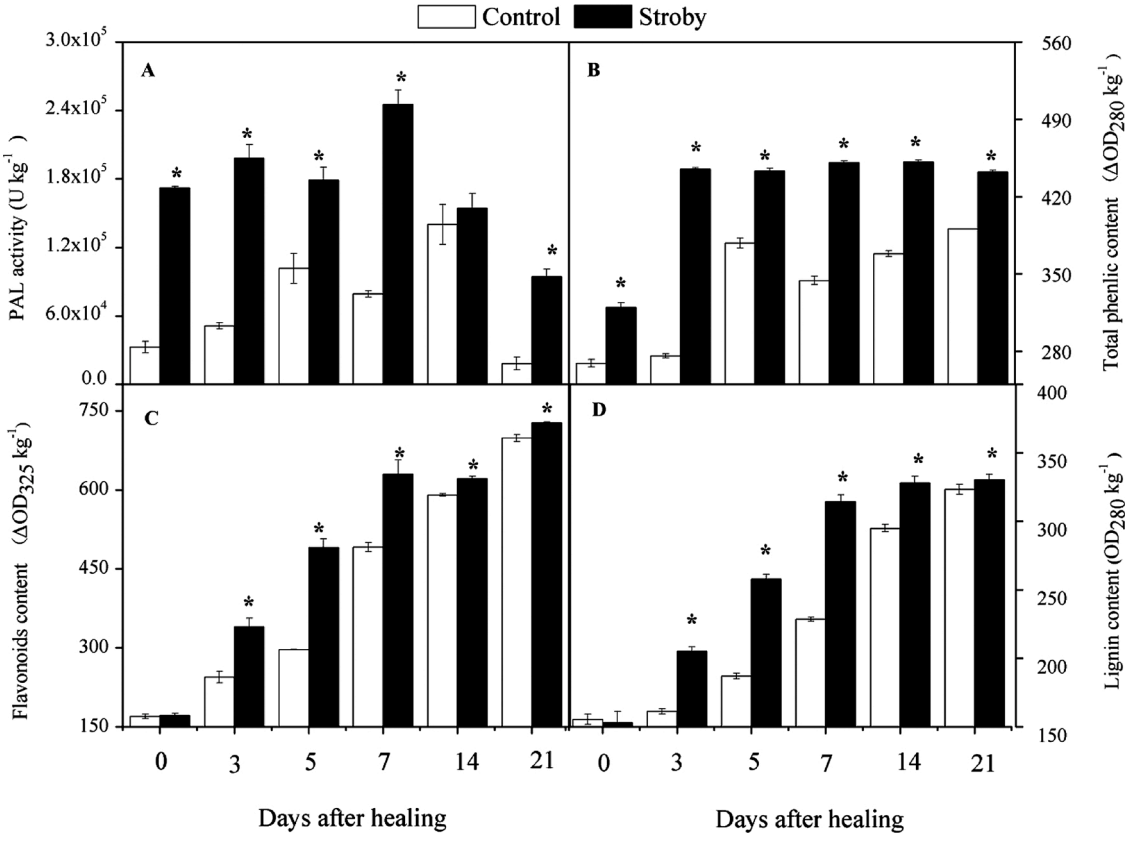


**Fig. 4.** Effect of preharvest multiple Stroby sprays on PAL activity (A), total phenolic (B), flavonoids (C) and lignin (D), contents at the wound sites of harvested potato tubers during wound healing. Bars indicate standard error (±SE). Asterisks denote significant differences ($P$ < 0.05).

treated tubers increased rapidly at early 7 d of healing and was significantly higher than the control (Fig. 4C, D). The flavonoids and lignin content of the treated tubers were 65.3 % and 37.9 % higher than the control on the 5th d of healing. The results indicated that Stroby sprays elicited PAL activity and increased the content of total phenols, flavonoids and lignin at wound sites of harvested potato tubers during healing.

The content of cinnamic acid in the treated and control tubers increased first and then decreased, and the treatment significantly enhanced the content of cinnamic acid except 0 d, which was 60 % higher than control on the 7th d of healing (Fig. 5A). At the 0 d of healing, the caffeic acid content in the treated tubers was significantly higher, which was 1.3 times higher than the control. The treated tubers kept a higher caffeic acid content during healing, which increased by 48.4 % when compared with the controls at 21 d of healing (Fig. 5B). There is no significant different in the ferulic acid content between the treated and the control at 0 d of healing. The treated tubers had more ferulic acid content during healing, which was 26.7 % and 46.3 % higher than the control at 14 d and 21 d of healing (Fig. 5C). Stroby sprays resulted in increase of *p*-coumaric acid content at harvest, which was 1.5 time higher than the control. The content of *p*-coumaric acid in treated tuber reached the maximum at 14 d of healing, which was significantly higher than the control except for 21 d of healing (Fig. 5D). The results indicated that Stroby sprays promoted accumulation of phenolic acids as substrates for SPP.

## 4. Discussion

In present study, the results showed that Stroby sprays could significantly reduce the weight loss and disease index of harvested potato tubers during healing (Fig. 1). The reduction of weight loss by Stroby sprays may be related to promoting the formation of healing periderm at wound sites to prevent the tubers from losing water (Fig. 2 and 3). Suberized tuber periderm yields a physical barrier against water evaporation and pathogen infection (Lulai, 2007). In addition, Stroby has been found to reduce stomatal aperture to control transpiration in wheat (Grossmann et al., 1999), which indicates Stroby could regulate lentical of tubers. Stroby sprays tubers had a lower disease index in tubers. On the one hand, that might be credited to efficaciously forming a physical barrier to inhibit the infection of pathogens (Fig. 2 and 3). On the other hand, Stroby has been proven to have the ability to strongly inhibit conidial germination and mycelial growth of *F. kyushuense* (Wang et al., 2016), which indicates directly inhibition on pathogen by Stroby residue in tubers. Stroby also could enhance plant defenses against pathogens by inducing pathogenesis-related proteins (Herms et al., 2002).

Suberin is a heteropolymer containing cell wall-bound SPP covalently linked to SPA that is deposited between the cell wall and plasma membrane (Woolfson et al., 2018). SPP includes polymerized phenolic substances and their derivatives, amides and esters (Han et al., 2018). SPA consists of fatty acid-derived aliphatic constituents including long chain 1-alkanols, ω-hydroxyalkanoic acids, α, ω-dioic acids, mid-chain octodecanoates, and shorter C18 oxidized fatty acids etc (Woolfson et al., 2018). Phenylpropanoid metabolism plays a significant role in wound healing of plants, which provides substrates for the formation of suberin and lignin (Thomas, 2010). In this study, we observed that Stroby sprays significantly activated PAL, enhanced content of total phenol, flavonoids, lignin and four phenolic acids at wound sites of tuber (Fig. 4 and 5). PAL is the rate-limiting enzyme of phenylpropanoid metabolism, which catalyzes the conversion of L-phenylalanine to trans-cinnamic acid, and it is the first step in the phenylpropanoid metabolism (Rivero et al., 2001). Trans-cinnamic acid undergoes a series of reactions to produce SPP precursors, such as *p*-coumaric acid, caffeic acid, ferulic acid, cinnamic acid, which are actively involved in the formation of SPP (Wei et al., 2017). Lignin is a biopolymer with a three-dimensional network structure formed by the metabolism of phenylpropanoids, which is generated by the linkage of ether bonds and carbon-carbon bonds to coumarin, sinyl alcohol and coniferyl alcohol.

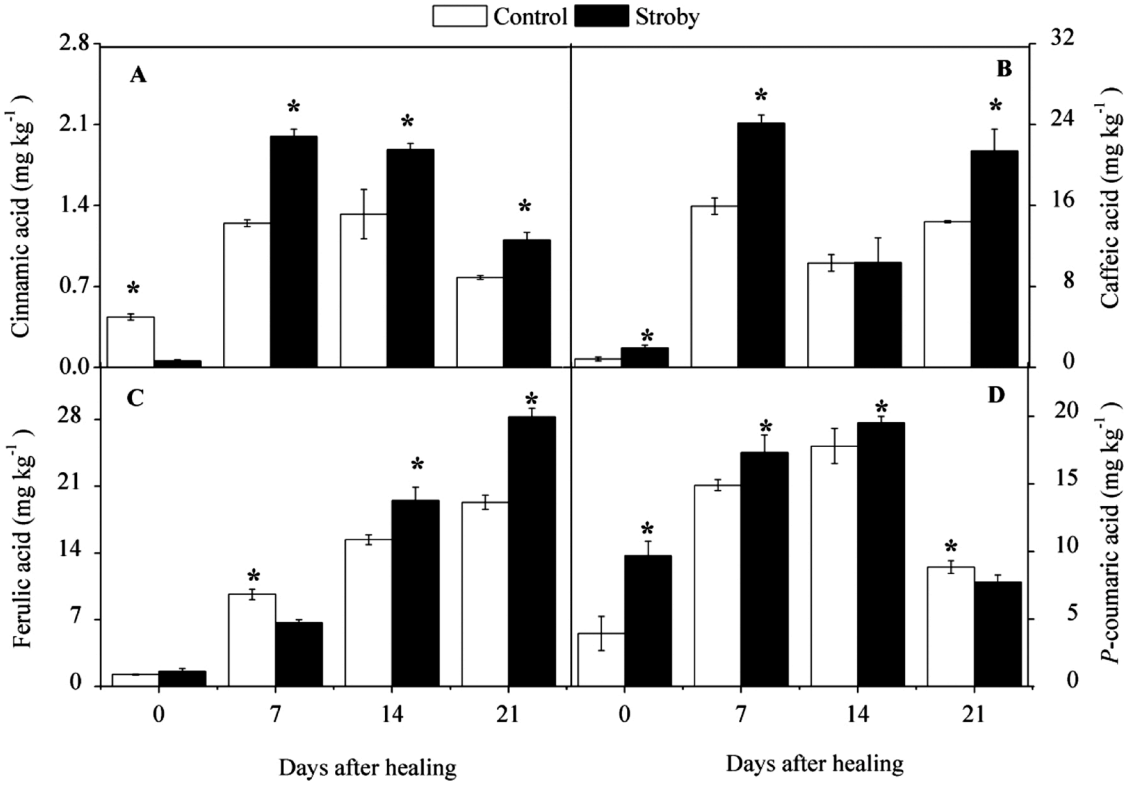


**Fig. 5.** Effect of preharvest multiple Stroby sprays on cinnamic acid (A), caffeic acid (B), ferulic acid (C) and *p*-coumaric acid (D) contents at the wound sites of harvested potato tubers during wound healing. Bars indicate standard error (±SE). Asterisks denote significant differences ($P < 0.05$).

When the phenolic acids forming SPP are produced by phenylpropanoid metabolism, these monomeric substances are thought to undergo polymerization mediated by peroxidase(s) and $H_2O_2$ in a process akin to that described for lignification (Arrieta-Baez and Stark, 2006). A suberization-associated, H2O2-dependent anionic peroxidase from potato tuber is related to the cross-linking of SPP monomers via preferential oxidation of hydroxycinnamates with a greater number of methoxy groups (ferulic acid and its derivatives to a greater degree than caffeic acid, followed by coumaric acid and sinapic acid) (Lulai, 2007). Once SPP synthesis and deposition into parenchyma cell walls is completed, the SPA monomers are synthesized, linked and deposited (Lulai, 2007).

Generally, suberin is one aspect of the overall wound-healing process that aids in sealing off damaged cells at the wound site. As a result, suberin could provide protection from water evaporation, oxidative deterioration and invading pathogens via forming a physical barrier, which is beneficial to maintain the physiological function of tuber (Vishwanath et al., 2015). Lulai and Corsini (1998) found that SPP deposited at the wound sites promoted bacterial cause of soft rot (*Erwinia carotovora* subsp. *carotovora*), but the phenolic matrix alone was not sufficient to prevent infection by a fungal pathogen, *Fusarium sambucinum*. In this study, the result shows that the Stroby treated-tubers accumulated more SPP and SPA at wound sites of tubers (Fig. 2 and 3), which indicated that Stroby spraying has the potential to induce rapid healing of potato wounds.

As substrates of suberin and lignin, phenolic compounds are closely related to the development of closing layer and periderm caused by wound stress (Dastmalchi et al., 2014). Additionally, the phenolic compounds also play an important role against pathogens infection and the higher the phenolic compounds, the better the disease resistance (Lulai and Corsini, 1998). Moreover, the phenolics could be further oxidized to quinones, producing direct toxicity to pathogen, furthermore phenolics are precursors of synthesis of lignin that can enhance the resistance of host by forming a physical barrier, thereby limiting the infection of pathogens (Shadle et al., 2003). Flavonoids are metabolites of phenylpropane pathway, which not only have strong antioxidant properties, but also show an antifungal activity (Stadnik and Buchenauer, 2000). As an antioxidant, the flavonoids effectively inhibit the expansion of the disease in potato (Dastmalchi et al., 2014). Lignin is also the main component of wound periderm and plays an important role in inhibiting water transpiration and maintaining cell structural stability (Borg-Olivier and Monties, 1993, Ramamurthy et al., 2000). In addition, lignification of cell wall increases the ability of plant cell walls to resist fungal penetration, limits the spread of extracellular enzymes and toxins secreted by pathogens in the host, and restricts the transfer of water and nutrients from plants to fungi (Faraji et al., 2018). Grossmann et al. (1999) found that Stroby treatment significantly increased ABA level in wheats, and ABA had been proven to promote tuber wound healing by activating phenylpropane metabolism (Kumar et al., 2010; Li et al., 2017). Therefore, we believe that Stroby-mediated ABA may be involved in potato tuber wound healing. But the specific mechanism remains to be revealed.

Preharvest spraying is considered to be a prospective technology, which significantly reduce the occurrence of decay in harvested fruits and vegetables (Lara, 2013). The sprayed chemicals could enter the interior of plants through the cuticle, microcracks in the cuticle and natural opens (Schönherr, 2006). Subsequently these substances are absorbed by the plant and transported to the developed tubers to produce an accumulation effect, which may be accompanied by plant growth and development. As a new broad-spectrum fungicide, Stroby effectively control crop diseases not only by inhibiting the growth of fungi (Cheng et al., 2015), but also by inducing resistance of plants (Herms et al., 2002). In this study, we observed that multiple sprays with Stroby during potato tuber development promoted wound healing of harvested tubers by activating phenylpropanoid metabolism, which suggested that Stroby sprays could be as a promising strategy for accelerating wound healing in harvested potato tubers.

## 5. Conclusions

Preharvest multiple sprays with Stroby could promote the synthesis of total phenols, flavonoids and lignin by inducing the PAL activity in the wound sites of harvested potato tubers. Moreover, Stroby sprays enhanced content of the phenolic acids, accelerated the accumulation of suberin and lignin at wound sites, and decreased the weight loss and disease index of wound tubers during healing, thus promoting wound healing of harvested potato tubers.

## CRediT authorship contribution statement

**Xiangzhen Ge:** Investigation, Methodology. **Yan Zhu:** Investigation, Methodology, Data curation, Funding acquisition. **Zhicheng Li:** Software, Validation. **Yang Bi:** Conceptualization, Supervision, Project administration. **Jing Yang:** Resources, Writing - original draft. **Junlian Zhang:** Formal analysis. **Dov Prusky:** Writing - review & editing.

## Declaration of Competing Interest

The authors report no declarations of interest.

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