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Evaluation the ACC and BABA hormones on the expression of resistance genes in apple fruit challenged with *Penicillium expansum*

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ABSTRACT

Purpose: Apple fruit widely suffers from different damages every year including post-harvest diseases such as blue mold (caused by *Penicillium expansum*). Recent studies have shown the role of hormones in different signaling pathways after a pathogen attack. Hormones may trigger different the expression of genes involved in the resistance mechanism and thus activating the immune system in plants. **Findings:** qRT-PCR results showed an increase in the expression of *PR5*, *ERF1* and *CHIB* genes after treatment with BABA in both Golden Delicious and Fuji cultivars. ACC treatment also significantly increased the expression of *ERF1* and *CHIB* genes after challenged with fungal spores. The expression of *PR5* and *ERF1* was relatively very similar in both cultivars after treatment with BABA and ACC, respectively. In contrast, the expression of *CHIB* was different in two cultivars, thus showing a 4 times higher expression in Golden delicious in compared to Fuji. **Originality/Value:** Using of new insecticides with new and widespread mode of action can be recommended against postharvest pest in the practical entomology. **Research limitations**: No limitations were found. **Originality/value**: The results of this study can be important in breeding studies and to select genes involved in the resistance mechanism. In addition, hormones with a positive impact to provide resistance to fungal diseases can be considered as a possible alternative to fungicides.

INTRODUCTION

Apple as a commercially important fruit has been cultivated in different parts of the world since ancient times and has a very consumption among temperate fruits. Genetic variation among apple cultivars has adapted this plant to withstand different environments, including cold and warm regions. Recently, fruit tree cultivation has spread to non-traditional areas in subtropical and tropical regions around the world, in where the climate is different from their natural habitat, with mild, dry winters and hot, rainy summers (Pio et al., 2018). Although the growing season is very short (about three months, i.e. late summer to mid-autumn), fruit can be marketed at a later time if kept in cold storage. During storage, apples are highly susceptible to a wide range of injuries and postharvest diseases (Wu, 2010), most of which are caused by fungi (Martins et al., 2013; Tahir & Nybom, 2013). One of the most widespread and destructive postharvest diseases is blue mold, which is caused by the fungus *Penicillium expansum*. Blue mold is considered as a very major concern, not only due to economic loss to fruit production but also to the fruit processing industry because of patulin production, a mycotoxin produced by Penicillium (Pianzzola et al., 2004; Sanzani et al., 2010). Synthetic fungicides are one of the most primary tools to control postharvest diseases. However, public concern for human health and the hazardous impact of fungicides on the environment has directed the research to search for alternative methods (Martins et al., 2013).

Understanding the mechanism of interaction between the plant defense system and the pathogen is an important step towards this aim. Plant disease resistance is generally divided into two types, qualitative and quantitative. Qualitative resistance is controlled by the major R genes while quantitative resistance is usually mediated by multiple genes with minor effects (Poland et al., 2009). Plant defense mechanisms against stress include many reactions that play an important role in adapting to specific stress conditions (Iqbal et al., 2021). Ethylene (ET) is a gaseous phytohormone involved in various processes that are required for plant germination, growth and development (Abiri et al., 2017). Ethylene response factor (*ERF*) proteins have important functions in regulating the transcription of a variety of biological processes related to growth and development as well as different responses to environmental stimuli. ERF proteins are involved in cellular processes such as signaling (Prasath et al., 2014), response to biological and abiotic stresses (Abiri et al., 2017), and the regulation of metabolism (Broun et al., 2004) and growth processes (Zhang et al., 2005). It has also been shown that ERF proteins interact with the DRE/CBF gene to increase plant stress response, but the regulatory mechanism is not well understood (Mosa et al., 2017).

Chitin is a natural glycopolymer, widely found in fungal cell walls, insect skeletons and crustaceans (Zhang & Huang, 2010; Cao & Tan, 2019). Chitin oligosaccharides induce a variety of immune responses through certain chitin-binding proteins in plants (Komi et al., 2018). Chitinase activity in many plants is activated by a tissue infection caused by a fungus or pathogen (Wang et al., 2009). By blocking mycelial cells and creating defense pathways, plant chitinases are produced in response to pathogens and fungi. Studies by Cao and Tan (2019) showed that overexpression of chitinase (*CHI*) and glucanase genes may increase the immunity against fungal attack in tobacco (Cao & Tan, 2019). Pathogenesis-related 5 (*PR5*) is a family of proteins (also known as thaumatin-like proteins) produced by plant pathogens in many plants. This gene family has a crucial role in plant defense against several necrotrophic fungi and oomycetes (Prasath et al., 2014). To date, various *PR5* genes have been cloned in various plants, including *PR5* from cherry tomatoes (Ren et al., 2011) and *PhOSM* from petunias (Prasath et al., 2014). Overexpression of the PR5 gene has been shown to increase pathogen resistance via hydrolysis of glucans, the major cell wall compounds of most oomycetes and fungi (Nair et al., 2010).

The plant defense system activates signals in various ways and through the use of transcripts of genes, which cause the production or non-production of substances within the plant and thus cause resistance. Plant hormones produce different signaling pathways that trigger different defense genes (Çevik et al., 2012). The elaboration of the different reaction steps of the methionine cycle in plants, now often referred to as the Yang-cycle, was mainly inspired by the biochemical similarities between the plant pathway and the methionine salvage cycle which was already known for prokaryotes, yeast, and mammalians. An-up-to-date overview of the methionine and SAM metabolism in plants is given (Sauter et al., 2013). The major discovery that made the methionine cycle in plants unique from all other organisms was the characterization of 1-aminocyclopropane-1-carboxylic acid (ACC) as the intermediate between SAM and ethylene (Van de Poel & Van Der Straeten, 2014). 1-aminocyclopropane-1-carboxylic acid (ACC) is a non-protein amino acid acting as the direct precursor of ethylene, a plant hormone regulating a wide variety of vegetative and developmental processes. ACC is the central molecule of ethylene biosynthesis (Vanderstraeten & Van Der Straeten, 2017). The rate of ACC formation differs in response to developmental, hormonal and environmental cues. ACC can be conjugated to three derivatives, metabolized in planta or by rhizobacteria using ACC deaminase, and is transported throughout the plant over short and long distances, remotely leading to ethylene responses (Vanderstraeten & Van Der Straeten, 2017).

In the present report, we have evaluated the effect of two hormones, i.e. ACC (1-Aminocyclopropane 1-carboxylic acid) and BABA (β-Aminobutyric acid) on the expression of *CHIB*, *ERF1* and *PR5* genes in apple fruit challenged with *Penicillium expansum*, and discussed the possible positive impact of these hormones on controlling this disease.

MATERIALS AND METHODS

Plant materials

In this experiment, fruit of two cultivars named Fuji and Golden Delicious were used. Apple fruits were randomly selected from the orchard collection in Horticultural Research Center in Karaj, Iran, and were immediately transported to the laboratory. Fruits at the same ripening stage and similar size with no pests and disease or any signs of physiological damage were chosen. Fruits were first washed with distilled water, and then they were surface sterilized with sodium hydroxide (2%) to remove any possible contaminant. For inoculation, *P. expansum* suspension with a concentration of 1×10^5 spores/ml was used. The fruits were inoculated on both opposite sides to a depth of 3 mm with 20 µl of fungus suspension, as described previously (Ahmadi-Afzadi et al., 2013). All fruit were randomly divided into three groups, each group consisting of 10 fruits. Two groups were treated with BABA and ACC hormones, and one group was left untreated (control treatment). 24 hours after inoculation, two first groups were sprayed on both sides of fruits by, respectively BABA and ACC hormones, at a concentration of 5 mM. Fruits were then placed in separate containers and were kept in growth chambers at room temperature for 14 days until symptoms developed.

Measurement of morphological traits and pathogenicity severity

After 14 days post-inoculation (dpi), fruit firmness (firmness at harvest) was measured with a penetrometer (model FT-327, Effigy, Alfonsine, Italy), with a plunger diameter of 11.1 mm, depth 7.9 mm, on the opposite, peeled sides of each fruit, and expressed as kg/m^2. In addition, ten fruit per cultivar were picked on the same day and stored in a cold room in regular air condition (2–4 °C and 90% RH) for 14 days to measure second firmness (firmness after storage). Three variables, i.e. 1) average fruit firmness at harvest, 2) firmness after storage,

and 3) fruit softening rate (difference between firmness at harvest and firmness after storage, showing loos of firmness during storage) were calculated for each cultivar. Experimental data were presented based on the average of 10 fruit.

To measure pathogenicity severity, the lesion area developed after 14 days, was evaluated by measuring the lesion diameter grown on both sides of the apple fruit. For each cultivar, the average contamination on both sides of the apple was measured for 10 fruit. Statistical analyses of these data were conducted using the R package (ver. 3.3.2), and the figures were constructed using Microsoft Excel 2016.

RNA extraction and cDNA synthesis

Total RNA was extracted from three grams of apple fruit tissue by Gasic et al. (2004). Spectrophotometry (ScanDrop 250-Analytikjena, Germany) was used to quantify RNA, and the quality of RNA was checked on a 1% TAE gel. In order to remove DNA contamination, DNase I enzyme (Fermentas, Lithuania) was used according to manufacturer's instruction. After removing the contamination from the sample RNAs, the quantity of RNA was checked and, then reversed transcribed using 1.5 µg RNA, 1 µL MMLV reverse transcriptase (Fermentas, Lithuania), 1 µL Rnase inhibitor, and 2.5 mM dNTP mix according to manufacturer's instruction (Fermentas, Lithuania).

Primer design and gene expression analysis

Specific primers for expression of PR5, *ERF1a* and *CHIB* genes were selected from the articles and then analyzed by Oligo analyzer software according to the information available on the GDR (apple genome) site (Jung et al., 2019). Three pairs of primers were selected and synthesized by Pishgam Co. to amplify the desired genes. Each primer pair was checked by agarose gel electrophoresis to ensure the amplification of specific fragments with the expected length. Two reference genes were used to normalize the expression of the desired genes. The sequence of primers and the length of the amplified fragment are shown in Table 1.

The qRT-PCR reaction was carried out in a total volume of 10 µL, including 2 µL of diluted cDNA, 0.2 µL of each primer (10 µM), 5 µL 2X SYBR green (Yekta Tajhiz Azma Co.) using the real-time PCR machine (Rotor gene 3000). The amplification condition was set to 30 s at 95 °C, 40 cycles of 5s at 95°C and 30s at 60°C. In the qRT-PCR, a negative control was used to ensure the specific amplification. To analyze the data and to evaluate the expression of the studied genes, the relative comparison method of Faffel was used (Pfaffl, 2001). The Ct (threshold cycle) of the reference gene and the main gene treated with hormone and without hormone (control) were extracted and used to calculate a ratio for analyses.

RESULTS

Evaluation the firmness and softening of fruit

The initial firmness was 10.7 kg cm^{-2} in Fuji, while Golden Delicious had an initial firmness of 10 kg/cm^2. Firmness after storage was 9.1 and 9.5 for Fiji and Golden Delicious, respectively. The softening rate during storage (difference between initial firmness and firmness after storage) was 5.4 and 15.1%, for Fuji and Golden Delicious, respectively. The softening rate showed a significant difference between Golden Delicious and Fuji cultivars (t= -26.08, p-value<0.001), while the difference between two cultivars was not significant for neither initial firmness (t= -2.36, p-value=0.1) nor the firmness after storage (t= 1.96, p-value=0.15). As a result, Fuji showed a relatively higher rate of softening than Golden Delicious cultivars (Fig. 1).

Table 1. Primers used in the present study. Primers are extracted from the litritures and were checked and blasted de novo with GDR database

Gene	Sequence length	Forward Primer	Reverse Primer	Reference
CHIB	181	CTCACTCAGGCATTCTTCG	CATGGGTGACATGAGCAAA	Shin et al. 2014.
ERF1	171	CAGTTGAAAGAGTCCGCAA	CAAACACCACCACATTCTCTA	Shin et al. 2014.
PR5	153	CATGTCCTCCCACAGAGTAC	ATATAATCCCATTTCGTGCTTATG	Zhang et al. 2010.
UBC	117	TTGCTGGTGATCTCTGCATC	AGACCCACCTACTCCCGTCT	Storch et al 2015
ACTIN	140	GGCTCTATTCCAACCATCCA	TAGAAGCAGTGCCACCACAC	Storch et al 2015

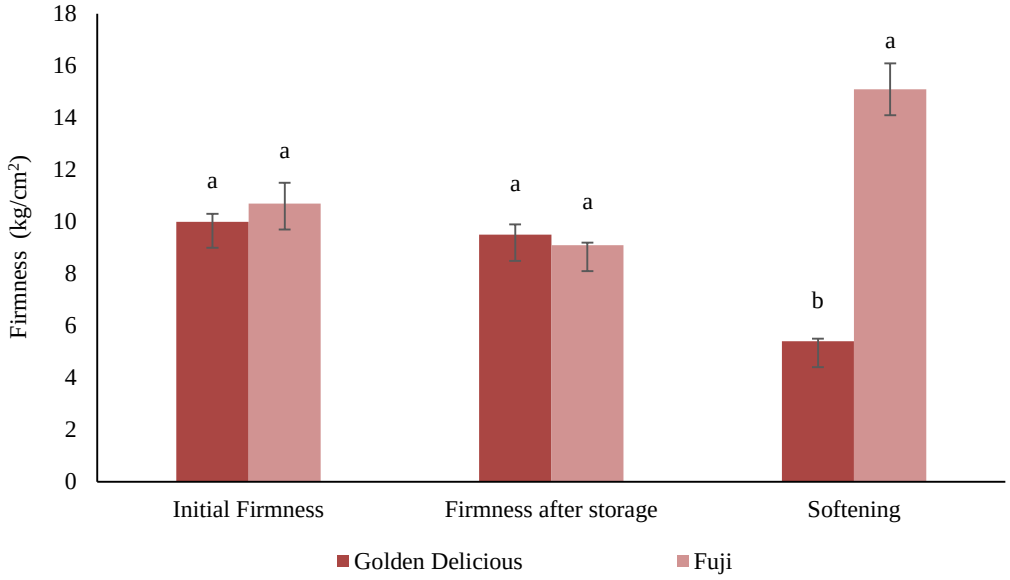

Fig. 1. Initial firmness, firmness after storage and softening rate of apple fruit in Golden Delicious and Fuji. The initial firmness and firmness after storage were not significantly different between cultivars, while Fuji has a significantly higher rate of softening in comparison with Golden Delisious (n=average of 10 fruit, $\alpha = 0.05$).

Assessing the level of infection severity in fruit

The results of inoculation evaluation showed that Golden Delicious produced more lesion area in all three treatments, i.e., 1) inoculated fruits-no hormone treatment, 2) inoculated fruits-ACC treatment and 3) inoculated fruits-BABA treatment, while Fuji had, in overall, smaller lesion area after inoculation. For the Golden Delicious fruit inoculated with fungal spores and treated with ACC, the highest infection severity was observed (lesion area = 29.4 mm) which was slightly higher than the lesion area for no hormone treatment (lesion area = 28.7 mm). The lesion area for the Golden Delicious fruit inoculated and treated with BABA (lesion area = 18.8 mm) was significantly smaller compared with two other treatments. For the Fuji cultivar, a similar result was obtained, where the treated with BABA showed the lowest level of contamination (16 mm). It can also be observed that treatment with BABA hormone was effective in reducing the level of infection in both cultivars (Fig. 2).

These results showed that in Fuji cultivar, despite the high softening percentage observed, but in terms of inhibiting the level of contamination compared to the Golden Delicious cultivar had a better performance.

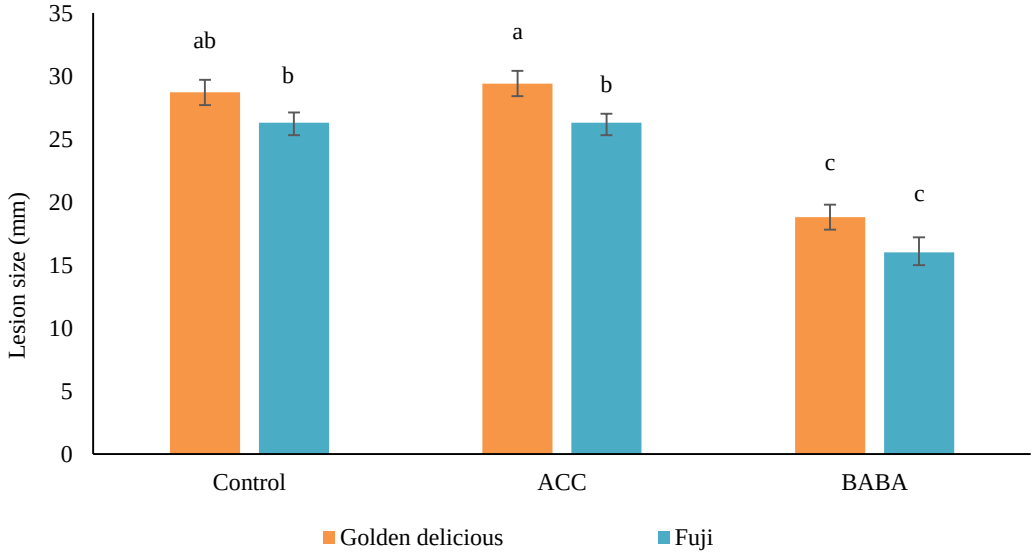

Fig. 2. Lesion area of blue mold in two Golden Delicious and Fuji apple in three tyreatments, i.e. control (no hormone treatment), ACC and BABA treatments. According to the results, treatment with BABA, reduced the development of disease in both cultivars.

Gene expression analysis

To check the specificity of primers, qRT-PCR melting curve analysis was performed. The results of the melting curve showed a single and specific amplification for all primers/samples, thus showing the accuracy of primes choose. Then to evaluate the expression of studied genes, a further qRT-PCR was performed. According to the results, the expression of all three genes, i.e. *CHIB*, *ERF1* and PR5 were significantly upregulated in both Golden Delicious and Fuji cultivars when ACC hormone treatment was compared to the control, i.e. no hormone treatment (Fig. 3).

Our results showed that the expression of *CHIB* was 5.9 times higher when inoculated fruits was treated with ACC. The *CHIB* gene showed a 1.6-fold change increases when Fuji fruit was treated with ACC. Similar results were obtained when the expression of *ERF1* gene was studied. In Golden delicious, a 6.2-fold change increase was observed when the ACC treated fruit was compared with their control. The expression of *EFR1* showed a 3.9 times higher expression in ACC treated in compared with control fruit. The upregulation of genes after treatment with ACC was significantly higher in Golden Delicious than Fuji cultivar, for both above-mentioned genes (Fig. 3).

For fruits treated with BABA hormone, the expression of the *PR5* gene showed a 6.9 times increases when BABA treated fruits were compared with the control fruits in the Golden Delicious cultivar. For Fuji cultivar, the same increase was observed thus a 10.4-fold change increase was observed when BABA treated fruits were compared with controls. As it can be seen from Figure 3, the upregulation of PR5 was higher in Fuji than Golden Delicious cultivar.

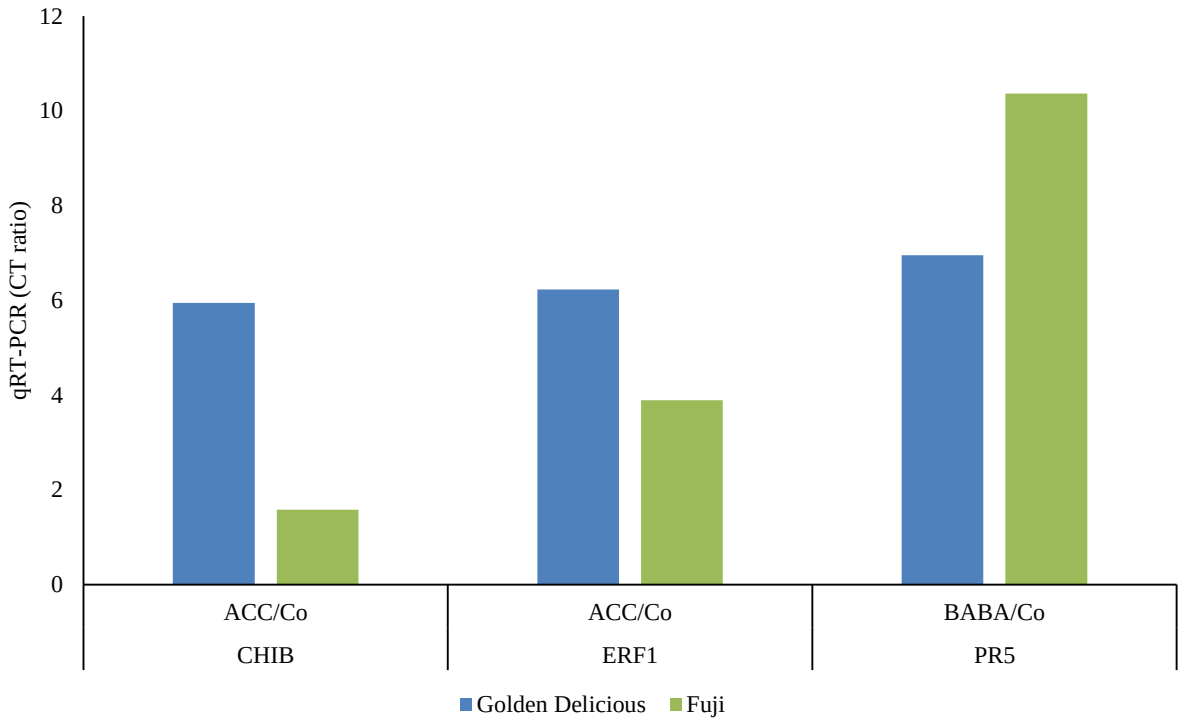

Fig. 3. Evaluation of CHIB, ERF1 and *PR5* gene expression after treatment with ACC and BABA in Golden Delicious and Fuji cultivars. The expression of all three genes was increased in two cultivars after hormone treatment. The *CHIB* and *ERF1* genes were more upregulated in Golden Delicious while the expression of PR5 was higher in Fuji than Golden Delicious.

DISCUSSION

Plant pathogen interaction is a well-understood mechanism that involves the activation of signaling pathways that result from a rapid defensive response to a set of plant pathogens. This response helps the host plant to prevent the further spread of the disease. Induction of plant defense signal involves the identification of specific pathogen effects by the products of host genes called resistance (R) genes (Iqbal et al., 2021). Fradin et al. (2009) investigated the positive role of R genes against *Verticillium* wilt in tomatoes. The plant defense system activates signals in various ways and through the use of transcripts copied from genes that these signals cause or stop the production of substances in the plant and thus resulting in resistance to diseases (Yang et al., 2013; Alazem et al., 2014). Different compounds can trigger the plant immunity system to respond to fungal attacks. One of the recently studied compounds is plant hormones that can produce different signal pathways that stimulate different defense genes (Çevik et al., 2012). Plant hormones are important signaling molecules, and they play an important role in regulating the immune response of plants to pathogens (Pieterse et al., 2012). The inhibitory impact of different plant hormones has already been reported on fungal disease. Reduction of symptoms caused by blue mold in apple cultivars after treatment with methyl jasmonate was first reported by Ahmadi-Afzadi et al. (2018). It was reported the activation of cell wall genes and the flavonoid pathway to blue mold resistance. In another study, the effectiveness of the two hormones methyl jasmonate and salicylic acid in sweet oranges was first reported. This report showed that SA has a significant effect on inhibiting germination and spore growth, and methyl jasmonate has a suppressive effect on fungal growth (Iqbal et al., 2012).

Impact of external application of hormone treatment on disease development

In the present study, treatment with BABA hormone could effectively reduce the lesion size on fruit challenged with fungal spores. In both cultivars, a significant reduction was observed when hormone-treated fruits were compared with controls (no hormone treatments). Similar research has already reported that hormones may effectively reduce the fungal disease severity. Hodge et al. (2005) found that BABA significantly reduced the growth of *Assyrotyphon pysum* in chickpeas, broad beans, green beans, red clover and alfalfa. It also increased aphid mortality, decreased the average relative growth of insects and decreased the intrinsic rate of the population. Li et al. (2019) showed that treatment with BABA hormone increases resistance to the pathogen *Kaltotrichum glasporida* and also leads to accumulation of salicylic acid in mango fruit from day four to eight days after treatment. According to studies by Wang et al. (2018), BABA treatment was initiated by inducing resistance to the immune response to control the growth of *rhizopus* rot in harvested peaches by increasing the expression of defense-related genes. BABA also stimulates the activity of enzymes involved in lignin biosynthesis and metabolic pathways, thereby maintaining cell wall strength in harvested peaches, which helps increase disease resistance to *rhizopus* rot (Wang et al., 2018). Quaglia et al. (2017) found that treatment with BABA hormone caused accumulation of *PR-1a* and *LOX-1a* gene transcripts and callus deposition in Golden Delicious fruit and increased resistance to blue mold.

In contrast, the application of ACC hormone did not significantly alter the lesion development of both Golden Delicious and Fuji fruits. It has already been reported that ACC hormone is responsible for the ethylene production pathway. Ethylene production has been described as one of the main factors influencing fruit maturation and softening. Due to the impact of ethylene on fruit ripening, it may be hypothesized that more ripe fruits may be more sensitive to fungal attack and development, resulting in larger lesion decay on fruits. The association between the rates of fruit firmness (less softening) with the level of resistance has already been reported in previous studies (Ahmadi-Afzadi, 2013). Thus, it can be concluded that the impact of ACC on fruit ripening may facilitate the pathogen development in fruits. However, more studies are needed to confirm this.

***CHIB* gene expression and resistance induction**

The results of *CHIB* (chitinase) gene expression showed the possibility that ACC hormone could have a positive effect on increasing the expression of this gene; because the results of the control samples showed that the expression of the CHIB gene was created when the infected cultivars were treated with ACC hormone. It was also observed that the expression was more upregulated in Golden Delicious cultivars compared to Fuji. It is reported that plant chitinases play an essential role in the plant's inherent resistance to pests and diseases (Kumar et al., 2018). Navarro-Gonzalez et al. (2019) investigated the resistance of transgenic sugarcane plants to the necrotrophic fungus *Butyris cinera*. In this study, it was shown that chitinase gene activity and resistance to necrotrophic fungi and pests were significantly increased in transgenic plants compared to wild plants. These enzymes form part of PR proteins in plants and have been widely used in modern biotechnology to study their potential for increasing plant resistance (Kumar et al., 2018). Das (2018) reported high chitinase activity in transgenic plants compared to non-transgenic plants that this increase was associated with resistance to powdery mildew pathogens. Cao and Tan (2019) reported that the rapid evolution of plant chitinases indicates a simultaneous interaction between plants and pathogens. In addition, changes in these proteins may explain the diversity of disease resistance. He also reported that the chitinases identified in tomatoes may be responsible for the host's defense against pathogens.

ERF gene expression and resistance induction

The results of qPCR showed that the *ERF1* gene is induced in samples treated with hormones compared to untreated samples. This finding may indicate that ACC hormone can have a positive effect on increasing the expression of *ERF1* gene. Similar to results of *CHS* gene, *EFR1* also was more upregulated in Golden Delicious than Fuji. *ERF* genes are the downstream components of the ET signaling pathway and are known as the various signaling pathways in plant hormones. The pathogenic attack leads to transcriptional and metabolic changes that result in adequate plant defense (Huang et al., 2016). Transcription factors (TFs) play a major role in the innate immunity of plants. Notably, ethylene responses (ERF) integrate hormonal pathways and are directly responsible for regulating the transcription of several Jasmonate (JA) / ethylene (ET) defense genes. Transcriptional activation or repression by ERF is achieved by binding to the JA / ET responsive gene promoter (Huang et al., 2016). Research by Shi et al. (2019) on post-harvest disease and ripening of wild pear species, showed that *PpEIN3b* gene expression was significantly increased under ACC treatment, and also showed that *PpEIN3b* may be a negative regulator in delaying fruit ripening and aging to increase fruit shelf life. In regards to the previous reports, our results also indicated that the ERF gene expression may be induced in response to the ACC treatment. On other hand, the inhibitory impact of ACC hormone was not significantly different from control fruits. Thus, we speculate that despite ERF gene induction, fruit softening due to ACC application may have a greater impact on disease development, resulting in a non-significant lesion decay reduction on both cultivars.

_PR5_ gene expression and resistance induction

BABA is one of the plant hormones that have been reported as an inducer component in providing resistance to biological and abiotic stresses in many plants (Satková et al., 2017). In the present study, BABA hormone could significantly reduce the fungal growth on fruits of both cultivars (35% and 40% on Golden Delicious and Fuji, respectively). The inhibitory impact of BABA has already been reported in tomato plants against powdery mildew infection (Worrall et al., 2012). Bengtsson et al. (2014) also reported that BABA can develop resistance in a wide range of plants against a variety of pathogens, including *Phytophthora* in potatoes. Their studies have shown that BABA reduces the growth of *Phytophthora* on potatoes and that BABA appears to affect plant hormone processes and amino acid metabolism.

In this study, the results showed that the PR5 resistance gene is expressed in both control and hormone-treated cultivars, but this expression is more upregulated in fruits treated with BABA hormone. The synthesis of pathogen-related proteins PR1 and PR5 is an example of induced defense mechanisms in which plants can resist pathogen attack and their expression is directly related to resistance (Engelbrecht & Van den Berg 2013). Lu et al. (2019) in their studies showed that the application of Salicylic Acid and Mesoporous Silica Nanoparticles mesoporous silica nanoparticles increased the expression of PR1 and PR5 genes in infected pineapple roots. They also showed that the expression level of PR1 and PR5 genes provide long-term protection against pathogenic attacks. According to the above studies, it can be assumed that the BABA hormone has a positive role in the expression of this resistance gene due to its ability to stimulate resistance to plant diseases and also to increase resistance to abiotic stresses on plants. Also, it can be involved in increasing the resistance of apple fruits to blue mold by the activity of the plant immune system.

CONCLUSION

Conventional methods of controlling diseases and fungi in horticultural crops may not be effectively useful to solve the postharvest problems for gardeners for a variety of reasons. These factors have motivated researchers to come up with better alternatives to the above-mentioned methods. Understanding the mechanism of the disease and the interaction between the disease and the plant defense system, the effect of biocontrol agents on pathogens provide important steps in defining resistance and producing tools for plant breeding and to breed resistant cultivars. The mechanism of resistance in plants is controlled by resistance genes. Plant hormones have important effects on signaling pathways and defense systems. These effects include the stimulation of various defense genes against pathogens. In this study, the effect of hormones on the expression of resistance genes was investigated. Overall, it can be concluded that plant hormones often lead to increased expression of genes involved in resistance and thus increase resistance in apple fruit against blue mold. BABA is known for its ability to stimulate resistance to plant diseases as well as increase resistance to non-living stresses on plants. Instead of having a direct effect on the plant pathogen, it activates the plant immune system and allows them to develop more effective resistance to infection. *PR5* resistance genes play an important role in plant resistance. ACC plays an important role in plant ethylene biosynthesis. This hormone regulates a wide range of growth and developmental processes. According to the results of this study and previous studies, ACC hormone may have a contradictory influence on resistance mechanisms. Although, it can induce the above-mentioned genes, it also mediates in the fruit ripening process resulting in softer fruit. Thus, fungal development and lesion decay can be facilitated on fruits treated with ACC.

Author contribution statement

Z.M. has performed and analyzed the experimental part, and has written the first draft of manuscript. MAA has designed the experiment and participated in writing and revising the manuscript. S.M., M.M and M.R. have read and participated in revising the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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