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Heat Shock Treatment Promoted Callus Formation on Postharvest Sweet Potato by Adjusting Active Oxygen and Phenylpropanoid Metabolism

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Abstract: This study aimed to investigate that rapid high-temperature treatment (RHT) at an appropriate temperature could accelerate callus formation by effectively promoting the necessary metabolic pathways in sweet potato callus. In this study, the callus of sweet potato was treated with heat shock at 50, 65, and 80 °C for 15 min. The callus formation was observed within 1, 3, and 5 days, and the accumulation of intermediates in the metabolism of phenylpropane and reactive oxygen species and changes in enzyme activities were determined. The results showed that appropriate RHT treatment at 65 °C stimulated the metabolism of reactive oxygen species at the injury site of sweet potato on the first day, and maintained a high level of reactive oxygen species production and scavenging within 5 days. The higher level of reactive oxygen species stimulated the phenylalanine ammonia-lyase (PAL), 4-coumarate-CoA ligase and cinnamate-4-hydroxylase activities of the phenylpropane metabolic pathway, and promoted the rapid synthesis of chlorogenic acid, *p*-coumaric acid, rutin, and caffeic acid at the injury site, which stacked to form callus. By Pearson's correlation analysis, catalase (CAT), PAL, and chlorogenic acid content were found to be strongly positively correlated with changes in all metabolites and enzymatic activities. Our results indicated that appropriate high-temperature rapid treatment could promote sweet potato callus by inducing reactive oxygen species and phenylpropane metabolism; moreover, CAT, PAL, and chlorogenic acid were key factors in promoting two metabolic pathways in sweet potato callus.

Keywords: Rapid high temperature; Phenylalanine ammonia-lyase; Catalase; Chlorogenic acid; Key metabolic mechanisms

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1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is now an important vegetable crop, as its yield is high and it is easily cultivated [1]. China is the leading global sweet potato producer. According to 2017 statistics, the total sweet potato planting area in China was 8.9373 million hm^2 and the total yield was 34.189 million tons. However, this root tuber crop is characterized by a thin epicarp, high water content, and poor storage performance. Sweet potato epicarp is readily damaged during mechanized or artificial harvesting and postharvest processing [2]. Consequently, sweet potato storage and transport are associated with storage decay by microbial pathogens, depletion of nutrients with high respiration rates, and some other quality losses [3]. The Ministry of Agriculture and Rural Affairs of China reported that the comprehensive loss of postharvest sweet potato roots caused

by improper storage exceeded 30% in 2019 [4]. Therefore, mitigation of postharvest loss during storage and transportation is of paramount importance.

Callus formation is an internal defense mechanism of injured plant tissues. It occurs in roots, stems, leaves, and fruit [5]. Calli help prevent water loss and microbial infection. Wounded plant tissues generate transduction signals that induce reactive oxygen, phenylpropanoid, and fatty acid metabolism. Oxidative cross-linking of related metabolites then occurs and calli gradually form around the wounds [6]. Numerous studies have been conducted on callus formation of root and tuber crops such as white potato. The mechanism of phellem formation in potato callus has been elucidated [7,8]. Other studies demonstrated that potato callus formation was influenced by crop variety [7], harvest maturity [9], temperature [10], relative humidity, ambient light conditions [11], gas composition [12], and chemical stimulation [6,13]. However, few studies have investigated the sweet potato callus healing process. Prior research on sweet potato callus formation focused mainly on improving sweet potato storability through callus healing. Low-temperature heating (30 °C–35 °C) under cold storage for 3–7 days promoted sweet potato callus formation [14]. Mwanga found that sweet potatoes subjected to continuous callus treatment at 32 °C and 90% relative humidity (RH) for 4 days were more durable than those without callus treatment [15]. Amand reported that 18 different sweet potato varieties subjected to callus treatment at 30 °C and 85% RH for 7 days more readily formed wound calli than untreated samples [16]. Nevertheless, sweet potato is associated with strong harvest seasonality, long callus formation time, high heating equipment costs, and environmentally unsustainable crop protection methods. For these reasons, postharvest callus healing cannot fully meet market and production demands. In addition, some preharvest studies have pointed out that multiple fungicide stroby spray treatment [17] and sodium nitroprusside treatment [18] during the preharvest process could promote the wound healing for postharvested potatoes. Long-term callus maintenance at low temperatures may result in uneven heating and callus formation, yield loss, and tuber decay [17]. Therefore, callus healing time should be shortened and the conditions of the ambient environment and heat treatment should be harmonized to improve the efficiency and stability of callus formation.

The present study endeavored to produce rapid, uniform, and efficient sweet potato calli through rapid heat callus treatment (RHT) at comparatively higher temperatures and shorter treatment times. We observed sweet potato callus formation; analyzed changes in active oxygen metabolism, phenylpropanoids, and enzyme activity; and identified the key factors affecting sweet potato callus responses to RHT. The findings of the present study provide theoretical and methodological bases for rapid callus induction in postharvest sweet potato root tubers.

2. Results

2.1. Effect of RHT Treatment on Callus Wound Healing in Sweet Potato

Sweet potato callus consists of suberin and lignin, and the former is its principal component [4]. Suberin comprises fatty acid polymers and phenolic monomers produced by the fatty acid and phenylpropanoid metabolism pathways, respectively. Phenolic monomers combined with the cations in toluidine blue and turned purplish blue, and this reaction was used to evaluate suberin stacking. Figure 1 shows that after callus staining, only scant blue fluorescence appeared in the sweet potato wound at the original stage. In contrast, different fluorescence intensities appeared in the other groups and at the other stages. Compared with CK, all RHT groups presented with higher fluorescence on day 5. Therefore, RHT promoted suberin accumulation in sweet potato. The sweet potato wound tissue treated with RHT at 65 °C showed relatively more significant suberin deposition on days 1, 3, and 5. Hence, RHT at 65 °C most strongly promoted sweet potato callus. After RHT at 65 °C, the callus on the wound surface was grayish white with no obvious browning. In contrast, the sweet potato calli formed after RHT at 50 °C and 80 °C presented with different degrees of browning.

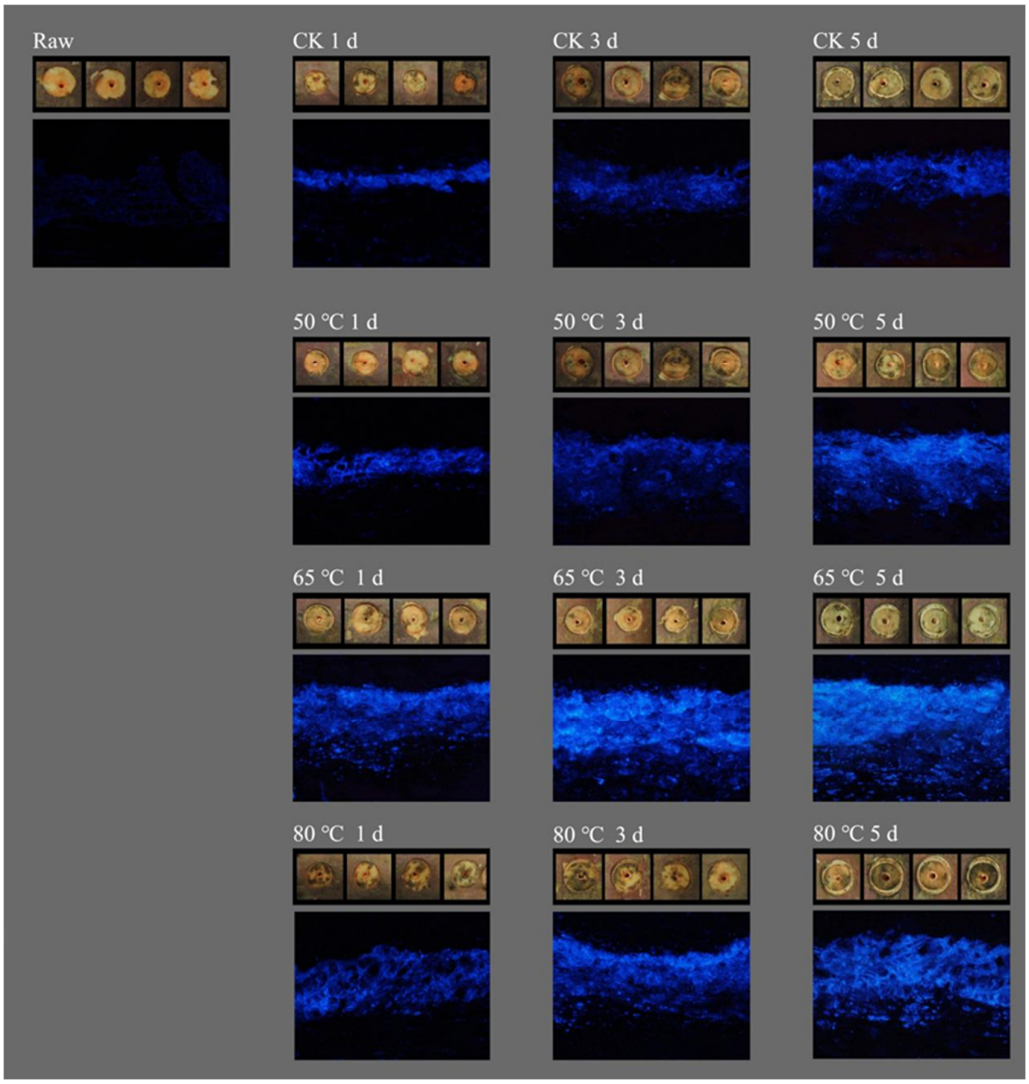

Figure 1. Effects of RHT treatments on suberin deposition in sweet potato tuber wound.

Lignin causes stacking and accumulates around the wound during callus healing in white potato and sweet potato [19]. Lignin is a glycerolipid polymer that forms during callus healing and is localized mainly to the cell walls and the plasma membranes. The composition and structure of lignin and wax are similar. Both prevent water and nutrient loss and resist bacterial infection in plants [20]. Therefore, the lignin content in healing tubers also affects callus formation. Figure 2A shows that from day 1, the lignin content in sweet potato had significantly increased compared with the raw samples under RHT and CK. However, on days 3 and 5, the lignin content was significantly higher under RHT at 65 °C and 80 °C than under CK and RHT at 50 °C. Figure 2B shows that callus deposition increased with lignin content and suberin stacking. The callus was composed of lignin and polyphenolsuberin. The higher the production of lignin and polyphenolsuberin, the thicker the callus. Thus, this finding and those illustrated in Figures 1 and 2A reveal that callus thickness was greatest under RHT at 65 °C. It reached 0.38 mm and 0.49 mm on days 3 and 5, respectively, and lignin–suberin stratification was higher than it was under the other treatments. The foregoing results indicate that the heat-shock treatment promoted lignin accumulation and suberin deposition in sweet potato wounds and that RHT at 65 °C might be the most effective stimulatory treatment for this purpose.

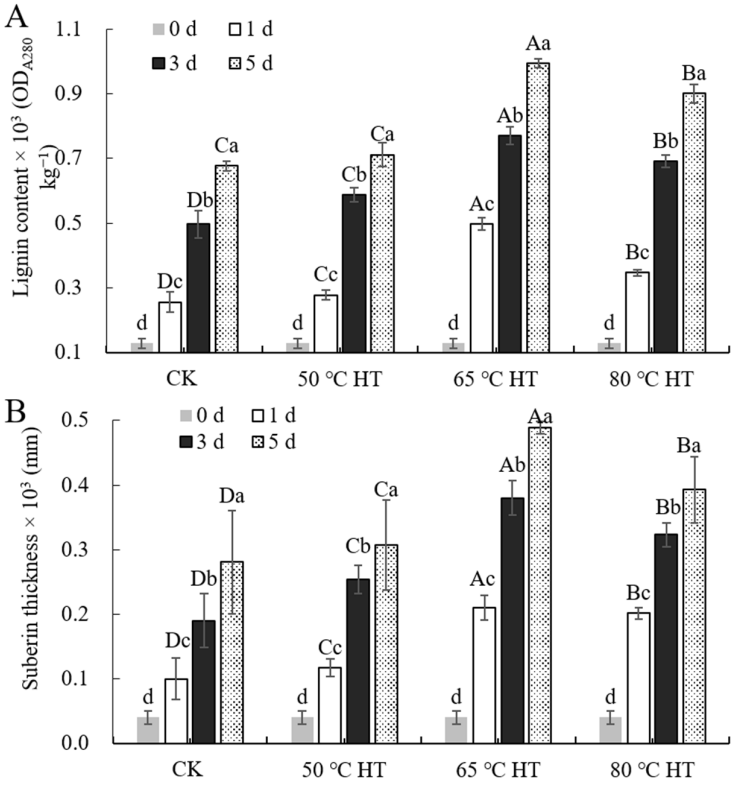

Figure 2. Effects of RHT treatment on suberin thickness and lignin content in sweet potato callus ((**A**): Liginn content; (**B**): Suberin thinckness. Note: Capital letters are comparisons at the same time at different temperatures, and lowercase letters are comparisons at different times at the same temperature).

2.2. Effects of RHT Treatment on Phenolic Compounds in Sweet Potato Callus

Phenolic compounds are precursors of suberin and lignin in sweet potato and the main components in sweet potato wounds and calli [3]. High-performance liquid chromatography (HPLC) was used to detect phenolic compounds in sweet potato callus. They were identified as *p*-coumaric acid, catechin, chlorogenic acid, and rutin. On day 5, the total phenol and flavonoid levels were higher in sweet potato subjected to RHT at 65 °C than in those subjected to other treatments. By contrast, the total phenol and flavonoid levels did not differ between sweet potatoes treated with RHT at 50 °C and those treated with RHT at 80 °C. For these reasons, the total phenols and flavonoids included substances implicated in suberin and lignin biosynthesis.

The chlorogenic acid content strongly responded to the temperature treatments and was higher in all RHT groups than in CK. From days 1–5, the chlorogenic acid content was the highest under the RHT at 80 °C (Figure 3C). The *p*-coumaric acid and rutin levels also substantially increased during callus healing. However, the changes in *p*-coumaric acid content were relatively more evident in response to RHT at 65 °C and 80 °C. The *p*-coumaric acid content in the sweet potato subjected to day 5 of RHT at 65 °C was twice that of the sweet potato under CK. Figure 3E shows no difference in rutin content under any treatment between days 1 and 3. By day 5, however, the rutin content was significantly higher under RHT at 65 °C than under the other treatments. Figure 3F shows no significant difference between the RHT at 50 °C and CK treatment at any sampling point in terms of catechin content. Therefore, RHT at 50 °C did not markedly induce catechin biosynthesis. There was no significant difference in the sweet potato subjected to RHT at 65 °C or RHT at 80 °C in terms of catechin content on day 3. By day 5, the catechin content was higher under RHT at 65 °C than under the other heat treatments. For these reasons, RHT at 65 °C and 80 °C promoted phenolic compound accumulation during sweet potato callus healing. Furthermore, the accumulation of phenolic compounds may help sweet potato wounds

rapidly heal and form callus tissue. However, RHT at 80 °C might block total phenol, total flavonoid, *p*-coumaric acid, and catechin synthesis. Excessively high temperatures might inhibit the enzymes involved in phenylpropanoid metabolism. The present study showed that RHT at 65 °C was the optimal treatment for inducing phenolic compound synthesis in sweet potato wounds.

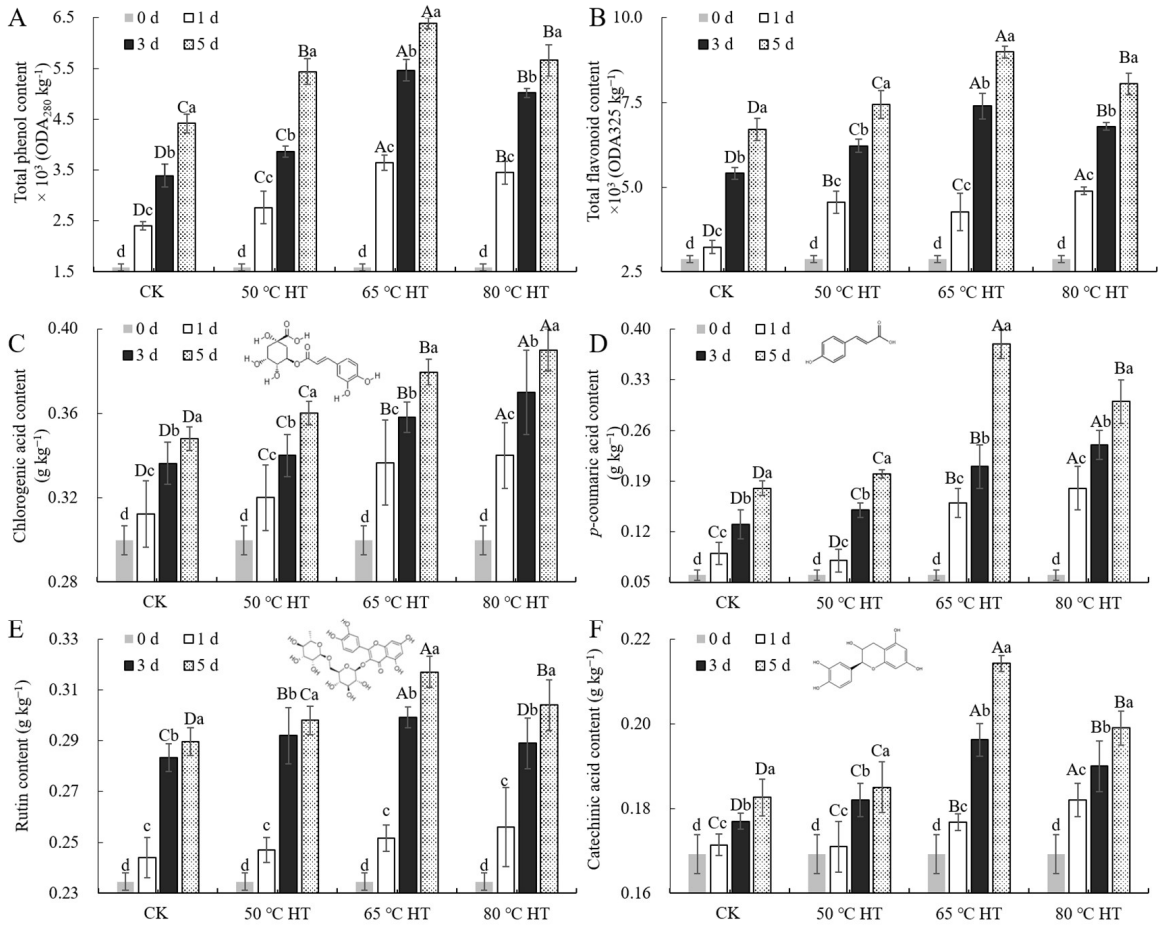

Figure 3. Effects of RHT treatment on total phenol, total flavonoid, chlorogenic acid, p-coumaric acid, rutin, and catechin content in sweet potato callus ((**A**): Total phenol content; (**B**): Total flavonoid content; (**C**): Chlorogenic acid content; (**D**): p-coumaric acid content; (**E**): Rutin content; (**F**): Catechinic acid content. Note: Capital letters are comparisons at the same time at different temperatures, and lowercase letters are comparisons at different times at the same temperature).

2.3. Effects of RHT Treatment on Phenylpropanoid Metabolic Enzyme Activity during Sweet Potato Callus Healing

Numerous studies demonstrated that plant callus formation is closely related to phenylpropanoid metabolism. Phenylalanine ammonia-lyase (PAL), *trans*-cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), and cinnamyl alcohol dehydrogenase (CAD) are the major intermediate metabolic enzymes in phenylpropanoid metabolism [21]. Figure 3 shows that compared with the origin, the activity levels of the foregoing enzymes in all groups were increased during callus healing. PAL is a key rate-limiting enzyme at the beginning of phenylpropanoid metabolism. Figure 4C shows that PAL activity significantly increased under all treatments on day 1 and was highest under RHT at 80 °C. On days 3 and 5, however, PAL activity declined under RHT at 80 °C but increased under the other three treatments. On day 5, PAL activity under RHT at 65 °C was more than twice that under CK. The 4CL and C4H activity levels significantly increased on day 1. RHT at 80 °C inhibited 4CL and C4H activity on days 3 and 5. C4H activity peaked on day 1 and remained high

under RHT at 65 °C on days 3 and 5. C4H activity significantly increased under RHT at 50 °C on day 5. Nevertheless, there was no significant difference between RHT at 65 °C and RHT at 50 °C in terms of C4H activity. The 4CL activity reached a peak on day 3 under RHT at 65 °C and was higher than that at other sampling points. CAD is implicated in lignin biosynthesis at the end of phenylpropanoid metabolism. Figure 3D shows that the change trend in CAD activity differed from those of the other three enzymes. CAD activity increased with time in all treatment groups. On days 3 and 5, there were no differences among RHT at 50 °C, 65 °C, and 80 °C in terms of CAD activity. In addition, CAD activity was high in the CK group at day 3. Hence, lignin content and phellem layer thickness also increased under CK in the absence of any high-temperature stimulus, and sweet potato injury may have promoted CAD activity. However, PAL, 4CL, and C4H were relatively more temperature sensitive. Appropriate RHT treatment may induce PAL, 4CL, and C4H in sweet potato. Several studies reported that wound injury and temperature stimulation promote ROS metabolism [4]. The influence of temperature on CAD, PAL, 4CL, and C4H may also be associated with ROS generation and clearance.

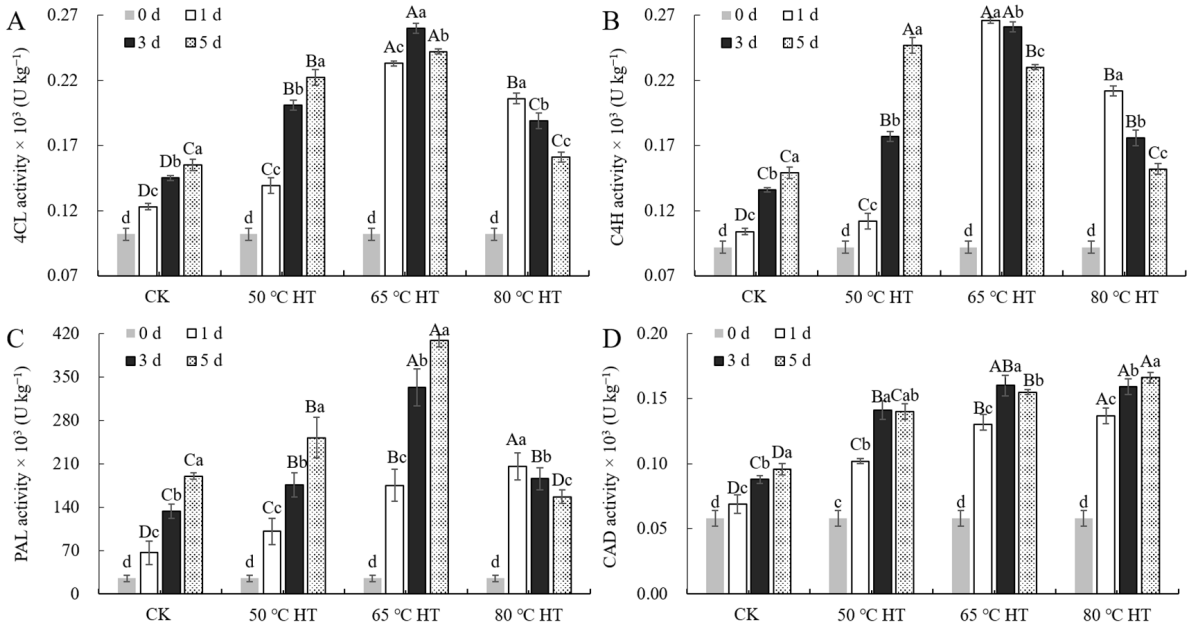

Figure 4. Effects of RHT treatment on PAL, C4H, 4CL, and CAD activity in sweet potato callus ((**A**): 4CL activity; (**B**): C4H activity; (**C**): PAL activity; (**D**): CAD activity. Note: Capital letters are comparisons at the same time at different temperatures, and lowercase letters are comparisons at different times at the same temperature).

2.4. Effects of RHT Treatment on ROS Production and Scavenging in Sweet Potato Callus

Neither low- nor high-temperature RHT treatment stimulated phenylpropanoid metabolism in sweet potato possibly because active oxygen metabolism occurred in the wounds [4]. Figure 5 shows the indices related to ROS generation and scavenging in sweet potato callus healing. Figure 5B,C shows ROS production capacity in sweet potato. ROS production was significantly higher under RHT at 80 °C than under other treatments. Therefore, temperature induces ROS generation in sweet potato. Figure 5A shows the ROS scavenging parameters during sweet potato callus healing. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay displayed the same trends for CK, RHT at 50 °C, and RHT at 65 °C and exhibited a gradual increase from days 0 to 5. For the sweet potatoes treated with RHT at 80 °C, DPPH scavenging peaked on day 1 and gradually decreased thereafter. On day 1, DPPH scavenging under RHT at 80 °C was 1.25 times higher than it was under RHT at 65 °C. Nevertheless, DPPH scavenging under RHT at 65 °C was higher than that under CK and RHT at 50 °C. These results suggest that high temperature

significantly induces free radical scavenging in sweet potato wounds. At day 3, DPPH scavenging significantly decreased in sweet potato under RHT at 80 °C possibly because of decreases in the activity of enzymes related to ROS scavenging, namely, catalase (CAT) and superoxide dismutase (SOD). Figure 5E,F shows that the changing trends were the same for CAT, SOD, and DPPH scavenging. RHT at 50 °C and RHT at 65 °C significantly induced CAT and SOD, whereas RHT at 80 °C significantly inhibited them and weakened the free radical-scavenging capacity of sweet potato. Maximum CAT and SOD activity levels were lower under RHT at 80 °C than under RHT at 65 °C on day 1. Peroxidase (POD) is another important ROS-scavenging enzyme (Figure 5D). POD activity was the same under both RHT at 80 °C and RHT at 65 °C. POD activity was essentially the same on days 1, 3, and 5. However, POD activity was significantly higher under RHT at 80 °C and RHT at 65 °C than under CK or RHT at 50 °C.

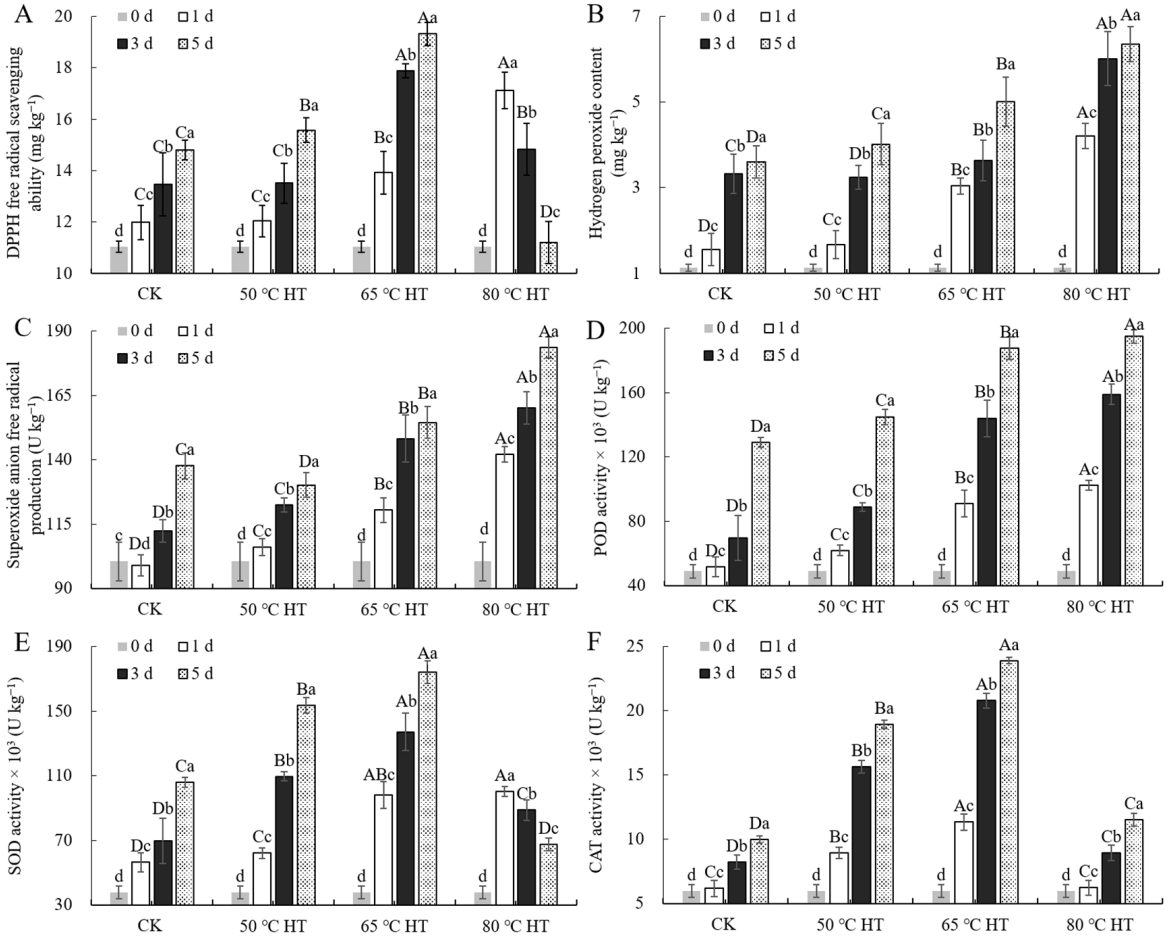

Figure 5. Effects of RHT treatment on DPPH, hydrogen peroxide, and superoxide anion scavenging and POD, SOD, and CAT activity in sweet potato callus ((**A**): DPPH free radical scavenging ability; (**B**): Hydrogen peroxide content; (**C**): Superoxide anion free radical production; (**D**): POD activity; (**E**): SOD activity; (**F**): CAT activity. Note: Capital letters are comparisons at the same time at different temperatures, and lowercase letters are comparisons at different times at the same temperature).

The foregoing results indicate that temperature had a significant impact on several active oxygen metabolism indices in sweet potato. Active oxygen metabolism, phenylpropane metabolism-related enzymes, phenolic compounds, and callus formation were also correlated. However, no valid conclusions could be drawn from direct data analysis. Hence, the key factors affecting sweet potato callus had to be identified through correlation data analysis.

2.5. Pearson's Correlation Analysis of Factors Affecting Sweet Potato Callus

Pearson's correlation analysis was conducted on all indices under all RHT treatments. The acceptance level was set to 0.01 to screen for key regulatory factors affecting the responses of sweet potato callus to RHT treatment. The results are shown in Figure 6. Compared with the lignin content, suberin stacking was more significantly correlated with other parameters in sweet potato callus healing. PAL and CAT were the most critical enzymes in phenylpropanoid and ROS metabolism, respectively, in terms of their effects on sweet potato callus formation. PAL and CAT activity levels were significantly correlated with suberin thickness. CAT activity was positively correlated with DPPH and superoxide anion scavenging as well as total phenol and total flavonoid production. PAL activity, lignin content, suberin thickness, and total phenol and total flavonoid production were also strongly positively correlated. The foregoing results suggest that CAT and PAL regulate ROS and phenylpropanoid metabolism, respectively; link these metabolic pathways; and improve sweet potato callus healing. In the study of sweet potato [22] and potato [23] on callus healing, it was pointed out that different physicochemical treatments can effectively stimulate the generation of reactive oxygen species in potato wounds, which can effectively promote potato root callus. In addition, Meng's [24] study on carrot pointed out that peroxidase in reactive oxygen species metabolism was the key enzyme in the carrot callus process and three peroxidases (DcPrx30, DcPrx32, and DcPrx62) were upregulated in the phloem of carrot.

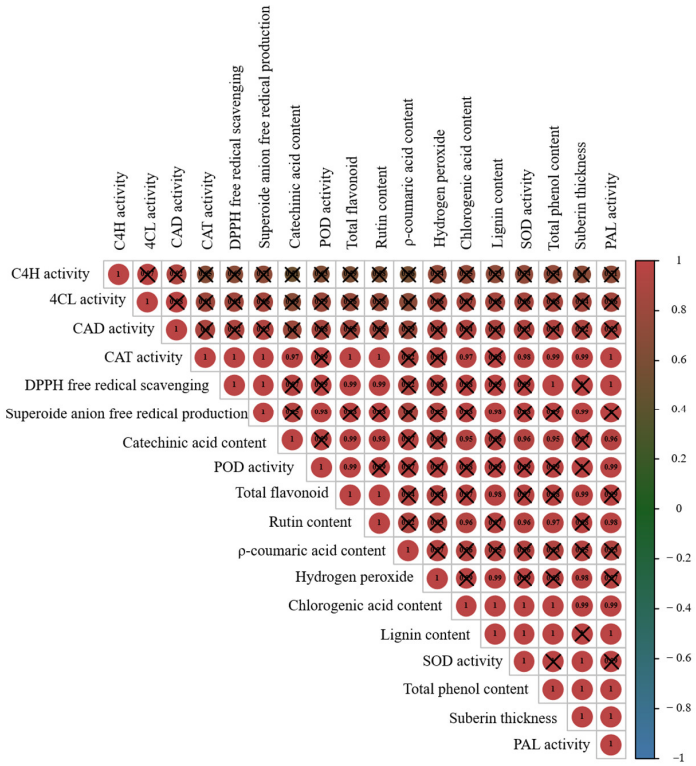

Figure 6. Correlation analysis of substances affecting sweet potato callus under different RHT treatments. Red: positive correlation. Blue: negative correlation. Numbers: significance of correlation. X: $p > 0.01$.

PAL and C4H are at the front end of phenylpropanoid metabolism and regulate phenolic compound biosynthesis [25]. Here, both enzymes were more strongly correlated with sweet potato callus than either 4CL or CAD. The latter enzymes were not significantly correlated with any index. In ROS metabolism, CAT and SOD scavenge free radicals [26]. Here they were significantly correlated with callus parameters. POD affects hydrogen peroxide production and was not significantly correlated with suberin stacking or lignin content. Thus, ROS production may not substantially contribute to sweet potato callus

induction. ROS were generated under RHT at 80 °C. Nevertheless, the influence of this treatment on callus formation was weaker than that of RHT at 60 °C. Phenolic compounds are key substrates in suberin stacking. Total phenols and flavonoids were strongly correlated with suberin stacking thickness. However, only the chlorogenic acid content was significantly correlated with suberin stacking thickness. Thus, chlorogenic acid may be the key phenolic compound affecting sweet potato callus. There were significant correlations among chlorogenic acid content, PAL and CAT activity, and DPPH scavenging.

3. Discussion

Callus is vital to the successful storage and transport of root crops. Root crops enter into direct contact with the soil during their growth and can transmit soil-borne microorganisms at harvest. Root crops often undergo severe decay during storage and transportation [27]. Postharvest callus formation involves cell proliferation and differentiation, signal transduction, disease resistance, secondary metabolism, and energy generation [7]. However, relatively few reports have been published on postharvest calli to date. Several studies indicated that ROS, fatty acid, and phenylpropanoid metabolic pathways occur in the calli of root crops such as white potato and sweet potato [28]. Hence, the enzymes associated with these pathways are implicated in the entire callus formation process.

ROS may act both as signal molecules and oxidants in white potato and sweet potato callus formation [29]. In the present study, ROS production responded rapidly when the sweet potatoes were subjected to RHT at 65 °C and RHT at 80 °C. Superoxide anion production capacity significantly increased in sweet potato callus subjected to RHT at 65 °C. Accumulation of superoxide anion, singlet oxygen, and other ROS disrupted active oxygen metabolism, which, in turn, activated SOD, CAT, POD, and other antioxidant enzymes in sweet potato. SOD then disproportionated the superoxide anions into H_2O_2. Therefore, RHT accelerated the induction of the key metabolic pathways associated with callus formation in damaged sweet potato tissue. Phenylpropanoid and fatty acid metabolism are the key pathways affecting sweet potato and white potato callus formation [30]. Phenylpropanoid metabolism induces the generation of numerous monophenols in injured plant tissues. Other enzymes catalyze the polymerization of these monophenols into polyphenol suberin (SPP). Monophenols also participate in lignin metabolism, which synthesizes lignin stacks around the calli [31]. In fatty acid metabolism, the damaged tissues produce numerous short-chain fatty acids (SCFAs) that are then polymerized to form suberin polyaliphatics (SPA). The terminal SPA in fatty acid metabolism and the SPD generated by phenylpropanoid metabolism participate in suberin polymerization. Suberin then deposits the final sealing callus layer known as the polyphenolic domain (SPPD) [32]. We found that RHT at 50 °C did not induce the rapid production of ROS such as superoxide anion and hydrogen peroxide and could not, therefore, initiate phenylpropanoid metabolism. Though RHT at 80 °C induced ROS production in the early sweet potato stages, it significantly inhibited CAT and SOD. Hence, RHT at 80 °C disrupted ROS production and clearance by days 3–5 and could not continuously promote callus formation in the wound tissue. In addition, ROS imbalance may cause fruit and vegetable browning [33]. We discovered substantial melanin accumulation in the callus surface of the sweet potato subjected to RHT at 80 °C. The melanin might have been derived from polyphenol oxidation.

Abundant hydrogen peroxide was produced under all RHT treatments and induced sweet potato callus formation through phenylpropanoid metabolism. RHT at 65 °C induced PAL and 4CL, and phenylpropanoid metabolism generated phenolic monomers. Zhu showed relatively higher PAL and 4CL activity and phenolic compound production around white potato wounds [33]. Compared with RHT at 50 °C and RHT at 80 °C, RHT at 65 °C significantly increased total flavonoid, rutin, chlorogenic acid, *p*-coumaric acid, and other phenolic compounds at days 0–5. At the appropriate temperatures, heat shock treatments induce phenylpropanoid metabolism and promote SPDD stacking. Compared with CK and RHT at 50 °C, RHT at 65 °C RHT and RHT at 80 °C significantly increased the lignin content. However, there were no significant differences among CK, RHT at 50 °C, RHT at

65 °C, or RHT at 80 °C in terms of lignin content by day 5. Therefore, RHT temperature may only be weakly correlated with lignin synthesis and accumulation. The terminal steps in lignin synthesis are associated with phenylpropanoid metabolism [34]. Santos stated that lignin synthesis may be correlated with low temperature, whereas lignin metabolism is relatively less affected by temperature fluctuation or high temperature [35]. Here, we measured the parameters of ROS and phenylpropanoid metabolism and found that both pathways induced sweet potato callus formation. Hence, there may be a strong correlation between these pathways in sweet potato callus.

Pearson's correlation analysis confirmed the foregoing speculations. Suberin stacking, CAT, PAL, and chlorogenic acid may be key factors in RHT-induced sweet potato callus formation. PAL is a key rate-limiting enzyme in phenylpropanoid metabolism. It catalyzes the deamination of *L*-phenylalanine to *trans*-cinnamic acid [36]. C4H then hydroxylates *trans*-cinnamic acid to *p*-coumaric, chlorogenic, ferulic, erucic, and other phenolic acids that may participate in SPPD formation. Furthermore, 4CL catalyzes the conversion of these phenolic acids to various phenolic acid-CoA. CAD then transforms the latter into lignin-derived substrates such as cinnamyl alcohol, coniferol, and sinucinol. At this stage, callus formation in sweet potato is complete [37]. The key metabolic pathways and key factors that RHT treatment can use to promote sweet potato callus are shown in Figure 7. The present study demonstrated that in sweet potato callus formation, ROS metabolism rapidly responds to RHT treatment and CAT produces and removes ROS. Abundant ROS induce PAL, which synthesizes and causes the accumulation of chlorogenic acid. Suberin is then quickly stacked at the sweet potato wound and promotes callus formation there.

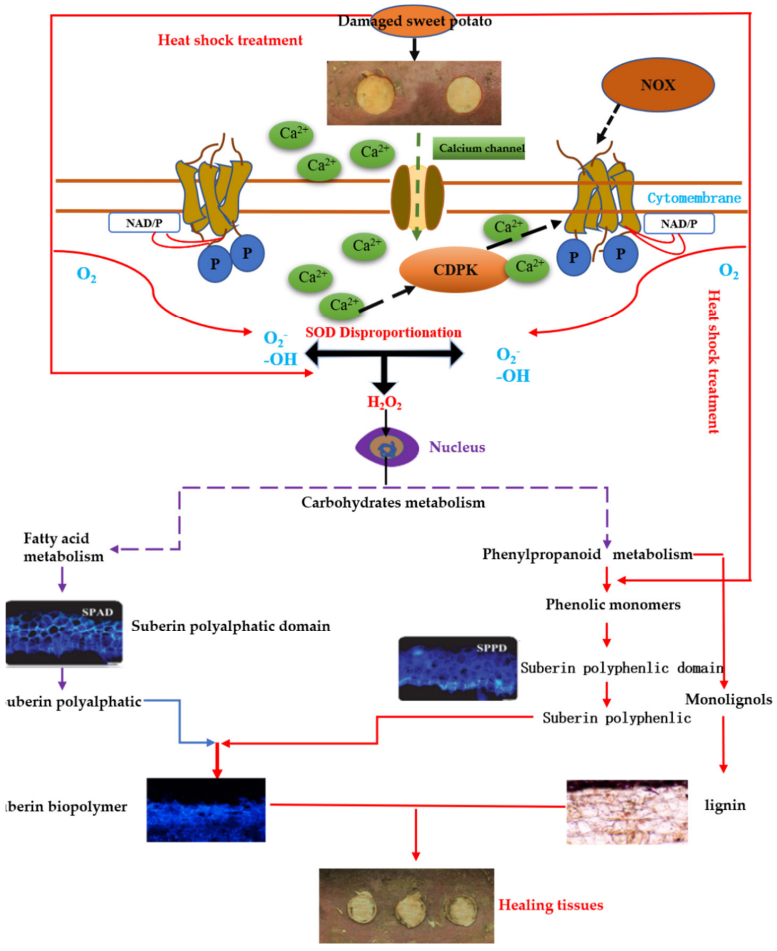

Figure 7. Pattern of metabolic pathways involved in callus of sweet potato tuber under heat shock treatment. CDPK: calcium-dependent protein kinase; NOX: NADPH oxidase; SOD: superoxide dismutase.

4. Materials and Methods

4.1. Sweet Potato Acquisition and Sample Preparation

Sweet potato (*Ipomoea batatas* (L.) Lam.). Xiguahong was purchased and transported from Fujian Province, China. Each tuber was packed in a bubble bag and transported within 1 day in a refrigerated truck (<13 ± 2 °C) from Fujian to Beijing. At the Beijing laboratory, tubers uniform in size; free of epicarp damage, disease, and insect pests; and weighing 250 ± 50 g were selected as the experimental materials.

4.2. Artificial Injury and Heat Shock Treatment of Sweet Potato Samples

The selected sweet potato roots were washed twice with tap water and distilled water and dried at room temperature (25 ± 1 °C). The knives (deli, Zhejiang, China) and hole (deli, Zhejiang, China) punches (15 mm diameter) used in the artificial wound experiment were disinfected with 95% (*v*/*v*) ethanol. Each tuber was perforated with the hole punches and 15 mm epicarp (deli, Zhejiang, China) disks were excised with the knives to a depth of 3 mm. Three wounds were artificially induced on two sides of each tuber.

The damaged sweet potatoes were arbitrarily divided into four groups. Unheated tubers served as the control (CK), whereas the other three groups were subjected to the RHT treatments.

The pretreatment method of sweet potato callus referred to the previous method in the laboratory [4]; the wounded tubers were placed in an independently designed sweet potato RHT machine and heated to 50 °C, 65 °C, or 80 °C for 15 min. After the RHT treatment, the tubers were cooled to 25 ± 1 °C and stored until the subsequent experiments.

All samples were transferred to cold storage (13 ± 1 °C; 55% RH) in the laboratory and wounds were allowed to heal for 5 days. The foregoing temperature and relative humidity were optimal for sweet potato storage [4]. The sample quantity of sweet potato in CK and the three RHT treatment groups were all more than 150. The sample healing preparation process was repeated thrice.

4.3. Lignin and Suberin Accumulation in Wounded Tissue

Sweet potato lignin staining was observed according to the method of Jiang with some modifications [6]. Tissue blocks with the injured surface were hand-sliced (0.4 mm–0.5 mm depth) vertically with a blade. 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 staining on a glass slide with a few drops of concentrated hydrochloric acid. After 5 min, images of red-stained deposited lignin were captured with a microscope (DM500, Leica Shanghai limited company, Shanghai, China) under 10 × magnification. Suberin deposition was microscopically detected by its autofluorescence according to Fugate et al. [20]. The autofluorescence of the suberin was analyzed using a microscope (DM500, Leica Shanghai limited company, Shanghai, China) with a fluorescence excitation filter at 280 nm and an emission filter at 620 nm. The prepared sections (0.3 mm–0.4 mm depth) were rinsed with distilled water 2–3 times before capturing images under 10× magnification. Six potato tubers for each group were used to observe staining and autofluorescence.

4.4. Determination of Lignin Content in Wound Callus of Sweet Potato

The determination method of lignin content by Zhou was improved [20]. At the end of the callus, samples were taken from the roots of sweet potato in each treatment group with a stainless-steel knife. During the determination, 1 g of frozen callus was taken, and 4 mL 95% ethanol precooled at 4 °C was added and beaten evenly with a beater. The callus was transferred to a 10 mL centrifuge tube and centrifuged at 4 °C at 10,000× *g* for 20 min. After the supernatant was discarded, 2 mL 95% ethanol was added, mixed, centrifuged (4 °C 10,000× *g* 10 min), and repeated 3 times, and then ethanol was used. After the precipitation was collected and dried to a constant weight, the dry matter was moved to a small test tube. Before the measurement, the water bath was placed in a fume hood in advance and the temperature was adjusted to 70 °C for preheating. First, 1 mL 25% acetyl bromide solution

was added to the test tube and mixed well. It was then immediately placed in a water bath for reaction for 30 min; 1 mL sodium hydroxide (2 mol·L^{-1}), 0.1 mL hydroxylamine hydrochloride (7.5 mol·L^{-1}), and 2 mL glacial acetic acid were added successively and centrifuged at 4 °C at 10,000× *g* for 20 min; and 0.5 mL supernatant was absorbed. The absorbance was measured at 280 nm with glacial acetic acid at a constant volume of 5 mL and repeated 3 times.

4.5. Determination of Phenylpropanoid Metabolism-Related Enzyme Activities in Sweet Potato Callus

The phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL) activity was evaluated spectrophotometrically according to the methodology of Jiang et al. [6] by using a UV–2600 spectrophotometer (Shimadzu, Kyoto, Japan), with some modifications. Protein (pro) content in the enzyme extract solution was measured by the Coomassie brilliant blue G250 method. The PAL, C4H, and 4CL activity was reported in U·Kg^{-1}.

Cinnamyl alcohol dehydrogenase (CAD) was determined by Sarni with some modification [38]. The 1.0 g frozen powder was homogenized in 3 mL TRIS-HCl buffer (pH 8.8, containing 40 g·L^{-1} PVP), 15 mmol·L^{-1} β-mercaptoethanol, 10% methylene, and 2% (*w*/*v*) PEG in an ice bath. After standing for 30 min and centrifugation at 12 000× *g* for 30 min (4 °C), the supernatant was a crude enzyme solution. Reaction system: 0.2 mL crude enzyme solution, 0.8 mL reaction solution (containing 10 mmol·L^{-1} nicotinamide adenine dinucleotide phosphorus (NADP) and 5 mmol·L^{-1} *trans*-cinnamic acid), water bath at 37 °C for 30 min, 1 mol·L^{-1} HCl to terminate the reaction (if precipitate exists, after centrifugation), absorbance value measured at 400 nm, and 0.2 mL PBS and 0.8 mL reaction solution used as control. The enzyme activity unit (U) was defined as 0.001 change of light absorption value per minute, and the CAD activity was expressed as U·Kg^{-1}.

4.6. Determination of Antioxidant Capacity and ROS Metabolism-Related Enzymes of Sweet Potato Callus

The activity of superoxide dismutase (SOD) was determined by azoblue tetrazole photoreduction method with some modifications [39]. The activity of catalase (CAT) was determined by colorimetric method [39]. Peroxidase (POD) activity was measured by referring to the method of Dastmalchi et al. [7]. The above enzyme activities were expressed by U·Kg^{-1}.

The determination of 1,1-dipheny1-2-picryl-hydrazyl (DPPH) clearance capacity referred to Oirschot and was modified [31]. A total of 5 g of frozen callus was taken and placed in a centrifuge tube (50 mL). The 20 mL ethanol (70%) solution was added and mixed, ultrasonic treatment was carried out for 1 h, centrifugation was carried out (4 °C 10,000× *g* 20 min), and the supernatant was taken as the extract. The 0.05 mL extract was absorbed and placed in a test tube. A total of mL DPPH ethanol solution with a concentration of 0.3 mmol·L^{-1} was first added and mixed. After a water bath at 30 °C for 1 h, the reaction was immediately cooled and terminated. DPPH radical scavenging capacity was calculated using trolox as standard equivalent, expressed in mg·kg^{-1}.

4.7. Determination of Phenols, Hydrogen Peroxide, and Superoxide Anion in Sweet Potato Callus

Extraction of phenolics was carried out according to Liu with some modifications [40]. Ten grams of sweet potato tissue were homogenized with liquid nitrogen, then mixed with 20 mL of 70% (*v*/*v*) ethanol and placed under ultrasound assisted at 30 °C for 0.5 h; thereafter, the mixture was centrifuged and the supernatant was collected and concentrated with a rotary vacuum evaporator (RE-52; Yarong Biochemistry Instrument Factory, Shanghai, China) at 30 °C. Identification and quantification of the phenolic compounds in the extract were carried out using an HPLC method (Liu et al., 2020) with Shimadzu liquid chromatography (pumps, LC-20AT; diode array detection, SPD-M20A) and an RP C18 column (Venusil ASB, 5 μm, 4.6 mm 9 250 mm; Agela Technologies Inc.,Tianjing, China). The operating conditions were as follows: mobile phase, 1% (*v*/*v*) acetic acid (A), and

methanol (B); gradient, 12–25% B from 0 min to 15 min, 25–35% B from 15 min to 25 min, 35–55% B from 25 min to 50 min, 55–65% B from 50 min to 60 min and 65–12% B from 60 min to 70 min; flow rate, 1.0 mL·min^{-1}; column temperature, 35 °C; injection volume, 20 L; and UV detection wavelength, 280 nm.

A total of 3.0 g of frozen sweet potato powder was extracted in 3 mL of cold acetone for 10 min before centrifugation at 4 °C and 10,000× *g* for 30 min; the supernatant was collected for H_2O_2 content determination. The reaction mixture contained 1 mL of supernatant, 100 μL of 20% $TiCL_4$, and 100 μL of concentrated ammonia. The mixture was reacted for 10 min and then centrifuged for 10 min. The resultant precipitate was washed three times with acetone and then dissolved with the addition of 2 mL of 1-mmol/L concentrated sulfuric acid. The OD value was measured at 410 nm, and H_2O_2 content was calculated from a standard curve. Thus, H_2O_2 content was expressed as mg·kg^{-1}.

Two grams of frozen callus were weighed and placed in a 10 mL centrifuge tube. The 5 mL 0.05 mol/L pH7.8 phosphoric acid buffer (containing 0.001 mol·L^{-1} ethylenediamine tetraacetic acid, 0.3% Triton X-100 and 2% polyvinylpyrrolidone) were added and mixed. Centrifugation (4 °C 10,000× *g* 20 min) was performed to collect supernatant. The content of superoxide anion (O^{2-}) was determined by using the Nanjing Jiancheng kit(Nanjing Jiancheng, Nanjing, China). A total of 0.8 mL supernatant was mixed with equal volume Tris-HCl buffer solution (50 mmol·L^{-1}, pH 8.2). The mixture was allowed to stand at 25 °C for 15 min, then 0.4 mL of 1.5 mmol·L^{-1} pyrogallic acid was added and thoroughly mixed. The absorbance value of the mixture was detected every 30 s at 550 nm, and the detection lasted for 5 min. Meanwhile, ascorbic acid was used as the positive control group, and 50 mmol·L^{-1} Tris-HCl buffer was used as the blank group. The content of superoxide anion in sweet potato was expressed as U·kg^{-1}.

4.8. Data Statistics and Analysis

The above experiments were repeated three times. Excel 2010 software (Microsoft Corporation, Washington, WA, USA) was used to make statistics on all data, calculate mean value and standard deviation, and plot. SPSS 26.0 software (IBM, New York, NY, USA) was used to conduct analysis of variance and multiple difference significance analysis of the experimental data; $p < 0.05$ indicated significant difference. Pearson's correlation heat map was analyzed and plotted using HIPLOT.

5. Conclusions

Damaged sweet potato tubers were subjected to RHT at 50 °C, 65 °C, and 80 °C. Changes in enzyme activity and intermediate accumulation in ROS and phenylpropanoid metabolism were evaluated. RHT at 65 °C significantly promoted callus formation in injured postharvest sweet potato root tubers. PAL in phenylpropanoid metabolism, CAT in ROS metabolism, and chlorogenic acid were the key factors inducing and developing callus in response to RHT treatment. The results of this study may provide theoretical and methodological bases for rapid callus induction in postharvest sweet potato. However, it remains to be established how PAL- and CAT-related genes respond as transduction signals to RHT treatment. Future research should determine how phenolic acids contribute to suberin stacking. Subsequent studies should aim to clarify the mechanisms by which heat shock promotes sweet potato callus and devise novel methods of reducing postharvest loss during sweet potato production, storage, and transport.

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