DNP and ATP regulate the pulp breakdown development in *Phomopsis longanae* Chi-infected longan fruit through modulating the ROS metabolism

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**ABSTRACT**

Compared with the *P. longanae*-infected longan, the DNP-treated *P. longanae*-infected fruit represented a higher pulp breakdown index, a higher O\(_2\) production rate, and a higher MDA content, but the lower activities of APX, SOD and CAT, the lower transcript levels of DlSOD1, DlSOD2, DlSOD3 and DlCAT1, the lower values of AsA, GSH, flavonoid and total phenolics, a lower scavenging ability of DPPH radical, and a lower value of reducing power. Whereas, the ATP-treated *P. longanae*-infected samples showed the contrary results. The above findings indicated that the DNP-promoted the pulp breakdown in *P. longanae*-infected longan was because DNP weakened the capacity of scavenging ROS, raised the O\(_2\) level, and accelerated the membrane lipids peroxidation. However, the ATP-suppressed the pulp breakdown in *P. longanae*-infected longan was because ATP improved the capacity of scavenging ROS, reduced the O\(_2\) level, and reduced the membrane lipids peroxidation.

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**1. Introduction**

Longan fruit has become one of the significant fruit in the world for its satisfaction of market value and quality attributes (Lin et al., 2014; Sun et al., 2022; Tang et al., 2021). However, the postharvest longan is storage life of fresh longan (Lin, Chen, et al., 2020; Sun et al., 2022). Specially, the pathogenic fungus-caused the pulp breakdown is one of the key factors to accelerate the quality deterioration of longan fruit (Chen et al., 2022). Furthermore, *Phomopsis longanae* Chi (*P. longanae*) is a dominant pathogenic fungus in postharvest fresh longan (Chen et al., 2014), and 28 °C is the optimum temperature for mycelial growth and spore germination of *P. longanae* (Zhang et al., 2013). The infection of *P. longanae* induces the pulp breakdown, and reduces the quality attributes of fresh longan (Chen et al., 2022). Hence, it is of great important to reveal the mechanisms of *P. longanae* infection-induced longan pulp breakdown, which help to develop an effective method for restraining the pathogen infection and reducing the pulp breakdown, and thus extend the storage time of fresh longan.

The metabolism of reactive oxygen species (ROS) plays an indispensable role in the quality properties of fresh produces (Xue et al., 2020). The dynamic equilibrium between ROS generation and ROS elimination can balance the ROS level, and thus avoid the oxidative damages and alleviate the membrane lipids peroxidation (Lin, Lin, Lin Fan, & Lin, 2021; Xue et al., 2020). ROS-scavenging enzymes (RSEs), such as CAT, APX and SOD (Lin et al., 2021), and antioxidant substances, including total phenolics, flavonoid, GSH, and AsA (Lin et al., 2021; Ng & Wang, 2021; Tang et al., 2021), play an important role in ROS metabolism. The abnormal ROS metabolism might disturb the ROS generation-elimination system, and then promote the ROS production and accelerate the membrane lipids peroxidation, and ultimately expedite the decay occurrence in fresh produces (Hao, Li, Xu, Hao, & Yang, 2019; Lin et al., 2021; Wei et al., 2019). On the contrary, the normal ROS...
metabolism could stabilize the levels of ROS generation-elimination, reduce the ROS level and membrane lipids peroxidation, and thus suppress the quality deterioration in fresh produces (Lin, Chen, et al., 2020; Lin, Lin, et al., 2020). Hence, the level of ROS metabolism was important for the quality attributes of fresh produces.

In addition, the energy level, which was crucial for the ROS metabolism, could regulate the ROS level and affect the quality attributes in fresh produces (Aghdam, Jannatizadeh, Luo, & Paliyath, 2018; He et al., 2021). Furthermore, pathogen infection might interfere the ROS metabolism and accelerate the massive accumulation of ROS, which promote the decay development in fresh products (Jiang et al., 2018). Our research showed that P. longanae infection might lower the ROS-scavenging capacity and increase the ROS production, and then lead to the decay development and shorten the storage time of fresh longan (Wang et al., 2018). Whereas, the energy level regulating the ROS metabolism is still unclear. Hence, the current work focused on the P. longanae-caused longan pulp breakdown and its relationship with the ROS metabolism, which is important for understanding the influence of two treatments of energy levels, DNP (an agent that inhibits energy production) and ATP, on the index of breakdown, the levels of O$_2^-$ or MDA, the activities of RSEs, the transcript levels of RSEs-related genes, the amounts of antioxidant substances, the DPPH radical scavenging ability, and the level of reducing power in pulp of P. longanae-inoculated fruit. The purposes of present work are to elucidate the role of ROS metabolism in P. longanae-induced longan pulp breakdown and its regulating mechanism, as well as provide the potential approach for suppressing the pathogen infection and reducing longan pulp breakdown, and thus extend the storage time of fresh longan.

2. Materials and methods

2.1. P. longanae spore suspension

The spore suspension of P. longanae was prepared referring to our previous procedure of Chen et al. (2014), and the final concentration of the above suspension with 1×10$^7$ spores L$^{-1}$ was obtained for the following inoculation.

2.2. Longan fruit and postharvest treatment

The longan fruit cv. Fuyan was picked from the longan orchard (Nan’an, Fujian, China) at the stage of commercial maturity (about 120 d after full bloom, and the values of chromaticity L*, a* and b* were 50.33 ± 0.16, 12.03 ± 0.22 and 28.66 ± 0.19, respectively). The gathered longans were shipped to our lab. The longans with uniformity in fruit integrity, shape, size, color, and without the visual blemish were selected. The selected longans were disinfected for 10 s with 0.5% (v/v) NaClO, then rinsed with sterile distilled water (SDW). The disinfected longans were used for the following treatment.

In preliminary experiment, the longan fruit were treated with 0 (the control), 0.1, 0.2, 0.3, 0.4, 0.5 or 0.6 mmol L$^{-1}$ DNP for 15 min, respectively, then stockpiled at 28°C. The experimental data indicated that there were not the obvious differences in the pulp breakdown index between longan fruit treated with 0 and 0.1 or 0.2 mmol L$^{-1}$ DNP. Furthermore, there were the notable differences in the index of pulp breakdown between 0 and 0.3, 0.4, 0.5, or 0.6 mmol L$^{-1}$ DNP-treated longan. Whereas, the 0.4 and 0.5 mmol L$^{-1}$ DNP-treated fruit, particularly the 0.6 mmol L$^{-1}$ DNP-treated fruit, exhibited a quick and serious development of pulp breakdown, which was not beneficial for assessing the pulp breakdown level and other related attributes. Thus, 0.3 mmol L$^{-1}$ DNP was used for the following experiment.

Additionally, the longan fruit were treated with 0 (the control), 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2 mmol L$^{-1}$ ATP for 15 min, respectively, then stockpiled at 28°C. The experimental data suggested that the 0.4 mmol L$^{-1}$ ATP-treated group displayed the lowest level of pulp breakdown than other groups. Hence, 0.4 mmol L$^{-1}$ ATP was chosen for the following experiment.

A total of 150 longans were employed for assessing fruit characteristics on storage day 0 (the harvest day). The remaining 12 000 longans were randomly divided into 4 groups with 3 000 longans each. Afterward, the two groups were soaked with SDW for 15 min, and the other two groups were soaked with the solution of 0.3 mmol L$^{-1}$ DNP and 0.4 mmol L$^{-1}$ ATP for 15 min, respectively, then the fruit were air-dried about one hour at 28°C, hereafter the treated-fruit were used for the following inoculation.

2.3. P. longanae inoculation

All the DNP-treated and ATP-treated longan, and one group of SDW-immersed longan were dipped into the above prepared spore suspension of P. longanae (1×10$^7$ spores L$^{-1}$) for 5 min, respectively. Another SDW-immersed group without the inoculation was used as the control group.

After the inoculation, all the fruit of the above-mentioned four groups were air-dried about one hour at 28°C, then were respectively packaged into the polyethylene film bags (15 μm thickness, fifty longan in each bag, 60 bags per group), afterwards stored at 28°C and 90% RH for the subsequent experiments. Three bags of longan were daily taken for assaying pulp breakdown index and the indicators related to the ROS metabolism.

2.4. Evaluation of pulp breakdown index

The 50 longan fruit were adopted to appraise the index of pulp breakdown referring to the detailed approach of Lin, Lin, et al. (2020).

2.5. Assay of O$_2^-$ production rate and MDA content

The pulp tissue (5 g) from 10 longans were adopted to assay the O$_2^-$ production rate and MDA content according to the detailed approaches of Lin et al. (2014) and Lin, Lin, et al. (2020). The above results were denoted as mmol kg$^{-1}$ min$^{-1}$ and μmol kg$^{-1}$, respectively.

2.6. Determination of RSEs activities

The pulp tissue (5 g) from 10 longans were employed to extract the enzyme solution of RSEs and measure the activities of RSEs, including SOD, APX and CAT, referring to the detailed means of Lin et al. (2014) and Lin, Lin, et al. (2020). The procedure of Bradford (1976) was adopted to assay the pulp protein content of the enzyme solution of RSEs. The results of the enzymes activities were denoted as U kg$^{-1}$ on the basis of protein mass.

2.7. Assay of antioxidant substances amounts

The pulp tissue (5 g) from 10 longans were adopted to assess the GSH and AsA contents basing on the previous detailed procedures of Lin et al. (2014) and Lin, Lin, et al. (2020). Results were indicated as g kg$^{-1}$.

The pulp tissue (5 g) from 10 longans were adopted to determine the amounts of total phenolics and flavonoid basing on the previous detailed means of Lin, Lin, et al. (2020), da Silva et al. (2020), and Mutlu-Ingok, Catakayla, Capanoglu, & Kubancoglu-Guler (2021). The g catechin equivalent (CE) kg$^{-1}$ and g gallic acid equivalent (GAE) kg$^{-1}$ were employed to represent the units of flavonoid and total phenolics, separately.

2.8. Determination of DPPH radical scavenging ability and reducing power

The pulp tissue (5 g) from 10 longans were adopted to assess the DPPH radical scavenging ability and reducing power basing on the detailed procedures of Lin, Lin, et al. (2020), Mutlu-Ingok et al. (2021).
Wang, Yang, & Li (2021), and Xiao, Xu, Lu, & Liu (2020). Their results were represented in % and g kg⁻¹, respectively.

### 2.9. RNA extraction

The longan pulp RNA was extracted using the TransZol Up RNA Kit (TransGen Biotech Co., Ltd., Beijing, China). The detailed protocols of Li, Zheng, et al. (2020) were employed to perform the concentration, integrity and quality of longan pulp RNA. The results found that, at storage days 0–4, the quality and integrity of longan pulp RNA were eligible for assaying the transcript levels. Whereas, on storage day 5, the extracted RNA of longan pulp were degraded seriously, which was not suitable for detecting the transcript levels.

### 2.10. Isolation and assay of RSEs-related genes

From the results of transcriptome sequencing, the full-length cDNA sequences of DlSOD, DlCAT and DlAPX in longan pulp were acquired, afterwards named as DlSOD1, DlSOD2, DlSOD3, DlCAT1 and DlAPX6 based on the phylogenetic features (Fig. S1-3). The genes of DlSOD, DlCAT and DlAPX corresponded to the sequence’s number of genome sequence library of longan (https://gigadb.org, GIGADB DATASEts), which were DlSOD1 (Dlo_030312.1), DlSOD2 (Dlo_030599.1), DlSOD3 (Dlo_014179.1), DlCAT1 (Dlo_028351.1) and DlAPX6 (Dlo_028545.1), respectively.

The purification and reverse transcription of RNA extractions, and the analyses of transcript levels of RSEs-related genes were assayed according to the previous detailed protocols of Li, Zheng, et al. (2020). The reference gene, DlActin (Dlo_013887.2), was used for the quantitative normalization. The primer sequences of DlSODs, DlCAT1 and DlAPX6 were designed via the software of Primer 5.0 (Table 1).

### 2.11. Bioinformatics analysis

The software of MEGA 5.0 was applied to draw the phylogenetic trees (Fig. S1-3). The software of DNAMAN 8 was adopted to assay the conserved sequences of amino acid sequences of DlSODs, DlCAT1 and DlAPX6 (Fig. S4-6). The website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was aligned the homologous sequences between the cDNA sequences of DlSODs, DlCAT1 and DlAPX6 and the other species (Table S1, Fig. S4-6). The website (https://web.expasy.org/protparam/) was applied for the analysis of the theoretical isoelectric point and mass value of DlSODs, DlCAT1 and DlAPX6 proteins (Table S2). In addition, the website (https://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) was employed to speculate the subcellular localization of the above proteins based on the translated amino acid sequences (Table S3).

### 2.12. Statistic analysis

The assessments of the above indicators were executed in triplicate. Each data in the figures were expressed as the mean ± standard error (n = 3). The SPSS software (IBM Corp., New York, USA) with the version 21.0 was used for assaying the experimental data.

### 3. Results

#### 3.1. Change in the index of pulp breakdown

The uprend of pulp breakdown index was shown in the four groups within the storage (Fig. 1A). Compared to the pulp breakdown index in control fruit, P. longanae-inoculated longan displayed a higher level, with the notable (P < 0.01) differences at days 2–5. In addition, compared to the pulp breakdown index in P. longanae-infected fruit, the DNP-treated P. longanae-infected fruit displayed a higher value, with the clear (P < 0.01) differences at days 2–5, but the ATP-treated P. longanae-infected group exhibited a lower level, with the prominent (P < 0.01) discrepancies at days 2–5.

Hence, P. longanae infection expedited the pulp breakdown development in postharvest fresh longan. Furthermore, DNP treatment exacerbated the above process, while ATP treatment restrained the above process.

#### 3.2. Changes in the levels of O₂⁻ and MDA

Fig. 1B exhibited that the production rate of pulp O₂⁻ in control group changed slightly at days 0–2, followed by a sharp increase at 2–3 d, then descended slowly at days 3–4, finally raised speedily within 4–5 d. But, the production rate of O₂⁻ in pulp of P. longanae-infected samples showed an increasing trend during storage. Furthermore, compared to pulp O₂⁻ production rate in control samples, P. longanae-infected samples displayed a higher level, with the prominent (P < 0.01) discrepancies at 1–5 d.

Fig. 1B also exhibited that O₂⁻ production rate in pulp of the DNP-treated P. longanae-infected group showed an increasing trend within storage. The rate of O₂⁻ production in pulp of the ATP-treated P. longanae-infected fruit rose sharply at 0–3 d, then declined speedily at 3–4 d, finally raised speedily at 4–5 d. Moreover, compared with the rate of O₂⁻ production in pulp of P. longanae-infected fruit, the ATP-treated P. longanae-infected fruit displayed a higher value, with the notable (P < 0.05) discrepancies within 1–5 d. But, the ATP-treated P. longanae-infected fruit showed a lower level, with the clear (P < 0.01) discrepancies on 1 d and at 3–5 d.

Fig. 1C indicated that pulp MDA amount in control group displayed the gradual uprend at 0–4 d, but exhibited the slow downtrend within 4–5 d. But, the pulp MDA content in P. longanae-infected samples increased sharply at 0–1 d, but dropped slightly at 1–2 d, and finally enhanced rapidly at 2–5 d. Moreover, compared to control fruit, P. longanae-infected group showed a higher pulp MDA level, with the clear (P < 0.01) discrepancies at 1–5 d.

Fig. 1C also indicated that the pulp MDA content in the DNP-treated P. longanae-inoculated samples raised sharply within 0–3 d, but reduced slowly at 3–4 d, finally increased rapidly at 4–5 d. While the pulp MDA content in the ATP-treated P. longanae-inoculated samples exhibited an increasing trend within 0–4 d, but changed slightly till 5 d. Furthermore, compared to the pulp MDA content of P. longanae-infected samples, the DNP-treated P. longanae-infected samples showed the clearly (P < 0.05) higher level at 1–5 d. Whereas, the ATP-treated P. longanae-infected samples displayed the conspicuously (P < 0.05) lower level at 3–5 d.

### Table 1

| Gene   | Forward primer (5′ to 3′)                                      | Reverse primer (5′ to 3′)                                      | Product (bp) |
|--------|--------------------------------------------------------------|--------------------------------------------------------------|--------------|
| DlSOD1 | GCCGTTGTCTCTCTCCACCA                                        | ACCCGCATGTCGACTTCTCAT                                         | 198          |
| DlSOD2 | ACCAGCCTCTTCATGCTTCCA                                        | CAGCATGCGCGCTCTGATCG                                          | 128          |
| DlSOD3 | GGCTACGGCGGCGAAAATCAT                                        | CGCCGTGAACCTTGATGCCA                                          | 157          |
| DlCAT1 | ACCCTTGCGCCAGGACAGACA                                        | GACTTTCGTCGGGCTGCAGACA                                        | 122          |
| DlAPX6 | GCCAATGCGCGCTCTGGA                                          | CTTCCGGCACTGTCGAGT                                             | 184          |
| DlActin| TGGTGTCCACACATGTCGCCG                                          | ATGCCGACAGTGCTCATCAC                                           | 203          |
Hence, *P. longanae* infection enhanced the levels of pulp \( \text{O}_2^- \) and MDA in harvested fresh longan. Furthermore, during storage, DNP treatment exacerbated the raised levels of pulp \( \text{O}_2^- \) and MDA in *P. longanae*-infected fruit, while ATP treatment retarded these processes.

### 3.3. Changes of RSEs activities

Fig. 2A exhibited that the pulp SOD activity of control group increased slightly at 0–1 d, then dropped sharply at 1–2 d, afterwards rose quickly at 2–4 d, finally decreased slightly at 4–5 d. But, the pulp SOD activity in *P. longanae*-inoculated fruit rose speedily within 0–1 d, followed by a slow decrease at 1–2 d, then increased quickly at 2–3 d, afterwards declined slowly at 3–4 d, finally dropped sharply till 5 d. In addition, *P. longanae*-inoculated group displayed the remarkably (\( P < 0.05 \)) higher activity of pulp SOD than control fruit at 1–4 d.

Fig. 2A also exhibited that the pulp SOD activity in the DNP-treated *P. longanae*-inoculated group raised slowly within 0–1 d, then dropped gradually at 1–2 d, followed by a gradual increase till 4 d, but declined sharply within 4–5 d. But, the pulp SOD activity in the ATP-treated *P. longanae*-inoculated group raised speedily during 0–1 d, then rose slightly at 1–2 d, afterwards went up quickly at 2–3 d, followed by a slow increase at 1–4 d, and dropped sharply at 4–5 d. Furthermore, compared to the pulp SOD activity in *P. longanae*-inoculated group, the DNP-treated *P. longanae*-infected group showed a lower activity, with the clear (\( P < 0.05 \)) differences within 2–5 d. But, the ATP-treated *P. longanae*-infected samples displayed a higher activity, with the notable (\( P < 0.05 \)) discrepancies at 1–5 d.

The results were also exhibited in Fig. 2E, the pulp CAT activity in control samples raised slightly at 0–1 d, then reduced speedily at days 1–2, followed by a quick increase at 2–3 d, but reduced slightly within 3–4 d, then rose slowly at 4–5 d. While, the pulp CAT activity in *P. longanae*-inoculated fruit raised quickly at 0–1 d, but dropped slightly within 1–2 d, then went up sharply at 2–3 d, finally declined sharply till 5 d. Furthermore, compared to the pulp CAT activity in control fruit, *P. longanae*-infected fruit showed the clearly (\( P < 0.01 \)) higher level at days 1–3, while displayed a notably (\( P < 0.01 \)) lower activity on 5 d.

The results were also exhibited in Fig. 2E, the ATP cat activity in the DNP-treated *P. longanae*-infected group rose speedily at 0–1 d, but decreased rapidly at 1–2 d, then enhanced speedily at 2–3 d, but dropped sharply till 5 d. While the pulp CAT activity in the ATP-treated *P. longanae*-infected group exhibited the sharp uptrend till day 3, but the quick downtrend till day 5. Furthermore, compared to the pulp CAT activity in *P. longanae*-infected fruit, the DNP-treated *P. longanae*-inoculated group displayed a lower value, with the notable (\( P < 0.05 \)) discrepancies within 2–5 d. Whereas, the ATP-treated *P. longanae*-infected fruit showed a higher level, with the clear (\( P < 0.05 \)) differences at 2–5 d.

Results were exhibited in Fig. 2G, the pulp APX activity in control samples raised speedily at 0–1 d, but dropped slightly at 1–2 d, then increased slowly till 4 d, finally decreased gradually at 4–5 d. But, the pulp APX activity in *P. longanae*-infected fruit raised speedily at 0–1 d, then dropped slowly within 1–2 d, afterward rose gradually at 2–3 d, but reduced sharply till 5 d. Moreover, *P. longanae*-inoculated group exhibited the notably (\( P < 0.05 \)) lower pulp APX level than the control group at 4–5 d.

Results were also exhibited in Fig. 2G, the pulp APX activity in the DNP-treated *P. longanae*-inoculated group changed slightly at 0–2 d, then reduced slowly at 2–3 d, afterward rose gradually at 3–4 d, finally reduced speedily till 5 d. But, the pulp APX activity in the ATP-treated *P. longanae*-inoculated fruit raised sharply at 0–1 d, then increased quickly at 1–3 d, afterwards declined speedily at 3–4 d, finally enhanced slowly during 4–5 d. Furthermore, compared to the pulp APX activity in *P. longanae*-infected samples, the DNP-treated *P. longanae*-inoculated group manifested a lower level, with the conspicuous (\( P < 0.05 \)) discrepancies at 1–5 d. But, the ATP-treated *P. longanae*-infected group showed a higher level, with the prominent (\( P < 0.05 \)) discrepancies.
Fig. 2. Effects of DNP and ATP on the activities of SOD (A), CAT (E) and APX (G), and the transcript levels of DlSOD1 (B), DlSOD2 (C), DlSOD3 (D), DlCAT1 (F) and DlAPX6 (H) in pulp of P. longanae-inoculated longan. Value in the figure represented as mean ± standard error (n = 3), vertical bar indicated the standard error. Compared to the P. longanae-inoculated longan, at the same storage day, the remarkable discrepancies in the control longan, in the DNP-treated P. longanae-inoculated longan, and in the ATP-treated P. longanae-inoculated longan are respectively indicated by * (P < 0.05) or ** (P < 0.01), (P < 0.05) or (P < 0.01), and (P < 0.05) or (P < 0.01). ○, control group; ●, P. longanae-inoculated group; ■, DNP + P. longanae-inoculated group; ▴, ATP + P. longanae-inoculated group.
within 1–5 d.

Hence, *P. longanae* infection displayed the higher activities of pulp RSEs (SOD, APX and CAT) in fresh longan at the early and middle stages of storage, but the lower levels of pulp RSEs of fresh longan at the late stage of storage. Moreover, compared to *P. longanae*-inoculated fruit, within storage, DNP treatment lowered the pulp RSEs activities in *P. longanae*-infected longan, while ATP treatment raised the pulp RSEs activities in *P. longanae*-infected longan.

### 3.4. Changes in the transcript levels of RSEs-related genes

Fig. 2B indicated that the transcript level of pulp *DlSOD1* in control group dropped quickly at 0–2 d, then enhanced rapidly after two storage days. But, the pulp *DlSOD1* transcript level in *P. longanae*-infected samples rose slightly within 0–1 d, then decreased slowly at 1–2 d, afterwards increased speedily within 2–3 d, but reduced sharply at 3–4 d. Moreover, *P. longanae*-inoculated fruit displayed the conspicuously (*P* < 0.01) higher pulp *DlSOD1* transcript level than control fruit within 1–3 d.

Fig. 2B also indicated that the transcript level of pulp *DlSOD1* in the DNP-treated *P. longanae*-inoculated group dropped quickly at 0–1 d, then rose slowly at 1–2 d, finally decreased rapidly till 4 d. While, the transcript level of pulp *DlSOD1* in the ATP-treated *P. longanae*-infected fruit rose rapidly at 0–3 d, but dropped sharply at 3–4 d. In addition, compared to the transcript level of pulp *DlSOD1* in *P. longanae*-infected samples, the DNP-treated *P. longanae*-inoculated group exhibited the conspicuously (*P* < 0.05) lower level within 1–4 d. But, the ATP-treated *P. longanae*-inoculated group rose sharply within 1–4 d.

Fig. 2C also indicated that the transcript level of pulp *DlSOD2* in *P. longanae*-infected fruit displayed the sharp uprend at 0–1 d, but the quick downturn till 4 d. Furthermore, compared to the transcript level of pulp *DlSOD2* in control group, *P. longanae*-inoculated fruit showed the remarkably (*P* < 0.05) higher level within 1–4 d.

Fig. 2D also indicated that the transcript level of pulp *DlSOD3* in control group dropped speedily at 0–2 d, but increased quickly at 2–3 d, then decreased slowly at 3–4 d. But, the pulp *DlSOD3* transcript level in *P. longanae*-infected fruit exhibited the quick uprend at 0–1 d, but the rapid downturn at 1–4 d. Furthermore, *P. longanae*-inoculated group displayed the conspicuously (*P* < 0.05) higher pulp *DlSOD3* transcript level than control group at 2–3 d.

Results were exhibited in Fig. 2F, the pulp *DlCAT1* transcript level in control samples raised slightly at 0–1 d, but declined quickly at 1–2 d, then raised sharply till 4 d. But, the pulp *DlCAT1* transcript level in *P. longanae*-infected fruit rose sharply at 0–2 d, but reduced speedily at 2–4 d. Furthermore, *P. longanae*-inoculated group displayed the notably
(P < 0.01) higher pulp DlCAT1 transcript level than control longan at 2–3 d.

Results were also indicated in Fig. 2F, the pulp DlCAT1 transcript level in the DNP-treated P. longanae-infected group rose gradually within 0–1 d, then dropped speedily at 1–3 d, finally went up slowly within 4–5 d. While, the pulp DlCAT1 transcript level in the ATP-treated P. longanae-infected group enhanced sharply within 0–1 d, then dropped slowly at 1–2 d, afterwards rose gradually at 2–3 d, finally reduced speedily till 4 d. Furthermore, compared to the pulp DlCAT1 transcript level in P. longanae-infected group, the DNP-treated P. longanae-infected fruit displayed the clearly (P < 0.01) lower level at 2–3 d. But, the ATP-treated P. longanae-infected group showed the conspicuously (P < 0.05) higher level on 1 d and at 3–4 d.

Fig. 3H displayed that the pulp DlAPX6 transcript level in control fruit dropped slowly at 0–1 d, then rose rapidly at 1–3 d, finally decreased speedily until 4 d. However, the transcript level of pulp DlAPX6 in P. longanae-infected group went up sharply at 0–1 d, but reduced rapidly till 4 d. In addition, compared to the pulp DlAPX6 transcript level in control fruit, P. longanae-infected group showed a conspicuously (P < 0.01) higher level on 1 d, but displayed a clearly (P < 0.01) lower value on 4 d.

Fig. 2H also displayed that the pulp DlAPX6 transcript level in the DNP-treated P. longanae-infected fruit declined quickly at 0–3 d, but rose slowly till 4 d. Whereas, the pulp DlAPX6 transcript level in the ATP-treated P. longanae-infected samples raised sharply within 0–1 d, then reduced slowly at 1–2 d, afterwards rose quickly at 2–3 d, but decreased sharply within 3–4 d. Furthermore, compared with the DlAPX6 transcript level in pulp of P. longanae-infected group, the DNP-treated P. longanae-infected fruit displayed the clearly (P < 0.01) lower level at 1–4 d. But, the ATP-treated P. longanae-infected fruit showed the conspicuously (P < 0.05) higher level on 1 d and at 3–4 d.

Hence, P. longanae infection up-regulated the transcript levels of DlSOD1, DlCAT1 and DlAPX6 at the early and middle stages of storage, but down-regulated the transcript levels of these genes in pulp of fresh longan at the late stage of storage. Besides, P. longanae infection up-regulated the transcript levels of pulp DlSOD2 and DlSOD3 in fresh longan within the whole storage period. Moreover, compared to P. longanae-inoculated fruit, during storage, DNP treatment down-regulated the transcript levels of pulp RSEs-related genes in P. longanae-infected longan, while ATP treatment up-regulated the transcript levels of pulp RSEs-related genes in P. longanae-infected longan.

3.5. Changes in the amounts of antioxidant substances

Fig. 3A indicated that the pulp AsA content in control group dropped slowly at 0–5 d. But, the AsA content in pulp of P. longanae-infected samples exhibited a quick downturn till 5 d. In addition, compared to the pulp AsA content in control fruit, P. longanae-infected fruit showed the notably (P < 0.01) lower level at 1–5 d.

Fig. 3A also indicated that the pulp AsA content in the DNP-treated P. longanae-infected samples exhibited the sharp downturn at 0–5 d. While, the pulp AsA content in the ATP-treated P. longanae-infected samples dropped speedily at 0–1 d, then changed slightly at 1–2 d, afterwards decreased slowly at 3–5 d. Furthermore, compared with the pulp AsA amount in P. longanae-infected fruit, the DNP-treated P. longanae-infected samples displayed the remarkably (P < 0.01) lower content at 3–4 d; but, the ATP-treated P. longanae-infected samples showed the notably (P < 0.01) higher amount at 3–5 d.

Fig. 3B indicated that the pulp GSH amount in control samples exhibited the slow downturn at 0–4 d, then decreased quickly till 5 d. But, the GSH amount in pulp of P. longanae-infected samples dropped speedily at 0–4 d, and reduced sharply at 4–5 d. In addition, compared to the pulp GSH amount in control fruit, P. longanae-infected fruit displayed the clearly (P < 0.05) lower level at 1–5 d.

Fig. 3B also displayed that the pulp GSH amount in the DNP-treated P. longanae-infected fruit declined sharply within 0–2 d, then changed slightly 2–3 d, finally reduced speedily till 5 d. While, the pulp GSH amount in the ATP-treated P. longanae-infected fruit exhibited the quick downturn within storage. Furthermore, compared with the pulp GSH content in P. longanae-infected samples, the DNP-treated P. longanae-infected group displayed the remarkably (P < 0.01) lower content at 1–4 d; while, the ATP-treated P. longanae-infected fruit showed the notably (P < 0.01) higher content at 2–3 d and on 5 d.

Fig. 3C exhibited that the flavonoid content in pulp of control fruit dropped quickly at 0–3 d, but changed slightly at 3–4 d, hereafter declined slowly till 5 d. But, the flavonoid content in pulp of P. longanae-infected samples reduced sharply within 0–3 d, then increased slightly at 3–4 d, finally dropped slowly till 5 d. Furthermore, compared to the flavonoid content in pulp of control fruit, P. longanae-infected fruit displayed the notably (P < 0.05) lower level at 2–5 d.

Fig. 3C also exhibited that the pulp flavonoid content in the DNP-treated P. longanae-infected samples displayed the sharp downturn throughout storage. But, the pulp flavonoid amount in the ATP-treated P. longanae-infected fruit dropped quickly at 0–3 d, but changed sharply 3–4 d, hereafter declined slowly till 5 d. But, the flavonoid content in pulp of P. longanae-infected samples reduced sharply within 0–3 d, then increased slightly at 3–4 d, finally dropped slowly till 5 d.
P. longanae-infected samples raised slightly at 0–1 d, but dropped quickly at 1–4 d, hereafter changed slightly at 4–5 d. Besides, compare to the pulp flavonoid content in P. longanae-infected fruit, the DNP-treated P. longanae-infected samples displayed the clearly (P < 0.05) lower value within 2–5 d, but, the ATP-treated P. longanae-infected fruit showed the conspicuously (P < 0.01) higher content at 2–3 d.

Fig. 3D indicated that the pulp total phenolics amount in control group reduced speedily at 0–1 d, but rose rapidly at 1–2 d, then dropped speedily within 2–3 d, afterwards enhanced slowly during 3–4 d, finally declined sharply till 5 d. Whereas, the pulp total phenolics content in P. longanae-infected fruit dropped speedily at 0–5 d. Furthermore, compared to the pulp total phenolics content in control group, P. longanae-infected fruit displayed the clearly (P < 0.05) lower level at 2–5 d.

Fig. 3D also indicated that the pulp total phenolics content in the DNP-treated P. longanae-infected fruit reduced sharply within 0–3 d, then decreased slowly till 5 d. But, the pulp total phenolics content in the ATP-treated P. longanae-infected longan increased slightly at 0–1 d, then decreased sharply at 1–3 d, but rose speedily at 3–4 d, then reduced quickly till 5 d. Moreover, compared with the total phenolics content in pulp of P. longanae-infected samples, the DNP-treated P. longanae-infected fruit showed the clearly (P < 0.05) lower level within 2–3 d; but, the ATP-treated P. longanae-infected fruit displayed the remarkably (P < 0.05) higher value at 3–4 d.

Hence, P. longanae infection accelerated the decreases in contents of antioxidant substances (GSH, flavonoid, AsA, and total phenolics) in pulp of postharvest fresh longan. Furthermore, during storage, DNP treatment exacerbated the decline of pulp antioxidant substances in P. longanae-infected fruit, while ATP treatment retarded these processes.

3.5. Changes in DPPH radical scavenging ability and reducing power

Fig. 4A indicated that pulp DPPH radical scavenging ability in control fruit raised slightly at 0–1 d, but displayed the gradual downtrend at 1–5 d. However, DPPH radical scavenging ability in P. longanae-infected fruit dropped rapidly at 0–3 d, but increased slowly at 3–4 d, then dropped speedily within 4–5 d. Besides, DPPH radical scavenging ability in P. longanae-infected fruit showed the prominently (P < 0.01) lower level than control fruit at 1–5 d.

Fig. 4A also exhibited that pulp DPPH radical scavenging ability in the DNP-treated P. longanae-infected samples dropped speedily during storage. But, DPPH radical scavenging ability in the ATP-treated P. longanae-infected samples reduced sharply at 0–1 d, but rose slightly at 1–2 d, then decreased quickly till 5 d. In addition, compared to the pulp DPPH radical scavenging ability in P. longanae-infected fruit, the DNP-treated P. longanae-infected fruit showed the prominently (P < 0.05) lower value at 3–5 d; while, the ATP-treated P. longanae-infected fruit displayed the conspicuously (P < 0.05) higher value at 1–5 d.

Fig. 4B indicated that pulp reducing power in control group dropped speedily during 0–4 d, but changed slightly within 4–5 d. While, the pulp reducing power in P. longanae-infected longan reduced speedily at 0–4 d, but rose slowly till 5 d. Besides, P. longanae-infected displayed the clearly (P < 0.01) lower pulp reducing power than control samples at days 1–5.

Fig. 4B also indicated that the pulp reducing power in the DNP-treated P. longanae-infected fruit decreased sharply at 0–3 d, but changed slightly within 3–4 d, finally dropped speedily within 4–5 d. However, the pulp reducing power in the ATP-treated P. longanae-infected fruit reduced speedily throughout the storage period. In addition, compared with the pulp reducing power in P. longanae-infected group, the DNP-treated P. longanae-infected fruit showed the remarkably (P < 0.05) lower value at 3–5 d; while, the ATP-treated P. longanae-infected fruit displayed a clearly (P < 0.01) higher level on 4 d.

Hence, P. longanae infection accelerated the decreases in DPPH radical scavenging ability and reducing power in pulp of postharvest fresh longan. Furthermore, during storage, DNP treatment exacerbated the decline of pulp DPPH radical scavenging ability and reducing power in P. longanae-infected fruit, while ATP treatment retarded these processes.

4. Discussion

4.1. DNP and ATP regulate the levels of O2\textsuperscript{-} and MDA in P. longanae-infected longan and their relation to pulp breakdown

The excessive generation of ROS can disorder the ROS metabolism, that is ROS production-elimination system, and then damage the cell membranes, and expedite the decay symptom of postharvest produces (Hao, Li, Xu, Huo, & Yang, 2019; Tang et al., 2021). The balanceable ROS metabolism can result in the reduced ROS level, the lower membrane lipids peroxidation, the mitigated damage of cell membranes, and the enhanced disease resistance (Tang et al., 2021), which can be adjusted by the ATP level (Lin et al., 2017). The O2\textsuperscript{-} production rate and MDA level are the vital indicators to estimate the levels of ROS and membrane lipids peroxidation, respectively (Lin et al., 2021; Sun et al., 2018). Moreover, the small level of ROS generated at early stage in host can serve as the signal molecule to stimulate the defense responses, and then resist the pathogen infection (Xue et al., 2020).

Some works indicated that the treatment of hydrogen peroxide (Lin et al., 2021) or the infection of pathogen (Sun et al., 2018) could lower the quality properties in fresh produces, which was owing to the higher levels of ROS and MDA. But, the treatments of chitosan (Jiang et al., 2018; Lin, Chen, et al., 2020), or hot water (Huan et al., 2017) could retain the quality attributes of fresh products, which was in connection with the lower ROS content and the reduced MDA amount. Therefore, the levels of ROS and MDA might affect the postharvest quality of fresh products.

In this study, during storage, the P. longanae-infected longan displayed the higher O2\textsuperscript{-} production rate (Fig. 1B), the higher MDA amount (Fig. 1C), and the higher breakdown index (Fig. 1A) in pulp than control longan. The correlation analyses manifested that, for P. longanae-inoculated samples, a raised pulp breakdown index (Fig. 1A) was clearly positively correlated with an increased O2\textsuperscript{-} production rate (Fig. 1B) and an enhanced MDA amount (Fig. 1C) in pulp, with the correlation coefficient r values of 0.832 and 0.901, respectively. Therefore, P. longanae infection might accelerate the ROS generation, and then aggravate the membrane lipids peroxidation, and thus destroy the cellular membranes structure, accordingly facilitate the longan pulp breakdown occurrence.

Additionally, in comparison with P. longanae-infected group, within storage, the DNP-treated P. longanae-infected group showed the higher rate of O2\textsuperscript{-} production (Fig. 1B), the higher amount of MDA (Fig. 1C), and the higher index of breakdown (Fig. 1A) in pulp. On the contrary, during storage, there were the lower O2\textsuperscript{-} production rate (Fig. 1B), the lower MDA amount (Fig. 1C), and the lower breakdown index (Fig. 1A) in pulp of the ATP-treated P. longanae-infected samples. Thus, the above results implied that DNP treatment for stimulating the pulp breakdown incidence in P. longanae-infected fruit was resulted from the DNP-accelerated the ROS generation, and then promoted the membrane lipids peroxidation, and thus damaged the cellular membranes. But, ATP treatment for mitigating pulp breakdown symptom in P. longanae-infected fruit was associated with ATP-suppressed the production of ROS, and thus reduced the peroxidation of membrane lipids, accordingly stabilized the structure of cell membranes.

4.2. DNP and ATP regulate the activities of RSEs in P. longanae-infected longan and their relation to pulp breakdown

The RSEs contain CAT, SOD and APX, which can regulate the levels of ROS generation and removal, and hereafter scavenge ROS, and lower ROS accumulation (Lin, Chen, et al., 2020). SOD is responsible for catalyzing O2\textsuperscript{-} to H2O2 by dismutation (Xue et al., 2020). An increment of SOD activity may lead to the decrease of O2\textsuperscript{-} amount (He et al., 2021;
Lin, Lin, et al., 2020). The degradation of H$_2$O$_2$ to H$_2$O may be acquired through the actions of CAT and APX (He et al., 2021). Furthermore, the RSEs activities can be regulated by the energy level (He et al., 2021; Lin et al., 2017; Liu et al., 2017).

Previous reports indicated that the pathogen-induced the increased RSEs activities might contribute to eliminate the excess ROS in fresh produces at the early stage of pathogen infection. Nevertheless, the RSEs activities reduced with the prolonged storage time, resulting in the raised ROS level, the increased membrane lipids peroxidation, and the lower quality in fresh products (Jiang et al., 2018; Sun et al., 2018). Moreover, the treatments of hydrogen peroxide (Lin et al., 2021) or acibenzolar-S-methyl (ASM) + dehydroepiandrosterone (DHEA) (Wei et al., 2019) might lower the RSEs activities, and then promote the ROS accumulation, and thus accelerate the decay development of harvested produces. But, the treatments of acetylsalicylic acid (ASA) (Xue et al., 2020), melatonin (Zhang et al., 2018), or propyl gallate (Lin et al., 2015) could raise the activities of RSEs, resulting in the lower level of ROS, and then maintain the quality of harvested products. Therefore, the RSEs activities played a vital role in regulating the ROS level, then affected the quality of postharvest products.

In current work, compared with control group, at the early and middle stages of storage, P. longanae-infected fruit displayed the higher activities of pulp RSEs including SOD, CAT and APX (Fig. 2A, E, G), which might be resulted from the pathogen infection-induced oxidative burst, that was the rapid increase of ROS like O$_2^–$ (Fig. 1B). These results implied that the P. longanae infection-increased the RSEs activities were beneficial for eliminating the excrecent ROS at the early and middle stages of storage. Whereas, during the late stage of storage, P. longanae-infected group exhibited the lower pulp RSEs activities (Fig. 2A, E, G), but the higher values of pulp O$_2^–$ (Fig. 1B) and MDA (Fig. 1C), and the higher pulp breakdown index (Fig. 1A) than control group. These results revealed that the P. longanae infection decreased the activities of RSEs (SOD, APX, CAT) to reduce the ROS-scavenging level, and enhanced the ROS level, and then aggravated the membrane lipids peroxidation and injured the cell membranes, and thus promoted the longan pulp breakdown.

Furthermore, compared with P. longanae-treated group, the DNP-treated P. longanae-infected samples manifested the lower activities of RSEs such as SOD, CAT and APX (Fig. 2A, E, G), but an enhanced O$_2^-$ production rate (Fig. 1B), a higher content of MDA (Fig. 1C), and a higher index of breakdown (Fig. 1A) in pulp within the storage. Whereas, the ATP-treated P. longanae-infected samples displayed the opposite results (Figs 1, 2A, E, G). These results implied that DNP treatment accelerated the P. longanae-infuced pulp breakdown of longan, which was because DNP lowered the RSEs activities to decrease the ROS-scavenging capacity, and then raised the ROS level, and thus facilitated the membrane lipids peroxidation and accelerated the cellular membranes damage. On the contrary, ATP treatment suppressed the P. longanae-caused longan pulp breakdown, which was due to ATP-increased the RSEs activities to enhance the ROS-scavenging level, and afterwards lowered the ROS production, and eventually alleviated the membrane lipids peroxidation and stabilized the cellular membranes structure.

4.3. DNP and ATP regulate the transcript levels of RSEs-related genes in P. longanae-infected longan and their relation to pulp breakdown

The levels of RSEs activities including APX, SOD and CAT can be regulated by the transcript levels of corresponding genes such as APX, SOD and CAT, and then affect the scavenging level of ROS (Chen et al., 2021; Lin et al., 2021). Some literatures indicated that the treatments of hydrogen peroxide (Lin et al., 2021) or ASM + DHEA (Li, Wei, et al., 2020) could down-regulate the transcript levels of RSEs-related genes, resulting in the lower activities of RSEs, and then led to the ROS accumulation and accelerated the quality loss in harvested fresh produces. Whereas, the treatments of nitric oxide (Zhang et al., 2019), propyl gallate (Lin, Lin, et al., 2020), sodium hydrosulphide (Liu et al., 2017) or ASM (Li, Wei, et al., 2020; Wei et al., 2019) could up-regulate the transcript levels of RSEs-related genes to enhance the RSEs activities, and ultimately reduced the ROS generation and stabilized the quality in harvested fresh produces. Hence, the transcript levels of RSEs-related genes could regulate the RSEs activities, which affected the ROS level and the quality in postharvest fresh products.

In current work, compared with control group, at the early and middle stages of storage, P. longanae-infected fruit displayed the higher transcript levels of pulp RSEs-related genes containing DISOD1, DISOD2, DISOD3, DlCAT1 and DlAPX6 (Fig. 2B-D, F, H), resulting from the P. longanae infection-induced the oxidative burst (Fig. 1B), which induced the boosted activities of RSEs (Fig. 2A, E, G) to eliminate ROS. Whereas, at the late stage of storage, P. longanae-infected samples showed the lower transcript levels of pulp DISOD1, DlCAT1 and DlAPX6 (Fig. 2B, F, H), the lower activities of pulp RSEs (Fig. 2A, E, G), but a higher producing rate of pulp O$_2^–$ (Fig. 1B), a higher level of pulp MDA (Fig. 1C), and a higher pulp breakdown index (Fig. 1A). These data indicated that P. longanae infection down-regulated the transcript levels of DISOD1, DlCAT1 and DlAPX6, which decreased the RSEs activities, and weakened the ROS-scavenging capacity, and thus enhanced the ROS content and aggravated the membrane lipids peroxidation, in turn, facilitated the longan pulp breakdown.

Moreover, compared to P. longanae-infected fruit, within storage, the DNP-treated P. longanae-infected samples showed the lower transcript levels of RSEs-related genes including DISOD1, DISOD2, DISOD3, DlCAT1 and DlAPX6 (Fig. 2B-D, F, H), the lower activities of RSEs (SOD, CAT, APX) (Fig. 2A, E, G), but the higher values of O$_2^–$ (Fig. 1B) and MDA (Fig. 1C), and the higher breakdown index (Fig. 1A) in pulp. On the contrary, the ATP-treated P. longanae-infected samples displayed the opposite results (Figs 1, 2). These data implied that DNP treatment down-regulated the transcript levels of RSEs-related genes to reduce the RSEs activities, and then declined the ROS-scavenging ability, and thus stimulated the ROS accumulation and membrane lipids peroxidation, in turn, expedited the P. longanae-caused longan pulp breakdown. Whereas, ATP treatment up-regulated the transcript levels of RSEs-related genes, resulting in the higher RSEs activities, and then boosted the ROS-scavenging capacity, and eventually decreased the ROS production and membrane lipids peroxidation, accordingly suppressed the P. longanae-induced longan pulp breakdown.

4.4. DNP and ATP regulate the contents of antioxidant substances in P. longanae-infected longan and their relation to pulp breakdown

The non-enzymic antioxidant substances, like GSH, flavonoid, AsA, and total phenolics, also play the vital roles in eliminating the ROS, which reduce the ROS level and alleviate the damage of cell membranes (Lin et al., 2017; Xue et al., 2020). AsA, belongings to a ROS-detoxifying compound, may promote the generation of H$_2$O from H$_2$O$_2$ via increasing the APX activity (Lin, Chen, et al., 2020). Furthermore, GSH can eliminate H$_2$O$_2$, and other ROS like -OH (Lin, Chen, et al., 2020). In addition, flavonoid and total phenolics are important for eliminating the excrecent ROS and preventing the ROS accumulation (Lin et al., 2021). Previous documents indicated that the treatments of hydrogen peroxide (Lin et al., 2021), ASM + DHEA (Wei et al., 2019), or the infection of pathogen (Jiang et al., 2018) could expedite the decreases in amounts of antioxidant substances, resulting in the lower ROS-scavenging ability, and thus promoted the decay development in harvested products. However, the treatments of chitosan (Lin, Chen, et al., 2020), melatonin (Zhang et al., 2018), AsA (Xue et al., 2020), or propyl gallate (Lin et al., 2015; Lin, Lin, et al., 2020) could increase the levels of antioxidant substances, and then enhance the capacity to eliminate ROS, and eventually stabilize the quality properties in postharvest produces. Thus, the amounts of antioxidant substances could influence the ROS level, which affected the quality properties in harvested products.

In this study, within the entire storage, in contrast to the control
samples, the lower contents of pulp AsA (Fig. 3A), GSH (Fig. 3B) and flavonoid (Fig. 3C), while a higher producing rate of pulp O$_2^-$ (Fig. 1B), a higher level of pulp MDA (Fig. 1C), and a higher pulp breakdown index (Fig. 1A) were found in the _P. longanae_-inoculated longan. Also, at 1–5 d of storage, the pulp total phenolics amount of _P. longanae_-inoculated group displayed a lower level than the control samples (Fig. 3D). These findings implied that _P. longanae_ infection reduced the contents of antioxidants containing AsA, GSH, flavonoid and total phenolics, which lowered the ROS-scavenging level to boost the ROS accumulation and facilitate the membrane lipids peroxidation, and then disordered the cell membranes structure, and induced the longan pulp breakdown.

Furthermore, within storage, compared with _P. longanae_-infected samples, the DNP-treated _P. longanae_-infected samples showed the lower levels of pulp AsA (Fig. 3A), GSH (Fig. 3B), flavonoid (Fig. 3C), and total phenolics (Fig. 3D), but the higher values of pulp O$_2^-$ (Fig. 1B) and MDA (Fig. 1C), and a higher index of pulp breakdown (Fig. 1A). Whereas, the contrary research results were shown in the ATP-treated _P. longanae_-infected samples (Figs. 1, 3). These results indicated that DNP treatment exacerbated the _P. longanae_-caused longan pulp breakdown, which was because DNP decreased the amounts of antioxidant substances, and then lowered the ROS-scavenging ability, promoted the ROS generation, and thus aggravated the membrane lipids peroxidation and damaged the cell membranes. Whereas, ATP treatment for suppressing the _P. longanae_-caused longan pulp breakdown was associated with ATP-retained the higher values of antioxidant substances, and then enhanced the capacity of eliminating ROS, decreased the ROS level, and ultimately alleviated the membrane lipids peroxidation and stabilized the cell membranes structure.

4.5. DNP and ATP regulate the DPPH radical scavenging ability and reducing power in _P. longanae_-infected longan and their relation to pulp breakdown

The scavenging activity of DPPH radical and the level of reducing power are the primary indicators to appraise the antioxidant ability in fresh produces (Wang et al., 2018), which may influence the generation-scavenging of ROS (Lin et al., 2021). Some documents showed that the treatment of hydrogen peroxide (Lin et al., 2021) or the pathogen infection (Sun et al., 2018) could lower the reducing power and the DPPH radical scavenging ability, and then raised the ROS accumulation, and ultimately aggravated the quality deterioration in fresh produces. However, the treatment of chitosan (Lin, Chen, et al., 2020) resulted in the higher reducing power or DPPH radical scavenging ability, and then reduced the ROS level, and thus suppressed the decay occurrence in fresh produces. Hence, the DPPH radical scavenging ability and reducing power could affect the ROS level, and ultimately regulate the quality in fresh produces.

In current work, within storage, in comparison to control longan, there were the lower DPPH radical scavenging ability (Fig. 4A), the lower reducing power (Fig. 4B), but the greater O$_2^-$ production rate (Fig. 1B), and the higher breakdown index (Fig. 1A) in pulp of _P. longanae_-infected samples. These data implied that the infection of _P. longanae_ lowered the DPPH radical scavenging ability and decreased the reducing power, then reduced the antioxidant capacity and raised the ROS level, and thus damaged the cell membranes, in turn, induced the longan pulp breakdown.

Furthermore, compared with _P. longanae_-infected samples, within storage, the DNP-treated _P. longanae_-infected samples showed a lower scavenging ability of DPPH radical (Fig. 4A), a lower level of reducing power (Fig. 4B), but the higher levels of O$_2^-$ (Fig. 1B) and breakdown index (Fig. 1A) in pulp. Whereas, the contrary research results were
Whereas, ATP treatment retarded the pulp breakdown occurrence in _P. longanae_-infected longan, which was because ATP treatment enhanced the DPPH radical scavenging ability and raised the reducing power, then increased the antioxidant capacity, and eventually reduced the ROS accumulation and stabilized the cell membranes structure. Therefore, according to the above results, the probable mechanisms of DNP and ATP modulating _P. longanae_-induced longan pulp breakdown through regulating ROS metabolism were illustrated in Fig. 5. However, the possible mechanisms, involving the biochemical and molecular pathways, of DNP and ATP regulating the pulp breakdown in longan fruit infected by _P. longanae_ are still unclear. Thus, the mechanisms of DNP and ATP regulating the pulp breakdown in _P. longanae_-infected longan should be further investigated through metabolomics and transcriptomic analyses.

5. Conclusions

In summary, the ROS metabolism played the crucial role in DNP and ATP-regulating the _P. longanae_-induced the longan pulp breakdown. DNP treatment promoted the pulp breakdown of _P. longanae_-infected longan, which was because DNP decreased the activities of RESs (SOD, APX, and CAT) through down-regulating the transcript levels of RES-related genes (DISOD1, DISOD2, DISOD3, DIAFPx6 and DICAⅡ), reduced the amounts of antioxidants (AsA, flavonoid, GSH, and total phenolics), dropped the scavenging ability of DPPH radical, and reduced the level of reducing power, which led to the lower ROS-scavenging ability and raised the ROS accumulation, and eventually aggravated the membrane lipids peroxidation and cell membranes damage. Whereas, ATP treatment retarded the pulp breakdown development in _P. longanae_-infected longan was due to the ATP-raised the ROS-scavenging ability, and afterwards lowered the ROS production, then increased the antioxidant capacity, and eventually reduced the ROS accumulation and stabilized the cell membranes structure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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