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ORIGINAL ARTICLE

MAPK5 and *MAPK10* overexpression influences strawberry fruit ripening, antioxidant capacity and resistance to *Botrytis cinerea*

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Abstract

Main conclusion ***FaMAPK5*** **and** ***FaMAPK10*** **genes were involved in ABA-mediated strawberry fruit ripening and could enhance the antioxidant capacity by increasing non-enzymatic components and enzymatic antioxidants.**

Abstract Mitogen-activated protein kinases (MAPKs) are the key proteins involved in plant stress response by activating an antioxidant defense system, which cooperates with plant hormones. However, the involvement of MAPKs in the regulation of strawberry fruit ripening and resistance is unclear. In this study, two genes, *FaMAPK5* and *FaMAPK10*, were isolated, and their expression pattern and function analysis were conducted. The results showed *FaMAPK5* and *FaMAPK10* were expressed in all tested tissue/organ types and reached the highest expression level at the white stage during strawberry fruit development and ripening. Transient overexpression of *FaMAPK5* and *FaMAPK10* increased the fruit anthocyanin, abscisic acid (ABA), total sugar, and glucose contents. ABA and especially hydrogen peroxide (H_2O_2) treatment induced the production of large amounts of H_2O_2 and noticeably increased the expression levels of *FaMAPK5* and *FaMAPK10* in strawberry fruit, while the reduced glutathione (GSH) had the opposite effect. The level of total phenol and activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) significantly increased in *FaMAPK5* overexpression fruit, and increased activities of SOD and CAT were observed in *FaMAPK10* overexpression fruit. In addition, *Botrytis cinerea* treatment showed that overexpression of *FaMAPK5* conferred retarded disease symptom development and enhanced fruit disease resistance. Our research revealed that *FaMAPK5* and *FaMAPK10* might participate in ABA-mediated H_2O_2 signaling in regulating strawberry fruit ripening and resistance.

Keywords Abscisic acid · Gene expression · Hydrogen peroxide · Mitogen-activated protein kinases · Strawberry

Abbreviations

CAT	Catalase
GSH	Glutathione
MAPK	Mitogen-activated protein kinase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Mitogen-activated protein kinases (MAPKs) are identified when cells are stimulated by mitogen. The basic components of MAPK signaling cascades are well conserved from yeast to human and plant, including MAPK kinase kinase (MAPKKK, MAP3K or MEKK), MAPK kinases (MAPKK, MAP2K, MKK or MEK) and MAPK (MPK) (Pitzschke et al. 2009). Each level of the three-kinase cascades has multiple members in a cell, which helps to transmit signal specificity. In recent years, screening and identification of *MAPK* gene family have been studied in several plants. For

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instance, about 110 genes encoding *MAPK* pathway components are identified in the *Arabidopsis* genome, including 20 *MAPKs*, 10 *MAPKKs* and more than 80 *MAPKKKs* (Colcombet and Hirt 2008). A total of 16 MAPKs, 5 MAPKKs and 89 MAPKKKs are present in tomato (Kong et al. 2012; Wu et al. 2014). Fourteen MAPKs, 5 MAPKKs and 62 MAPKKKs can be found in grapevine (Cakir and Kılıçkaya 2015). Recently, 12 *FvMAPKs*, 7 *FvMAPKK*s, 73 *FvMAPKKK*s and 1 *FvMAPKKKK* genes are identified in the strawberry (*Fragaria vesca*) genome (Zhou et al. 2017). Therefore, the interlinked MAPK cascades have thousands of different MAPKKK–MAPKK–MAPK combinations. However, only a limited number of entire MAPK cascades have been deeply investigated in model plants. For example, AtMEKK1–AtMKK4/5–AtMPK3/6 involves the flagellin-mediated innate immune response (Asai et al. 2002), and AtMEKK1–AtMKK2–AtMPK4/6 regulates tolerance to freezing and other abiotic stresses in *Arabidopsis thaliana* (Kovtun et al. 2000; Teige et al. 2004).

The MAPK controls plant development and various stress responses through effects on phosphorylation of transcription factors and other signaling components (Meng and Zhang 2013). It has been reported that MAPKs are involved in these biological processes by cooperating with plant hormones and reactive oxygen species (ROS). ROS, especially hydrogen peroxide (H_2O_2) initiates the MAPK cascade to respond to oxidative stresses, a highly conserved pathway in plant (Liu and He 2017). In *Arabidopsis thaliana*, AtMPK6 and AtMPK3 can be activated by various concentration of H_2O_2 (Kovtun et al. 2000). In tomato, H_2O_2 application might enhance chilling tolerance by activating gene expressions of *SlMAPK1/2/3* and *SlCBF1*, and by increasing activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), and by regulating the concentrations of phytohormones (Wang et al. 2017). H_2O_2 production is critical for ABA-induced stress tolerance in plants (Zhou et al. 2014). ABA-induced H_2O_2 generation activated calcium channels of guard cells and promoted the stomatal closure (Pei et al. 2000). Recently, MAPK cascades have been shown to participate in ABA signaling in response to biotic and abiotic stress (Jammes et al. 2011; Liu 2012; Danquah et al. 2014; de Zelicourt et al. 2016). Several lines of evidence indicate that there is a crosstalk among ABA, H_2O_2 and MAPKs (Smékalová et al. 2014). In Arabidopsis, AtMKK1–AtMPK6 could regulate *CAT1* expression in ABA-induced H_2O_2 production (Xing et al. 2008).

In our previous study, *FaMAPK5* and *FaMAPK10* expression levels were increased as a result of ABA application and ABA-mediated H_2O_2 production accelerated strawberry ripening (Luo et al. 2020). Given that MAPKs have been implicated in plant development and defense by mediating ABA and H_2O_2 signaling, we sought to investigate the role of *FaMAPK5* and *FaMAPK10* in strawberry fruit ripening and resistance by gene transient overexpression and *Botrytis cinerea* inoculation experiments, which provide promising candidate genes for strawberry breeding.

Materials and methods

Plant materials

Strawberry (*Fragaria*×*ananassa* cv. Benihoppe) was grown in a plastic greenhouse under natural conditions in Chengdu, China. Fruits were harvested at different stages: small green (SG), large green (LG), de-greening (DG), white (WT), initial red (IR) and full red (FR). In addition, roots, shoot, leaves and flowers of strawberry were collected. All plant tissues were harvested 100 days after strawberry planting. These materials were quickly frozen in liquid nitrogen and stored at − 80 °C until use.

Cloning of the *FaMAPK5* and *FaMAPK10* genes

Total RNA was extracted using a modified CTAB protocol (Jia et al. 2008). Approximately, 1 μg of total RNA was reverse transcribed to cDNA using a SMART™ RACE cDNA Synthesis Kit (Clontech, Mountain View, CA, USA). The primers used for *FaMAPK5* and *FaMAPK10* gene cloning are listed in Table S1. The PCR reaction protocol was as follows: 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 58 ºC for 30 s and 72 ºC for 2 min, with a final extension at 72 °C for 10 min.

Plasmid construction

The primers used for plasmid construction are listed in Table S2. For overexpression, the open reading frames (ORF) of *FaMAPK5* and *FaMAPK10* were, respectively, inserted into the modified pCAMBIA1301 (Lin et al. 2018) under the control of the cauliflower mosaic virus (CaMV) 35S promoter using the restriction enzymes *Bam*H I and *Xba* I, referred to as 35S::MAPK5 and 35S::MAPK10. These constructs and empty vector pC1301-35s-Nos were transformed into *Agrobacterium tumefaciens* strain GV3101 by the freeze–thaw method (Jia et al. 2011).

Transient gene expression in strawberry fruit

Transient gene expression assays in strawberry fruit were performed as described by Jia et al. (2011). A single *Agrobacterium* colony carrying the plasmid of interest was inoculated in 5-mL Luria–Bertani (LB) medium supplemented with appropriate antibiotics, and grown overnight on a rotary shaker at 28 °C. Then, 0.1 mL of overnight cultures was

added into 50 mL of the same medium and incubated at 28 °C overnight again. The cells were harvested by centrifugation at 4000 g for 15 min at room temperature, and subsequently resuspended in infiltration buffer (10 mM $MgCl_2$, 10 mM MES, 200 mM acetosyringone) to reach an OD_{600} of 0.8–1.0. Detached fruits at the DG stage were injected with about 800 µL of *Agrobacterium* suspension using a 1-mL syringe. To do this, the needle tip was inserted into the fruit center from the top, and then the *Agrobacterium* suspension was slowly and evenly injected into the fruits. After injection, the fruits were incubated at 23 °C with a 16 h light, 8 h dark photoperiod and 80–90% humidity. Ten individual fruits were sampled 5 days later for each infiltration. The experiment was repeated three times.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed on the CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). The primer sequences used for qRT-PCR are shown in Table S3. The amplification program consisted of one cycle at 95 °C for 2 min, followed by 40 cycles of 94 °C for 20 s, 54 °C for 20 s, and 72 °C for 30 s. *FaActin* gene (AB116565) was used as the internal control. The qRT-PCR experiment was performed on three independent biological replicates with technical replicates. The relative expression levels of the target genes were calculated according to the $2^{-\Delta\Delta Ct}$ algorithm.

Determination of total anthocyanin, ABA, total sugar, sucrose, glucose, fructose, total phenol, H_2O_2, and reduced glutathione (GSH)

The total anthocyanin determination was conducted using the pH differential method (Cheng and Breen 1991). Briefly, the extraction was conducted from 0.5 g strawberry fruit homogenized with 1.8 mL of cold 1% HCl-ethanol. Then, the homogenate was centrifuged at 8000 g for 25 min at 4 °C. Next, the supernatants were used for measuring the total anthocyanin content. Anthocyanin concentration was expressed as mg pelargonidin 3-glucoside equivalents/100 g of fresh weight.

Total sugar, sucrose, glucose, fructose, total phenol and GSH content were, respectively, determined by the commercial assay kits purchased from Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China). The strawberry fruit (0.2 g) was extracted with the respective extract provided in the kit and centrifuged at 8000 g for 15 min, and then the supernatants were used for the determination of corresponding substances according to the manufacturer's protocol.

The ABA content was measured by the commercial determination kit purchased from Shanghai MLBIO Biotechnology Co., Ltd. (Shanghai, China). The strawberry fruit (0.2 g) was extracted with 1.8-mL PBS (phosphate buffer saline) for 30 min and centrifuged at 5000 g for 20 min, and then the supernatants were used for measuring the ABA content according to the manufacturer's protocol by enzyme-linked immunosorbent assay.

H_2O_2 was measured as described by Cao et al. (2007). Briefly, strawberry fruits (0.5 g) were extracted in 5 mL pre-cooled acetone, followed by centrifugation at 12,000 g for 20 min at 4 °C. The 1-mL supernatant was mixed with 0.1 mL of 10% $TiCl_4$–HCl (v/v) and 0.2 mL of concentrated ammonia. The mixture was kept for 5 min and then centrifuged at 12,000 g for 15 min at 4 °C. The sediment was dissolved by 3 mL of 10% H_2SO_4 (v/v) and then measured spectrophotometrically at 412 nm to determine the H_2O_2 content.

Determination of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzyme activity

The activities of antioxidant enzymes were determined according to the method of Qin et al. (2015). All operations were done at 4 °C. The strawberry fruit (0.5 g) was homogenized in a mortar with 3 mL of 50 mM phosphate buffer at pH 7. Homogenates were filtered through two layers of Miracloth and the filtrate was centrifuged at 15,000 g for 15 min, at 4 °C. The resulting supernatant was stored at −80 °C. The CAT, POD, and SOD enzyme activities were measured spectrophotometrically. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. The POD activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2. The SOD activity in apoplastic fractions was estimated by recording the decrease in optical density of nitroblue tetrazolium dye at 560 nm by the enzyme.

Infection experiment by *Botrytis cinerea*

The strawberry fruits at the initial red stage were randomly divided into 3 groups and each group had 20 fruits. The sepals were removed from the fruits, and the fruit surface was cleaned with distilled water and dried in the air. Three groups were, respectively, injected with the *Agrobacterium* suspension harboring the 35S::MAPK5, 35S::MAPK10 and pC1301-35s-Nos plasmids. Three days later, strawberry fruits of each group were all inoculated with *Botrytis cinerea*. The original *Botrytis cinerea* was isolated from an infected strawberry fruit. Prior to each experiment, *Botrytis cinerea* was cultured on PDA (potato dextrose agar) medium at 25 °C for 12 days. The spore suspension was prepared according to the method described as

Zhang et al. (2020a). Next, 30 µL of spore suspension was evenly sprayed on the surface of the fruit, and then fruits were placed in artificial climate box, which environment condition was 20 °C, 16/8 h day/night cycle and 90% relative humility. The number of mildewed fruits and the infected area was recorded on the third and fourth day after inoculation.

Diaminobenzidine (DAB) staining

De-greening strawberry fruit discs were treated with H_2O, 25 mg/L ABA, 100 mM sucrose, 100 mM GSH or 100 mM H_2O_2 by immersing discs in these solutions for 6 h, respectively. After that, the treated discs were immediately washed in distilled water and dried by absorbent paper. Subsequently, these discs were stained with DAB for detection of H_2O_2, according to the method described by Zhang et al. (2020b).

Results

Spatiotemporal expression profiles of *FaMAPK5* and *FaMAPK10*

The expression profiles of *FaMAPK5* and *FaMAPK10* at different tissues/organs and different stages of fruit development were detected by qRT-PCR. The results showed that *FaMAPK5* and *FaMAPK10* constitutively expressed in all tested tissues/organs, but they displayed different expression patterns. The most abundant expression levels of *FaMAPK5* were detected in leaves, followed by flower, stem, root and fruit (Fig. 1a). The highest transcripts of *FaMAPK10* were detected in fruit, followed by flower, root, stem and leaves (Fig. 1b). Clearly, the expression profiles of *FaMAPK5* and *FaMAPK10* were similar during the fruit development. They displayed low transcript abundance at SG, LG and DG stages, and had a significant transcript accumulation at WT, IR and FR stages (Fig. 1c, 1d).

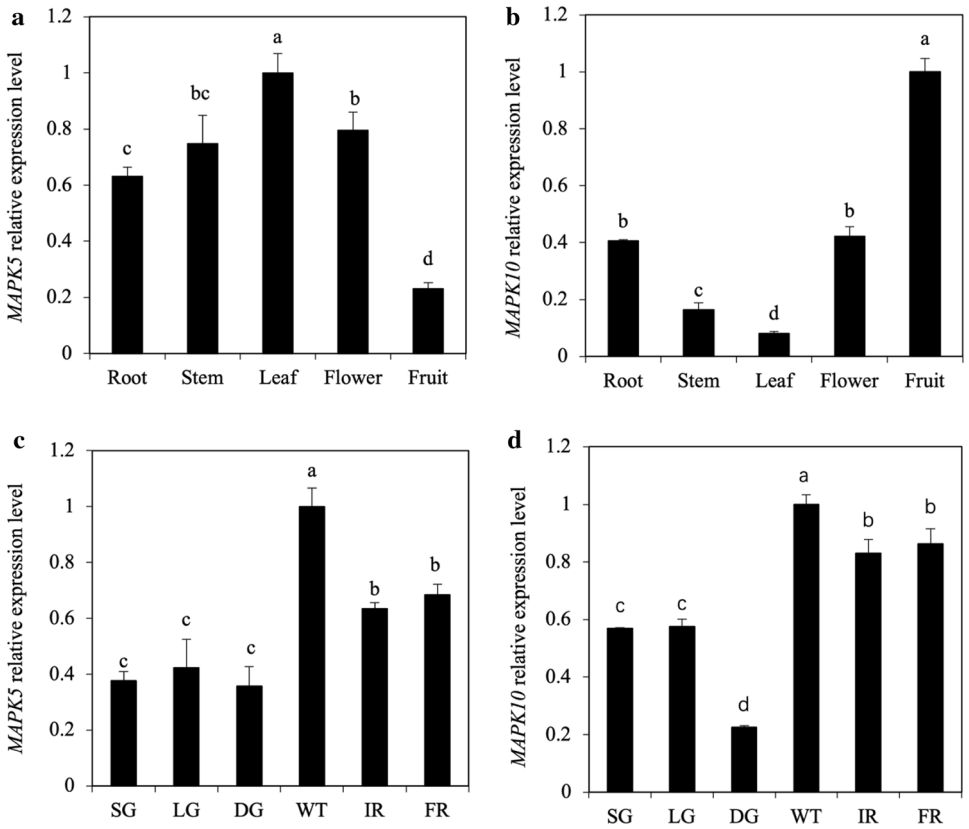

Fig. 1 Transcription levels of *FaMAPK5* and *FaMAPK10* in different tissues/organs and developmental stages. **a** Expression patterns of *FaMAPAPK5* gene in different tissues of strawberry. The fruits were sampled at full red. **b** Expression patterns of *FaMAPK10* gene in different tissues of strawberry. The fruits were sampled at full red. **c** Expression patterns of *FaMAPK5* gene at different developmental stages of strawberry fruit. **d** Expression patterns of *FaMAPK10* gene at different developmental stages of strawberry fruit. Small green (SG, 7 days after anthesis), large green (LG, 14 days after anthesis), de-greening (DG, 18 days after anthesis), white (WT, 20 days after anthesis), initial red (IR, 23 days after anthesis) and full red (FR, 28 days after anthesis). Data are expressed as means ± standard error of three biological replicates. Different lower case letters indicate significant difference ($P < 0.05$, ANOVA followed by the Duncan test)

Overexpression of *FaMAPK5* and *FaMAPK10* affects the levels of ABA, sugar and anthocyanin

To test the role of *FaMAPK5* and *FaMAPK10* in fruit ripening, 35S::*MAPK5 and 35S*::*MAPK10* were generated, and respectively, infiltrated into the strawberry fruits. The expression of *FaMAPK5* and *FaMAPK10* significantly increased in the overexpression fruits compared to the control (Fig. 2a, b). ABA is a critical signal molecule in regulating strawberry fruit development and ripening. The results showed that ABA content increased to about 1.12-fold and 1.19-fold as a result of *FaMAPK5* and *FaMAPK10* overexpression (Fig. 2c). Moreover, overexpression of *FaMAPK5* and *FaMAPK10* promoted strawberry fruit coloration and anthocyanin accumulation (Fig. 2d). The contents of sucrose and fructose did not significantly increase in the *FaMAPK5* overexpression fruits, yet they significantly decreased in the *FaMAPK10* overexpression fruits (Fig. 2e, f). However, the contents of glucose and total sugar increased significantly in the *FaMAPK5* and *FaMAPK10* overexpression fruit compared with that of the control (Fig. 2g, h). These results suggested that alteration of *FaMAPK5* and *FaMAPK10* transcripts could influence ABA, sugar and anthocyanin contents of strawberry fruit.

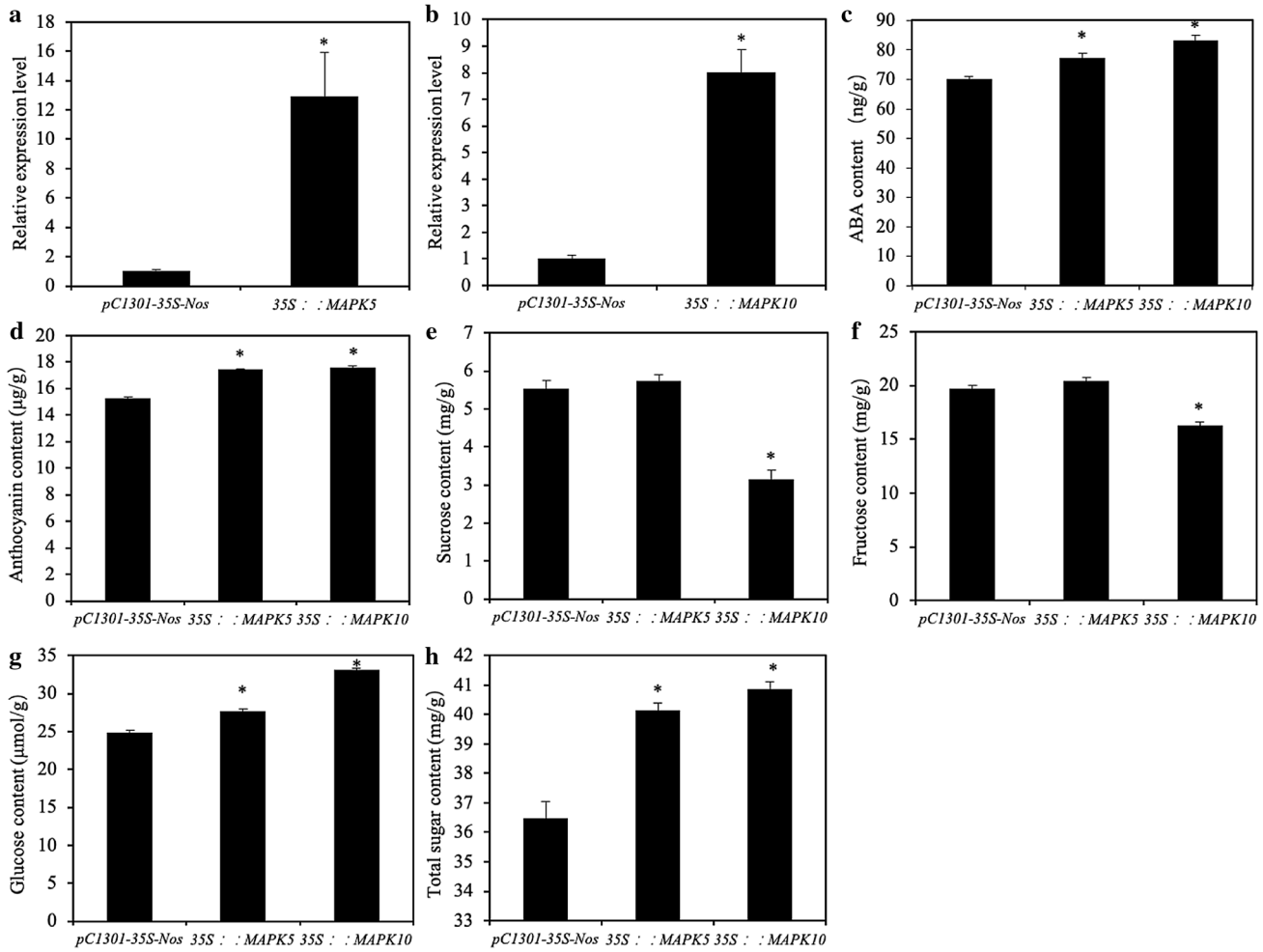

Fig. 2 Overexpression for the *FaMAPK5* and *FaMAPK10* genes in strawberry fruits. **a** Expression levels of *FaMAPK5* in the *FaMAPK5* overexpression fruit and control by qRT-PCR. **b** Expression levels of *FaMAPK10* in the *FaMAPK10* overexpression fruit and control by qRT-PCR. **c** ABA content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **d** Anthocyanin content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **e** Sucrose content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **f** Fructose content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **g** Glucose content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **h** Total sugar content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. Data are expressed as means ± standard error of three biological replicates. Asterisk indicates significant difference from empty vector ($P < 0.05$, ANOVA followed by the Duncan test)

Oxidative stress responses of *FaMAPK5* and *FaMAPK10*

MAPK and H_2O_2 are involved in the stress response process; thus, the relationship between them is particularly important under oxidative stress. Diaminobenzidine (DAB) staining can visually detect the content of H_2O_2 in plants. The peroxidase in cells can release oxygen from hydrogen peroxide, and then oxidize DAB to form brown deposit located at the active site of peroxidase. Normally, the darker the brown deposit, the more hydrogen peroxide is accumulated. In this experiment, the strawberry fruit discs were, respectively, treated with distilled water, 25 mg/L ABA, 100 mM sucrose, 100 mM GSH and 100 mM H_2O_2 for 6 h and then stained with DAB. The results showed that the darker brown color was observed in ABA-, H_2O_2- and sucrose-treated fruits, while a lighter brown color was observed in the GSH-treated fruits compared with the control (Fig. 3a). To further verify the results, the content of H_2O_2 in these treated strawberry fruit discs was determined. Compared with the control, the level of H_2O_2 was higher in ABA-, sucrose- and H_2O_2-treated fruit discs, especially in H_2O_2-treated fruit discs, and the content of H_2O_2 was lower in GSH-treated fruit discs (Fig. 3b), which was consistent with the DAB staining. Taken together, these results indicated that ABA, sucrose, H_2O_2 and GSH treatment induced or relieved oxidative stress by regulating the H_2O_2 level of strawberry fruit discs.

To explore the response characteristics of *FaMAPK5* and *FaMAPK10* gene to different levels of oxidative stress, the expression levels of *FaMAPK5* and *FaMAPK10* in strawberry fruit discs were detected under the above treatments. The qRT-PCR results showed that transcript levels of *FaMAPK5* and *FaMAPK10* in strawberry fruit discs were significantly upregulated after ABA and H_2O_2 treatments, while there was no significant change after sucrose treatment, and the expression levels were significant downregulated after GSH treatment (Fig. 3c, d). These results indicated that *FaMAPK5* and *FaMAPK10* might act as an important regulator in ABA and H_2O_2-mediated or GSH-mediated redox signaling.

Overexpression of *FaMAPK5* and *FaMAPK10* affects the levels of H_2O_2, GSH, total phenol and activity of antioxidant enzymes

Plant maintains a delicate balance between generation and removal of ROS, which is principally accomplished by the antioxidant defense system that includes enzymatic antioxidants and reduced non-enzymatic components. The H_2O_2 content did not significantly change in *FaMAPK5* and *FaMAPK10* overexpression fruits compared with the control (Fig. 4a). The GSH content was significantly increased in overexpressed *FaMAPK5* and *FaMAPK10* fruit (Fig. 4b). In addition, the level of total phenol and the activities of CAT, SOD and POD increased significantly in

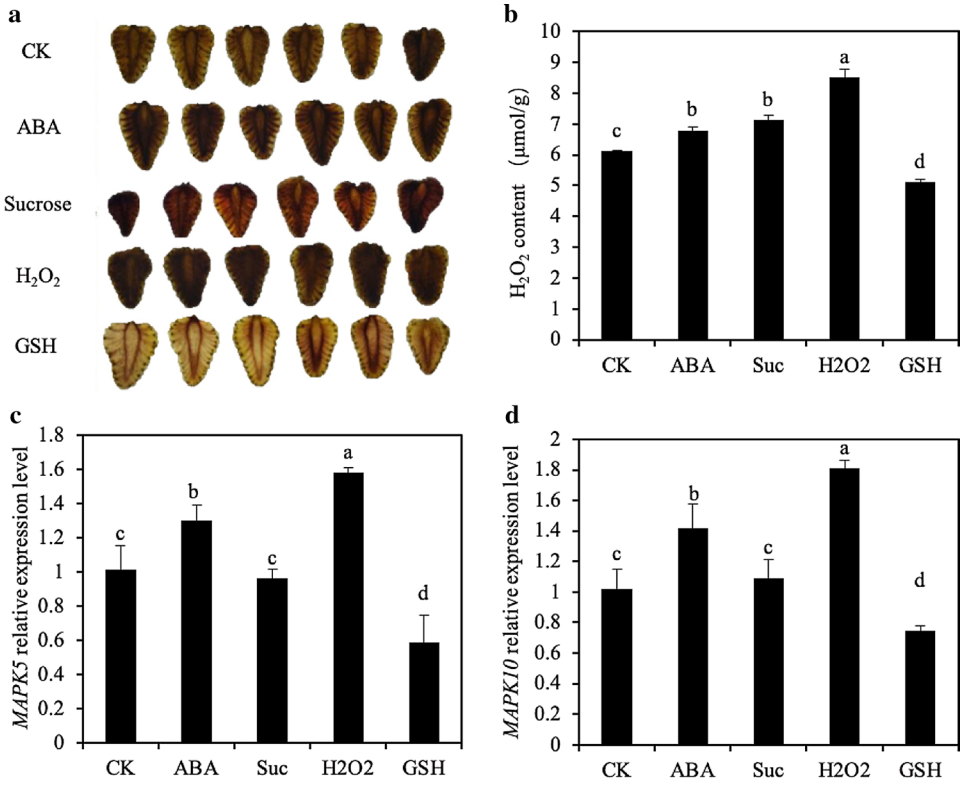

Fig. 3 Effect of ABA, sucrose, H_2O_2 and GSH on H_2O_2 content and expression level of *FaMAPK5* and *FaMAPK10*. **a** Diaminobenzidine (DAB) staining of the control (H_2O, CK), ABA-, sucrose-, H_2O_2- and GSH-treated strawberry fruit discs. **b** The corresponding H_2O_2 content in the control (H_2O, CK), ABA-, sucrose-, H_2O_2- and GSH-treated strawberry fruit discs. **c** Transcription level of *FaMAPK5* at the control (H_2O, CK), ABA, sucrose, H_2O_2 and GSH treatments. **d** Transcription level of *FaMAPK10* at the control (H_2O, CK), ABA, sucrose, H_2O_2 and GSH treatments. Suc: sucrose. Data are expressed as means ± standard error of three biological replicates. Different lower-case letters indicate significant difference ($P < 0.05$, ANOVA followed by the Duncan test)

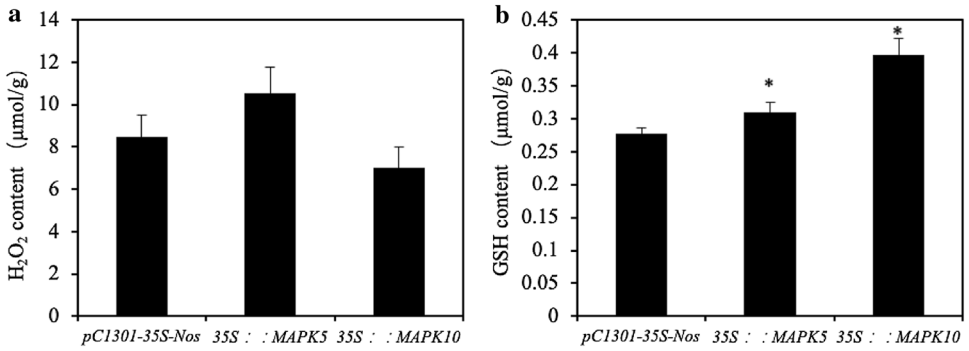

Fig. 4 Overexpression of *FaMAPK5* and *FaMAPK10* influences the levels of H_2O_2 and GSH. **a** H_2O_2 content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **b** GSH content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. Data are expressed as means ± standard error of three biological replicates. Asterisk indicates significant difference from empty vector ($P < 0.05$, ANOVA followed by the Duncan test)

overexpressing *FaMAPK5* fruit, but increases in total phenol content and POD activity were not observed in overexpressing *FaMAPK10* fruit (Fig. 5). These results indicated that overexpression of *FaMAPK5* and *FaMAPK10* could affect the redox state of strawberry fruit by regulating the levels of endogenous H_2O_2, non-enzymatic components (GSH and total phenol) and enzymatic antioxidants (CAT, SOD and POD).

Overexpression of *FaMAPK5* and *FaMAPK10* affects resistance to *Botrytis cinerea*

Due to antioxidant and redox regulation of *FaMAPK5* and *FaMAPK10* genes, a pathogen infection experiment in vitro was designed to verify whether the genes were involved in strawberry fruit defense against *Botrytis cinerea.* The results showed that incidence of the gray mold decay was 45%, 10% and 25% in the empty plasmid, overexpressing *FaMAPK5* and *FaMAPK10* fruits on the third day of inoculation with *Botrytis cinerea*, respectively. On the 4th day, the decay incidence was 65%, 25% and 65% in above treatments, respectively (Table 1, Fig. 6). The area of disease

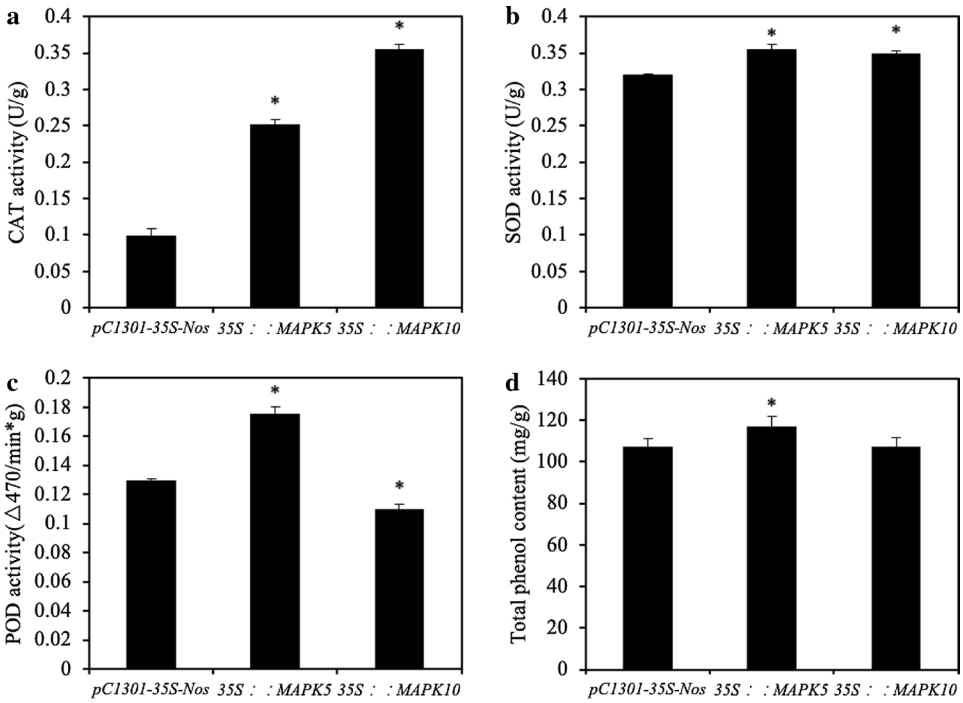

Fig. 5 Effects of transient overexpression of *FaMAPK5* and *FaMAPK10* on the antioxidant enzyme activities and total phenol content. **a** CAT activity of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **b** SOD activity of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **c** POD activity of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. **d** Total phenol content of strawberry fruit after overexpression of *FaMAPK5* and *FaMAPK10*. Data are expressed as means ± standard error of three biological replicates. Asterisk indicates significant difference from empty vector ($P < 0.05$, ANOVA followed by the Duncan test)

Table 1 Incidence of the gray mold decay

Samples	pC1301-35S-Nos	35S::MAPK5	35S::MAPK10
3 days after inoculation	45%	10%	25%
4 days after inoculation	65%	25%	65%

symptom development in *FaMAPK5* overexpressing fruits was smaller than that in fruits of other treatments (Fig. 6). Taken together, overexpression of the *FaMAPK5* gene showed a remarkable decrease susceptibility to *Botrytis cinerea* infection in strawberry fruit, while overexpression of *FaMAPK10* did not show an obvious effect.

Discussion

It is well known that MAPK cascades are important components for integrating responses to intra- and extracellular signaling in plants (Taj et al. 2010). Each level of MAPK cascades has multiple members, which confers their specific functions in various signal transduction process. Spatial and temporal expression profiles of gene are used for the early analysis of gene function. However, little is known about MAPK expression profiles during fruit development. In our study, the results showed that the expressions of *FaMAPK5* and *FaMAPK10* were detected in all tested tissues/organs types, and both of which had a significant transcript accumulation at fruit coloration stages (Fig. 1), indicating that *FaMAPK5* and *FaMAPK10* may play a certain role in strawberry fruit ripening. Color is always used as the index of fruit maturity, and the degree of red coloration in strawberry is determined by the content and composition of anthocyanins (da Silva et al. 2007). Anthocyanins have antioxidant properties, which contribute to free radical scavenging, anti-aging, anti-cancer and immunity boosting in human body (Pojer et al. 2013). Xia et al. (2009) reported that anthocyanins can inhibit apoptosis of many cells by inhibiting the activation of MAPK. In the present study, transient overexpression of *FaMAPK5* and *FaMAPK10* increased anthocyanin content significantly, which indicated a relationship between anthocyanin accumulation and MAPK expression (Fig. 2d). In addition, fruit sweetness is another good indicator of fruit ripening and flavor that influences consumers' purchase decision (Schwieterman et al. 2014). Sucrose, fructose and glucose are the main sources of sugar in strawberry fruit. Our results showed that overexpression of *FaMAPK5* and *FaMAPK10* could remarkably increase the total sugar and glucose content of strawberry fruit, suggesting these two genes increased the total sugar content mainly depending on the glucose content, especially for *FaMAPK10* (Fig. 2e–h). More importantly, the enhanced expression of *FaMAPK5* and *FaMAPK10* caused the increase of ABA content (Fig. 2c). It has been documented that ABA is associated with non-climacteric fruit ripening and it can promote

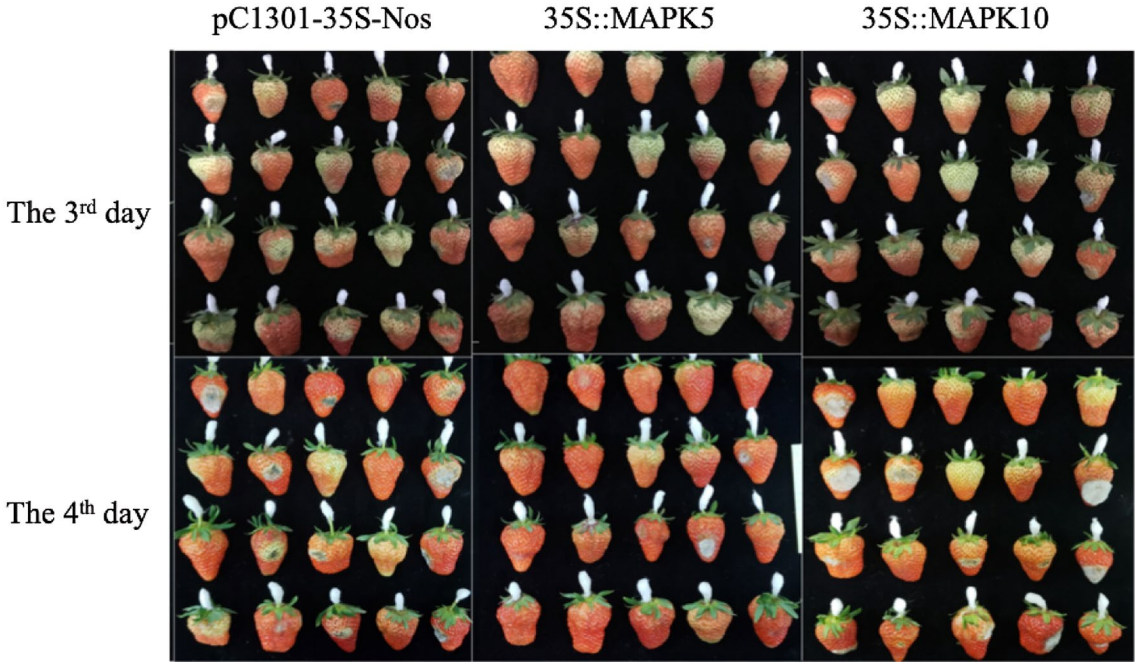

Fig. 6 Effect of overexpression of *FaMAPK5* and *FaMAPK10* on strawberry fruit disease resistance against *Botrytis cinerea*. The phenotype of strawberry fruits in the first row shows the third day after infection with *Botrytis cinerea*, and the phenotype of strawberry fruits in the second row shows the fourth day after infection with *Botrytis cinerea*

sugar, anthocyanin metabolism and accumulation in fleshy fruits (Jia et al. 2011; Luo et al. 2020). Hence, *FaMAPK5* and *FaMAPK10* was related to strawberry fruit ripening by regulating the content of anthocyanin, sugar and ABA.

The production of ROS and especially H_2O_2 is acknowledged as a signal for activation of defense mechanism under various stress conditions, in addition to the toxicity that could lead to oxidative damages in plant. It has been reported that H_2O_2 interacts with other signaling molecules such as plant hormones and MAPKs to respond to abiotic and biotic stresses (Xia et al. 2014; Saxena et al. 2016; Lee and Back 2017). Here, exogenous ABA and H_2O_2 application caused a significant increase of H_2O_2, which also noticeably enhanced the transcription of *FaMAPK5* and *FaMAPK10*. However, GSH treatment decreased the H_2O_2 content and inhibited the expression of *FaMAPK5* and *FaMAPK10* (Fig. 3). These results indicated that *FaMAPK5* and *FaMAPK10* might function in strawberry redox signaling, which was related to the endogenous H_2O_2 level. As a signal molecule, the generation of H_2O_2 in stress response is induced by ABA. In response to osmotic stress, ABA-triggered H_2O_2 accumulation promotes stomatal closure in plant leaves (Li et al. 2015), whereas reducing H_2O_2 level inhibits ABA-induced stomatal closure (An et al. 2016). Several evidences suggest that MAPKs are involved in ABA and H_2O_2 signaling (Jammes et al. 2009; Saxena et al. 2016). ABA-induced H_2O_2 generation activates MAPK and results in upregulation of the expression and activities of antioxidant enzymes. The activation of MAPK also enhances the H_2O_2 production, forming a positive feedback loop (Zhang et al. 2006, 2007). The exogenous H_2O_2 activates MAPK cascade, which in turn is mediated by ABA and other hormones (Cristina et al. 2010; Sinha et al. 2011). In this study, increased expression of *FaMAPK5* and *FaMAPK10* notably upregulated the ABA content, whereas they did not significantly influence the H_2O_2 content, which was probably a result of a marked increase of non-enzymatic components (GSH and total phenol) and enzymatic antioxidants (CAT, SOD and POD) (Figs. 4, 5). It is speculated that overexpression of *FaMAPK5* and *FaMAPK10* can enhance the antioxidant capacity in strawberry fruit.

Gray mold caused by *Botrytis cinerea* is one of the most destructive strawberry diseases worldwide and can cause more than 50% yield losses in severe cases (Cordova et al. 2017). Increasing evidences have shown that MAPKs are indeed involved in pathogen defense (Adachi et al. 2016; Bi and Zhou 2017; Zhang et al. 2018). In citrus, *CsMAPK1* plays an important role in defense response to the citrus canker pathogen by inducing defense gene expression and ROS accumulation (de Oliveira et al. 2013). In tomato, *SlMPK1/2/3* are involved in nitric oxide-induced disease resistance against *Botrytis cinerea*. Meanwhile, plant hormones and ROS participate in the disease response of SlMPK1/2/3 (Zheng et al. 2014). In apple, MAPK cascade is closely related to ROS signaling, which together regulate disease resistance against blue mold infection (Cheng et al. 2020). Recent researches have identified that a few MAPKs in strawberry implicate in pathogen response (Wei et al. 2017; Zhang et al. 2020a). In our experiment, overexpression of *FaMAPK5* and *FaMAPK10* significantly upregulated the ABA content, and activated the ROS scavenging system, and thus inhibited the ROS burst, indicating that the genes may play a regulatory role in strawberry fruit resistance against *Botrytis cinerea*. However, only *FaMAPK5* overexpression outstandingly retarded disease symptom development and conferred enhanced resistance to *Botrytis cinerea*, while *FaMAPK10* overexpression failed in doing that (Fig. 6). Whether *FaMAPK10* gene is effective in inhibiting the growth of other pathogens needs further studies.

In conclusion, it was found that *FaMAPK5* and *FaMAPK10* genes are involved in ABA-mediated strawberry fruit ripening. The expression level of *FaMAPK5* and *FaMAPK10* is jointly mediated by ABA and H_2O_2, and they have a feedback regulation on the content of ABA. Overexpression of *FaMAPK5* and *FaMAPK10* enhanced the antioxidant capacity by increasing non-enzymatic components (GSH and total phenol) and enzymatic antioxidants (CAT, SOD and POD). In addition, *FaMAPK5* can improve the disease resistance of strawberry fruit against *Botrytis cinerea*.

Author contribution statement YTZ, YL and HRT conceived and designed the study. YTZ wrote the paper. YL, YTL, MY, LXW and XYL conducted experiments and analyzed data. YZ, QC, MYL and YXL reviewed drafts of the paper. All the authors read and approved the final version.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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