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Research Article

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Effect of abscisic acid on rice defense mechanism against *Fusarium oxysporum*

Guo Peng, Yang Ming Xiu, Li Yun Peng, Haseeb Younis, Ni Zhe, Xu Xiao Feng, Liu Qing Ran, Xiong Tian Liang, Zhang Jun Hua*

College of Agriculture, Northeast Agricultural University, Harbin 150030, China

ABSTRACT

Fusarium oxysporum is one of the most destructive pathogens which causes rice seedling blight. ABA is part of a large signaling system that provides an effective system against microbial and environmental manipulations. The role of ABA in plant defense mechanisms is not clear. In this experiment, we prove the role of abscisic acid (ABA) in boosting rice plant resistance against *F. oxysporum* and optimizing ABA concentrations against *F. oxysporum*. This study is divided into two experiments. In the first experiment, we used various ABA concentrations of 0.0, 0.05, 0.1, 0.2, and 0.25 mmol/L under *F. oxysporum* stress. In the second experiment, we use Fluridone FLD as an ABA inhibitor with the following treatments, (F) is only applied with *F. oxysporum* (ABA+F), abscisic acid with *F. oxysporum* (ABAI+F), ABA inhibitor Fluridone with *F. oxysporum* (ABAI), where only ABA inhibitor Fluridone was applied and CK was used as a control. The results revealed that all the plants treated with ABA exhibit better performance against *F. oxysporum*, except those treated without ABA. ABA concentrations of 0.2 mmol/L effectively decreased the disease index and disease incidence rate as well as improved the quality of seedlings. ABA effectively increased the activity of defense-related enzymes like PPO, POD, PAL and SOD. ABA also lowers down the MDA content which proves its effectiveness against *F. oxysporum*. ABA resistance was also proved by plants treated with the abscisic acid inhibitor ABAI (Fluridone FLD). The ABA inhibitor reduced the rice resistance to *F. oxysporum*, by conforming the expression of defense-related genes PRB1-3, PRBI-2 and *Xa39(t)*. These gene expressions indicate the involvement of ABA in plant defense system.

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***Corresponding Author:**
Zhang Jun Hua
E-mail: podozjh@163.com

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. For many countries, rice is the staple food and major source of energy. Plants in natural environments face numerous biotic and abiotic stresses, requiring a strong immune system to survive and combat pathogens.

Fusarium species, particularly *Fusarium oxysporum*, are a highly destructive group of plant pathogens, causing significant economic damage (Aoki *et al.*, 2014). It causes great economic losses in global agriculture production. This pathogen is primarily responsible for Rice seedling blight. It is a soil-borne fungal pathogen that causes necrosis and wilting symptoms in rice plants due to the colonization of xylem tissue, making it the main disease at the seedling stage (Tjamos, 1989). To fight against *Fusarium*, farmers use various chemical fungicides. These fungicides are very harmful to the environment, and pathogenic variability often restricts their efficiency (Swarupa *et al.*, 2014). Against these types of pathogens, plants develop different types of strategies like the production of defense-related enzymes and hormones. Phytohormones such as abscisic acid (ABA), cytokinins (CKs), gibberellic acids (GAs), jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) are well known for their role in plant defense regulation (Di *et al.*, 2016). These hormones are also known for plant growth development and triggering of vital plant signaling when the plant comes under biotic and abiotic stress (Peleg & Blumwald, 2001; Vleesschauwer *et al.*, 2010; Spence *et al.*, 2015).

ABA is a key phytohormone involved in many metabolic processes like plant defense, adaptation to environmental stress, seed dormancy, and inter-species communication (Hauser *et al.*, 2011; Lievens *et al.*, 2017). ABA is part of a larger signaling system that provides an effective system against microbial and environmental manipulations (Lim & Lee, 2015; Di *et al.*, 2016). The role of ABA in plant defense mechanisms is remains inconsistent. For instance, ABA has shown some

roles in stomatal closure and opening, redox homeostasis and controlling some expression of defense-related genes (Agarwal & Jha, 2010). Furthermore, lower concentrations of ABA induce an antioxidant defense response against oxidative damage, but higher concentrations of ABA on the other side lead to the excessive generation of reactive oxygen species, which cause oxidative damage in plant cells (Jiang & Zhang, 2001). A recent study reported that in the early hours of *F. oxysporum* infection in flex, the non-mevalonate pathway is activated and there is a redirection of metabolites towards ABA synthesis. This increased ABA synthesis correlates with flax resistance (Boba *et al.*, 2020). Mycotoxins production has anti-pathogen activity and it was changed with the induction of ABA (Xu *et al.*, 2018).

Two distinct mechanisms by which ABA increases plant resistance to pathogens are callose priming and the regulation of defense-related gene expression through the activation of JA biosynthesis (Adie *et al.*, 2007; García-Andrade *et al.*, 2011). Transcriptome analysis of tomatoes exposed to exogenous ABA also revealed the expression of numerous genes involved in plant defense against infections. These genes regulate plant signaling networks, enzymatic activity, and free radical regulation (Anderson *et al.*, 2004; Wang *et al.*, 2013).

Several enzymes are involved in plant defense mechanisms such as L-phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD). PAL is the major enzyme of the phenylpropanoid pathway which transforms L-phenylalanine into trans-cinnamic acid which ultimately leads to the synthesis of lignin which is the main product of phenylpropanoid metabolism. This mechanism is vital for protection against pathogen invasion (Geetha *et al.*, 2005). PPO has a vital role in plant-defense resistant cultivars and showed higher PPO activity in comparison to susceptible cultivars against pathogens (Das *et al.*, 2004). POD also played a significant role in plant defense. In this study, we will determine the effect of ABA on plant defense mechanisms by evaluating defense-related enzymes like PAL, PPO, POD, superoxide dismutase (SOD), and malondialdehyde (MDA) content in rice leaves. The expression levels of some resistance genes, such as (PRB1-3, PRBI-2, *Xa39(t)*), were also analyzed. Genes PRB1-3 and PRBI-2 were reported to have functions in response to pathogen infection and defense-related mechanisms. These genes encode pathogenic-related proteins (Xu *et al.*, 2011; Liu *et al.*, 2017). Gene *Xa39(t)* is a novel dominant gene in rice conferring broad-spectrum resistance throughout the whole host growth cycle (Zhang *et al.*, 2015). Because of their significant role in plant defense systems, these genes were selected.

Some scientists also reported the negative effect of ABA on plant resistance (Kim *et al.*, 2011; Spence *et al.*, 2015). The role of ABA can either be positive or negative, depending on the pathogen encountered (Ton *et al.*, 2009; Robert-Seilaniantz *et al.*, 2011). Therefore, a comprehensive study is required to define the exact role of ABA in plant defense. We believe that this study will provide a new base for the plant disease resistance induced by exogenous ABA and open new horizons for controlling fungal diseases.

MATERIALS AND METHODS

To conduct this research, we used the rice variety Kongyu 131 and the *F. oxysporum* strain F02016038, which were provided by the Department of Plant Pathology, College of Agriculture, Northeast Agricultural University. ABA and ABA inhibitor (Fluridone FLD) were purchased from Henan Boming Biological Company.

Culturing of Plant Pathogenic *F. oxysporum*

F. oxysporum strain F02016038 was grown in petri plates on potato dextrose agar (PDA) at 28 ℃ for 3 days. After the fungus was grown, we took 5 pieces of fungus cake with a diameter of 5 mm from the fungus colony and placed them in liquid PDB media containing 100 mL of potato dextrose in a 250 mL triangular flask. This flask was then placed in a shaking incubator at 28 ℃ and 120 rpm for 5 days. Following that, we centrifuged it at 5000 rpm for 10 minutes. The precipitated spores were re-suspended in sterile water to produce a pathogen spore suspension. The spore count was measured using a hemocytometer and adjusted to 1×105 spores/mL.

Application of Different Concentrations of ABA on Rice Seedling Blight

ABA solution of 0.0, 0.05, 0.1, 0.2, and 0.25 mmol/L concentrations was sprayed on rice seedlings at the trefoil stage. Each treatment was replicated three times. After 24 h of ABA treatments, 10 mL of spore suspension with a concentration of 1x106 CFU/mL was applied to the roots of all the plants. After 6 days of inoculation, the disease condition was recorded, and the disease incidence rate and disease index were calculated.

Application of ABA Inhibitor FLD on Rice Seedling Blight

In this experiment, we examined the effects of ABA and the ABA inhibitor FLD on rice seedling blight. This experiment includes five treatments. The treatments are as follows: 0.2 mmol/L ABA inhibitor FLD with *F. oxysporum* (ABAI+F), ABA inhibitor FLD without *F. oxysporum* (ABAI), 0.2 mmol/L ABA with *F. oxysporum* (ABA+F), and one group treated only with *F. oxysporum* without ABA and ABAI (F), with the CK group serving as the control. All these treatments were applied through foliar application at the trefoil stage each treatment was replicated three times. After 24 h of these treatments, we applied a 10 mL spore suspension with a concentration of 1x106 CFU/mL to the roots of all the plants.

Evaluation of Disease Incidence Rate and Disease Index

After 6 days of inoculation, the disease condition was recorded, and the disease incidence rate and disease index were calculated.

Incidence rate = (Number of diseased plants/Total number of plants × 100%

Disease index =(Number of disease grade plants × Disease grade/(Total number of plants × Highest disease grade)× 100%

Determination of Fresh Weight, Dry Weight, Plant Height and Root Length

To determine the fresh weight of roots and shoots we use an electronic weight balance. After measuring the fresh weight, we put the roots and shoots in paper bags separately and place them in the oven at 105 °C for 72 h to determine the dry weight. Plant height and root length were measured by using a measuring tape.

Determination of Enzyme Activity and MDA Content

To determine the role of exogenous ABA in plant resistance, we conducted two experiments. In the first experiment, we used different concentrations of exogenous ABA. These are 0.0, 0.05, 0.1, 0.2, and 0.25 mmol/L. In experiment two, we have five treatments, including 0.2 mmol/L ABA inhibitor FLD with *F. oxysporum* (ABAI+F), ABA inhibitor FLD without *F. oxysporum* (ABAI), 0.2 mmol/L ABA with *F. oxysporum* (ABA), only *F. oxysporum* (F), and the CK. To determine enzyme activity and MDA content, we collected the samples at 0, 24, 48, 72, 96, 120, and 144 h after inoculating them with *F. oxysporum* and stored them at -80 °C. Enzymatic activities such as POD were determined by using the methods described by (Rao *et al*., 1996), SOD (Giannopolitis & Ries, 1977), PAL (Şirin & Aslım, 2019), PPO (Soliva *et al*., 2000), and MDA (Wang *et al*., 2015).

Analysis of Gene Expression by Quantitative Real-time PCR (qRTPCR)

To analyze the gene expression by qRTPCR, plant samples were taken at 0, 24, 48, 72, 96, 120, and 144 h after inoculation with *F. oxysporum*. The total RNA of samples was extracted by the TRIzol method (Liao *et al*., 2014), the RNA extraction kit was obtained from (Beijing ComWin Biotech Co., Ltd, China) and the first strand of cDNA was synthesized by reverse transcription with the M-MuLV enzyme. The cDNA was synthesized using cDNA synthesis Kit (Beijing DiNing Biotechnology. China).

The quality of the RNA extracted from rice seedlings was detected by 1% agarose gel electrophoresis. The total three RNA bands detected are 28S, 18S, and 5S, among them 28S and 18S are bright and clear, and 5S rRNA bands are dim (Figure 1), which indicates that total RNA extracted from rice seedlings is of high quality and free from impurities such as protein. The total RNA of rice seedlings was determined by using an ultraviolet spectrophotometer, and their A260/A230 values were between 2.0 and 2.2 and the A260/A280 values were between 1.8 and 2.15, which can be used for subsequent experiments such as reverse transcription and fluorescence quantification.

The expression of rice resistance genes (PRB1-3, PRBI-2, *Xa39(t)*) under the stress of *F. oxysporum* was analyzed by the RNA-seq technique. To calculate transcript level β-actin gene was used as a reference gene. The sequence of primers was described in Table 1. For this purpose, we used the test kit SGExcel FastSYBR Mixture (Plus) (Shenggong Bioengineering Co., Ltd.).

Gene expression in rice seedlings was detected by qRT-PCR. It can be seen from the primer melting curve (Figure 1) that the primers of the three genes can produce obvious single peaks, and there are no redundant miscellaneous peaks, which indicates that the data obtained from the gene expression experiment by qRT-PCR technology is reliable.

Statistical Analysis

All the treatments were replicated three times, and statistical analyses were performed using SPSS software (version 9.1, SPSS Institute Inc., Cary, NC, USA). All data were expressed as means±SD. The Tukey test was used ($p \leq 0.05$) for analyzing the differences among the exogenous ABA concentrations.

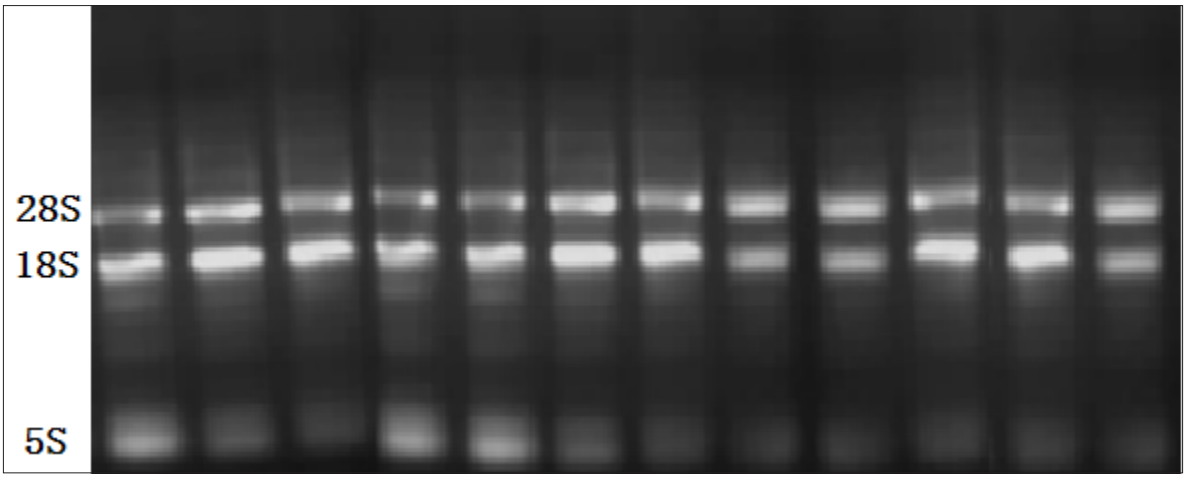

Figure 1: Electrophoresis results of total RNA of partial rice samples

RESULTS

Effects of different ABA Concentrations on Rice Seedling Blight

Our results indicated that exogenous ABA has a positive effect against rice seedling blight (Table 2). On the 6th day of inoculation, most of the rice seedlings in the group without ABA withered or died, and the incidence rate was close to 100%. The disease incidence rate and disease index decrease with an increase in ABA concentrations. Compared with 0.0 mmol/L ABA, the incidence rate and disease index of ABA treatment groups with concentrations of 0.05 mmol/L, 0.1 mmol/L, and 0.2 mmol/L ABA were 75.12%, 60.05%, and 45.19%, respectively. However, the incidence rate and disease index of rice seedlings treated with 0.25 mmol/L ABA were 45.76% and 21.33, respectively, which were higher than those treated with 0.2 mmol/L ABA.

Effect of ABA on Fresh Weight, Dry Weight, Plant Height and Root Length

Plants treated with ABA have significantly higher fresh weight, dry weight, plant height, and root length in comparison to the plant group treated without ABA. The highest plant height (16.3 cm) and root length (8.61 cm) were achieved by the plants treated with 0.2 mmol/L exogenous ABA. This treatment is followed by plants treated with 0.25 mmol/L exogenous ABA. This treatment has 16.15 cm plant height and 16.15 cm root length, respectively. The plants where no ABA was applied showed the lowest plant height of 13.11 cm and root length of 6.23 respectively.

Fresh weight and dry weight also show similar trends. The highest fresh weight of shoots (19.91 g) and roots (6.02 g) was achieved by plants treated with 0.2 mmol/L exogenous ABA, and the lowest was produced by plants treated without ABA having shoots and root fresh weight of 16.77 and 4.61 g respectively. Plants treated with 0.2 mmol/L exogenous ABA have the highest shoot and root dry weight of 4.12 g and 3.11 g, respectively, and the lowest dry weight of shoot and root was produced by plants treated without ABA. Details of these results are presented in Table 3. From these results, we can conclude that exogenous ABA has a significant impact on rice plants, and ABA treatment of 0.2 mmol/L proves that above this concentration, the quality of seedlings started to deteriorate.

Effects of ABA on Defense-related Enzymes and MDA Content in Rice

The application of ABA shows a significant effect on enzymatic activity. Under the stress of *F. oxysporum*, the POD activity of rice seedlings in all the treatments increased at first and then decreased. Within 24 h after the inoculation, the POD activity started to increase. This indicates that ROS (reactive oxygen species) began to damage rice cells, and POD activity was increased to alleviate this damage. POD activity reached its peak after 72 h of inoculation in all the treatments. At this point, the rice seedlings treated with ABA concentrations of 0.05 mmol/L, 0.1 mmol/L and 0.2 mmol/L increased by 3.03%, 17.58% and 24.50%, respectively, in comparison to the plants treated without ABA. After 72 h the POD activity started to decrease, possibly due to the deepening of damage caused by ROS. After 144 h the POD activity of the plant treated with 0.05 mmol/L of exogenous ABA was close to that of group where no ABA was applied, while the plant treated with 0.1 mmol/L and 0.2 mmol/L of exogenous ABA had significantly higher POD activity than that of the control group. A detailed comparison of treatments is shown in (Figure 2A). These results indicate that the plants treated with exogenous ABA can effectively regulate

Table 1: Primer sequences for PCR

Gene Symbol	Gene Name	Primer Sequence	Note
LOC4342317	PRB1-3	F: GGTGTCGGAGAAGCAGTGGTAC R: GGCGAGTAGTTGCAGGTGATGAA	Pathogenesis-related protein
LOC4342313	PRBI-2	F: CGGTTGGCTTGTGGATGGAGGA R: TGAGGACATCGCCGTTGTTGC	Pathogenesis-related protein
LOC9267585	*Xa39(t)*	F: TCCTCCTCAGATGTCTCACTCACT R: GTTCTTGGCTTCTTGCTGCTCTTG	Disease resistance protein RGA5-like
LOC4349863	*β-actin*	F: CTGGTATCGTGTTGGACTCTGG R: CCCGTTCAGCAGTGGTAGTG	Reference gene

Table 2: The effect of exogenous ABA and ABAI on Rice Seedling Blight Disease

	Treatments (mmol L^{-1})	Incidence Rate (%)	Significant Difference P<0.05	Disease Index	Significant Difference P<0.05
ABAI Comparison	CK	0	E	0	E
	F	89.27	A	76.25	A
	ABA (0.05)+F	75.12	B	50.27	B
	ABA (0.1)+F	60.05	C	31.13	C
	ABA (0.2)+F	45.19	E	20.05	E
	ABA (0.25)+F	45.76	D	21.33	D
	CK	0	D	0	D
	F	89.27	B	76.25	B
	ABA (0.2)+F	45.19	C	20.05	C
	ABAI+F	93.57	A	83.37	A

Table 3: Effects of exogenous ABA on seedling quality under *Fusarium oxysporum*

ABA Concentration (mmol L^{-1})	Fresh wt (g)		Dry wt (g)		Plant Height (cm)	Root Length (cm)
	Above Ground	Underground	Above Ground	Underground		
0	16.77[e]	4.61[e]	3.12[e]	2.12[e]	13.11[e]	6.23[e]
0.05	17.12[d]	5.42[d]	3.43[c]	2.98[c]	14.81[c]	7.37[c]
0.1	17.51[c]	5.64[c]	3.42[c]	3.01[c]	15.26[c]	7.77[c]
0.2	19.91[a]	6.02[a]	4.12[a]	3.11[a]	16.3[a]	8.61[a]
0.25	19.34[b]	5.83[b]	3.98[b]	3.07[b]	16.15[b]	8.37[b]

the activity of POD in plants to alleviate the cell damage caused by *F. oxysporum*.

Under the stress of *F. oxysporum*, the SOD activity started to increase within 24 h of inoculation at first, it increased rapidly, but after 120 h it started to decline in all the treatments. This indicates that SOD activity was increased under *F. oxysporum* stress. SOD activity was used to remove reactive oxygen species. All the treatments reached their peak at 120 h after the inoculation. At this point, the SOD activity of rice seedlings treated with exogenous ABA with the concentrations of 0.05 mmol/L, 0.1 mmol/L, and 0.2 mmol/L increased by 1.38%, 23.40%, and 31.65%, respectively, in comparison to plants without ABA. After 120 h of continuous development of the disease, the antioxidant system of the plant started to collapse, and the ability to regulate SOD began to decline, which led to the decline of SOD activity. At 144 h, the SOD activity of the exogenous ABA treatment group was significantly higher than that of no ABA group. SOD activity was described in (Figure 2B).

The PAL activity of rice increased steadily under *F. oxysporum* stress in all the treatments. After inoculation, the PAL activity of each treatment group began to increase slowly after 24 h. All the treatments reached their peak after 96 h except the treatment at 0.05 mmol/L. This treatment showed two peaks, the first peak appeared at 48h with an increase of 22.48%, and the second peak appeared at 96 h with an increase of 12.28% in comparison to the group without ABA. Plants treated with ABA concentrations of 0.1 mmol/L and 0.2 mmo/L had have higher PAL activity since the beginning when we compared them with the no ABA group. PAL activity is increased by 5.26% and 46.78%, respectively. These results indicate that exogenous ABA treatment can significantly increase PAL activity in plants. PAL activity was briefly explained in (Figure 2C).

After the initial inoculation, PPO activity of rice seedlings showed almost the same results their enzymatic activity did not change significantly. Plants treated with exogenous ABA exhibit higher PPO activity in comparison to the control group. After reaching its peak at 96 h PPO activity started to decrease due to the deepening of the destruction of the antioxidant system. At 144 h, the PPO activity of the exogenous ABA treatment group was close to that of the control group. PPO activity was briefly explained in (Figure 2D).

When plants come under the *F. oxysporum* stress, MDA content in all the treatments starts to increase. Initially, MDA content increased slowly from 0 to 48 h. After 72 h, the degree of cell membrane peroxidation began to increase gradually, and MDA content began to rise rapidly. MDA content is significantly higher in the plants treated without ABA than in the plants treated with exogenous ABA. This can be seen in (Figure 2E). With the increase in ABA concentration, the MDA content decreased significantly. Therefore, exogenous ABA can reduce the degree of membrane lipid peroxidation in rice seedlings under the stress of *F. oxysporum*, thus improving the disease resistance of rice.

Effects of ABA Inhibitor FLD on Defense-related Enzymes and MDA Content in Rice

In this experiment, the role of ABA in plant resistance is proved by ABA inhibitor FLD. We determine the effect of ABA inhibitor and ABA on defense-related enzymes. In these treatments, POD activity started to increase after 24 hours of inoculation and reached its peak after 72 hours of inoculation. At this point, group ABA+F has the highest POD activity, and plant group ABAI has the lowest. When we compare ABA+F with other treatments, it has (24.5, 71.8, and 99.1%) higher POD activity than treatments F, ABAI+F, and ABAI respectively (Figure 3A).

The results of SOD show almost similar trends. The activity of SOD started to increase after 24 hours of inoculation and reached its peak value at 72 hours of inoculation. This peak was maintained at 120 hours of inoculation after this, it started to decline. Plants treated with ABA+F have the highest SOD activity in comparison to other treatments. The lowest SOD activity was recorded in plants treated with ABAI. Plants treated with ABA+F has (32.5, 42.2, 55.9%) higher SOD activity in comparison to other treatments F, ABAI+F, and ABAI, respectively. The details of the results are presented in Figure 3B.

The PAL activity for ABA+F started to increase from the beginning and reached its peak after 96 hours of inoculation, after this PAL activity started to decline. Plants treated with ABAI had the lowest PAL activity. Plants treated with ABA+F have higher PAL activity in comparison to other treatments ABA+F has (50.9, 70.5, and 93%) more PAL activity in comparison to treatments F, ABAI+F, and ABAI respectively (Figure 3C).

PPO activity for all the treatments did change significantly. However, after 72 hours of inoculation, the plants treated with ABA+F started to increase and reached their peak after 96 hours of inoculation, but all other treatments almost remained the same (Figure 3D)

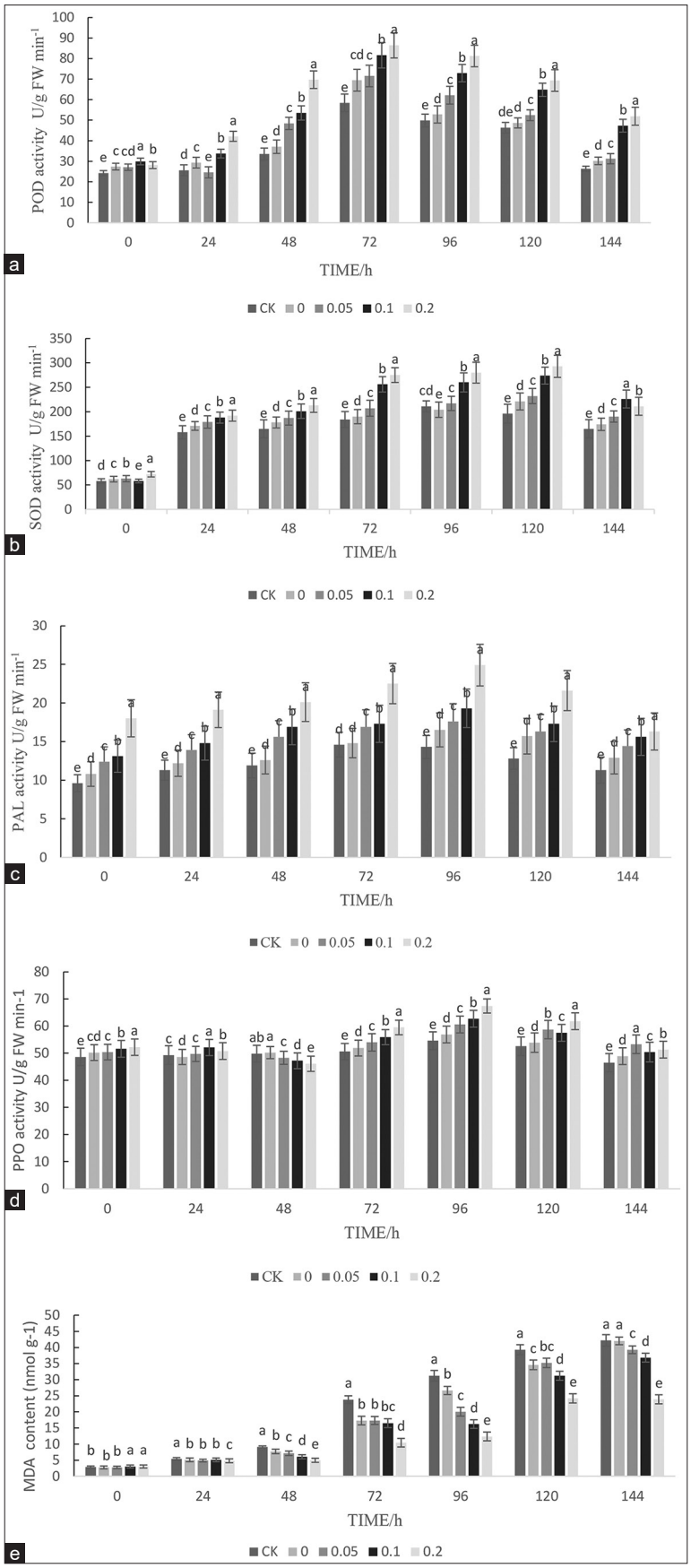

Figure 2: Effects of abscisic acid with different concentrations on a) POD, b) SOD, c) PAL and d) PPO activity and e) MDA content in rice leaves under the *F. oxysporum* stress

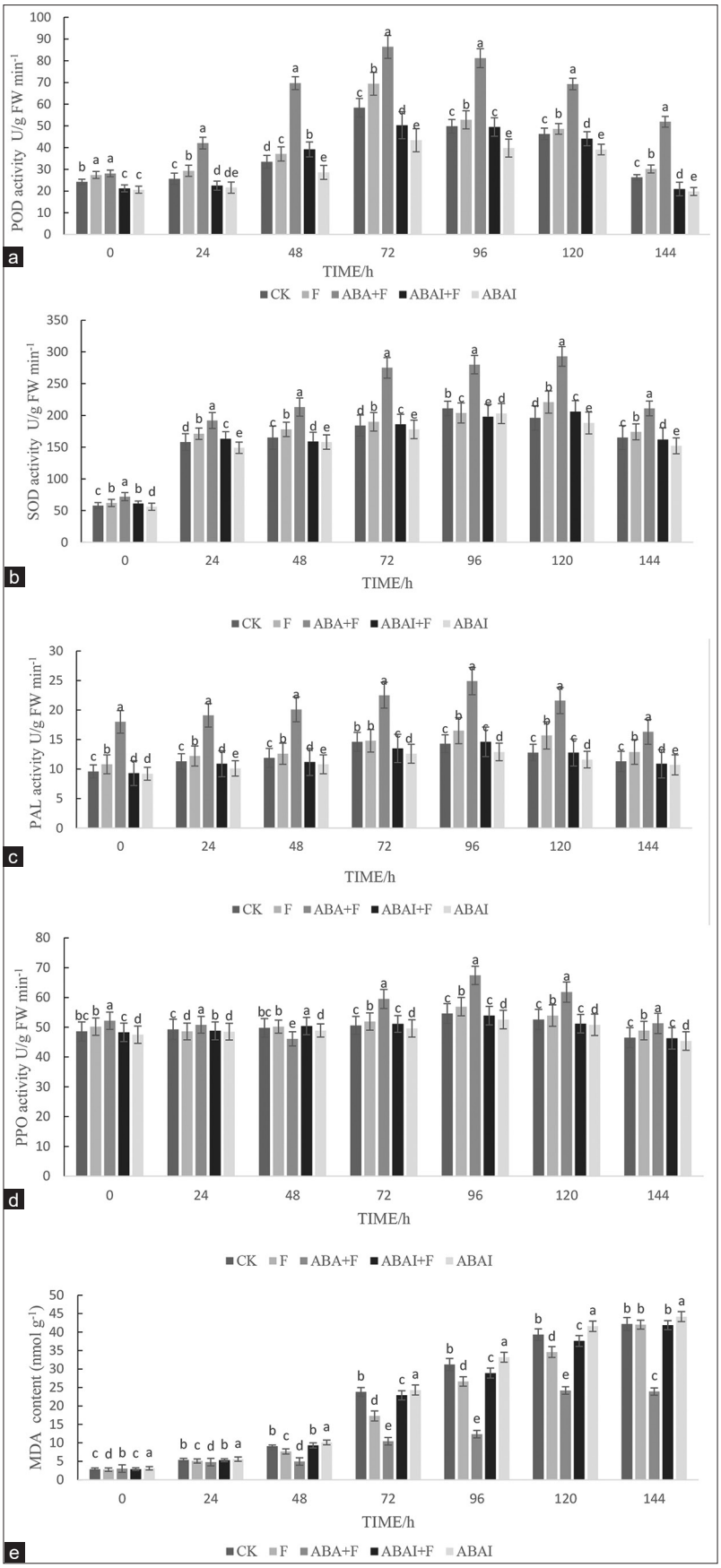

Figure 3: Effect of ABA inhibitor FLD and ABA on a) POD, b) SOD, c) PAL and d) PPO activity and e) MDA content on rice leaves under *F. oxysporum*

MDA content does not increase at the beginning for all the treatments. After 48 hours of treatment application, MDA content started to increase gradually and reached its peak after 144 hours. Plants treated with ABAI+F have the highest MDA content, followed by plant groups F and ABAI. These groups are statistically equal with each other. The lowest MDA content was observed in the CK group, which is treated only with water. Plants treated with ABAI+F have the highest MDA content among CK, F, ABA+F, and ABAI. These results (Figure 3E) indicate an increase in plant resistance due to ABA. Plants treated with ABAI+F have the highest MDA content, possibly due to the non-availability of ABA. This is also proved by plants treated with ABA+F this group has the lowest MDA content. The only difference between these two groups is ABA. This indicates the role of ABA in plant resistance.

Effect of Exogenous ABA on Expression Levels of Defence Related Genes

To determine the gene expression qRT-PCR was employed. According to qRT-PCR analysis under the stress of *F. oxysporum*, the expressions of three defense-related genes, PRB1-3, PRBI-2, and *Xa39(t)* in rice seedlings did not change significantly from 0 to 72 h, after 72 h their expressions started to increase, and after 96 h compared to the control group, the expression levels of three genes increased significantly in the exogenous ABA treatment group. The expression levels of these three genes in exogenous ABA treatment groups with concentrations of 0.05 mmol/L, 0.1 mmol/L, and 0.2 mmol/L were higher than the control group, with the increase in ABA concentration, the expression level of genes increased.

Gene PRB1-3 encodes pathogenic-related proteins. The expression level of this gene increased significantly at the beginning, and its expression was the same but after 96 h of inoculation with *F. oxysporum*, the level of expression started to increase and reached its peak after 144 hours. Plants treated with 0.2 mmol/L of ABA concentration showed the highest level of gene expression, and plants treated without exogenous ABA had the lowest level of gene expression (Figure 4A).

PRBI-2 also encodes pathogenic-related proteins. The expression level of this gene started to increase after 72 hours of inoculation and reached its peak after 144 hours of inoculation. When we compared the different ABA concentrations under *F. oxysporum* stress, we found that a concentration of 0.2 mmol/L had the highest level of gene expression, followed by a concentration of 0.1 mmol/L and the lowest gene expression was observed in the control group where we did not apply any exogenous ABA (Figure 4B).

In rice plants, gene *Xa39(t)* confers broad-spectrum resistance. Plants treated with the highest level of exogenous ABA concentration 0.2 mmol/L, started to increase after 48 hours of inoculation at this point all other concentrations remained dormant. They started to increase after 96 hours of inoculation and reach their peak at 144 hours after the inoculation at this point the concentration of 0.2 mmol/L had the highest level of gene expression, and the lowest gene expression was observed where there is no application of ABA (Figure 4C). These results prove that ABA plays an important role in plant defense and can significantly impact defense related genes.

Effects of ABA Inhibitor FLD on Expression Levels Defense Related Genes

The expressions of three defense-related genes, PRB1-3, PRBI-2, and *Xa39(t)* in rice seedlings did not change significantly at the beginning, but later they increased significantly. The expression level of gene PRB1-3 did not change significantly at the beginning after 96 hours of inoculation, but the plants treated with ABA with fungus (ABA+F) started to increase and reach their peak after 144 hours of inoculation. In other treatments that were treated without ABA, their expression levels did not change significantly (Figure 5A).

Gene PRBI-2 also shows similar trends. Plants treated with ABA and fungus (ABA+F) have the highest level of gene expression it started to increase after 72 hours of inoculation and reached its peak after 144 hours of inoculation. All other treatments did not change significantly, and the lowest levels of gene expression were observed in plants treated with ABAI (Figure 5B).

Xa39(t) is a broad-spectrum resistant gene. Plant group ABA+F has the highest gene expression it started to increase after 48 hours of inoculation and reached its peak after 144 hours of inoculation. In the beginning, all other treatments did not change significantly, but after 96 hours of inoculation, they also started to increase. The lowest level of gene expression was observed in plants treated with ABAI. These results can be seen in Figure 5C. Results of gene expression indicate that ABA has a significant role in plant resistant. Plants treated with ABA show higher levels of gene expression in comparison to ABAI, F, and CK. ABAI has lower levels of gene expression which indicates the role of ABA in plant resistance.

DISCUSSION

The role of ABA in abiotic stresses is a well-established fact, but its function and role in pathogenic stress are not so well established. In this study, our results showed that exogenous ABA has a significant effect on rice plants against *F. oxysporum*. We applied various levels of exogenous ABA to rice plants. ABA concentrations of 0.2 mmol/L exhibit the best results. This treatment effectively decreases the disease index, the disease incidence rate, and potentially improves the quality of seedlings. Above this concentration, the seedling quality started to deteriorate, and the disease index and incidence rate also started to increase. This means that excess ABA concentrations can negatively affect the rice plants against *F. oxysporum*, so we optimize ABA concentrations against *F. oxysporum*.

The antioxidant enzyme system in plants is an important way for plants to resist external biotic and abiotic stresses. When plants come under stress, a large number of antioxidant enzymes will be synthesized and accumulated, and their activities will

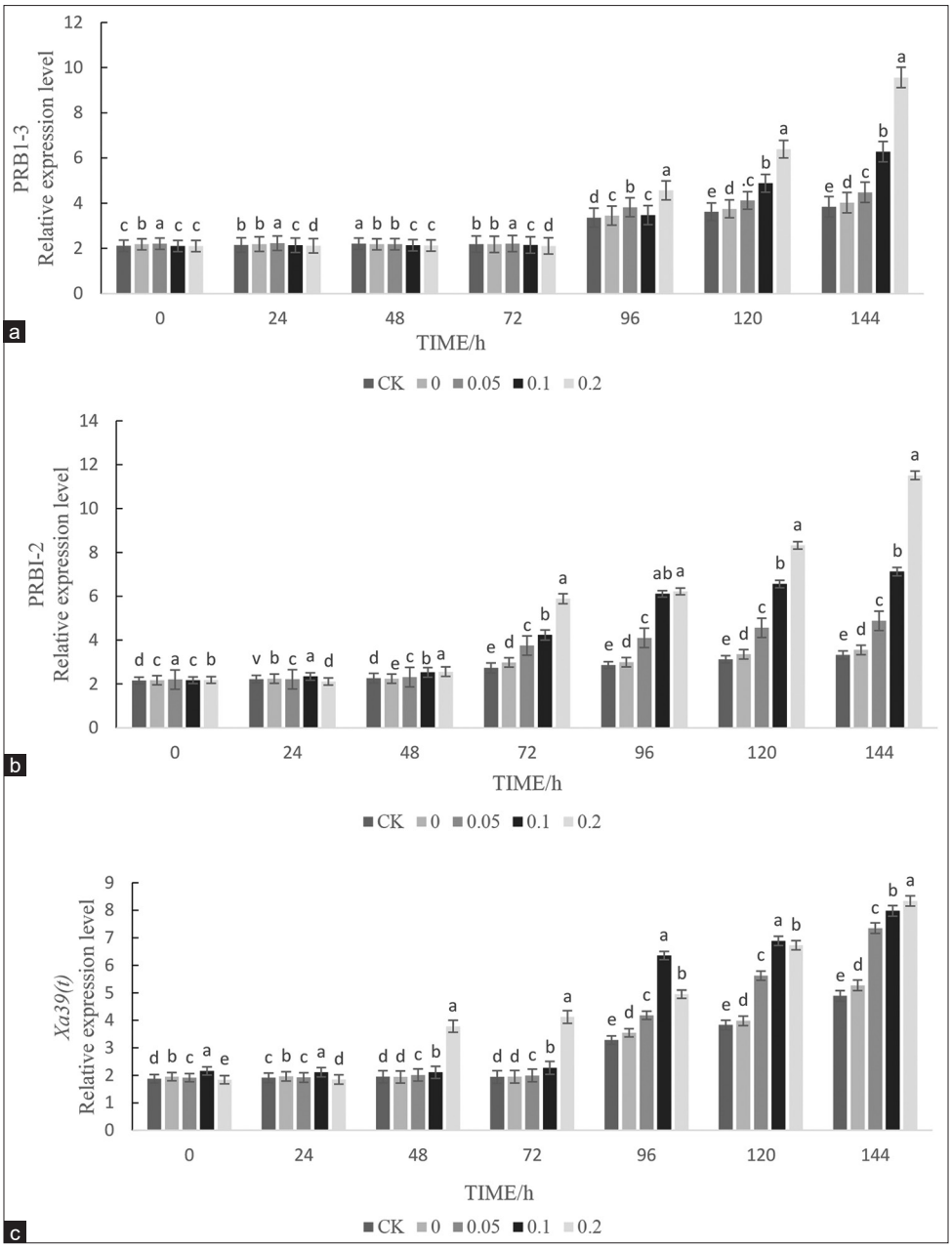

Figure 4: Effects of ABA with different concentrations on the expression of a) PRB1-3, b) PRBI-2 and c) *Xa39(t)* under *F. oxysporum* stress

increase significantly. These antioxidant enzymes are vital for tackling reactive oxygen species. Our results showed that exogenous ABA significantly increases the activity of antioxidant enzymes. ABA increased plant resistance against pathogens mostly through the formation and restoration of the cell wall and callose deposition (Zeyen *et al*., 2002), this led to the formation of plant papillae, which worked as a barrier against pathogen infections and induction of PAL (Ryerson *et al*., 1993). Papillae slow down the spread of disease and buy vital time for activating more complex and efficient plant defense mechanisms like the formation of phenolic compounds, activation of the antioxidant enzymatic system, and reactive oxygen species (Voigt, 2014). The increased antioxidant enzymatic activity also indicates the defense response to cellular damage caused by the pathogen (Chandrakar *et al*., 2018). Due to this cellular damage, MDA content is increased, as we already know that ABA is involved in the formation and restoration of the cell wall and callose deposition. That is why ABA is able to significantly decrease the MDA content. ABA is an essential part of the plant signaling network, which activates plant defense against pathogens. ABA has a complex network between SA, ET, and JA. These phytohormones are vital for plant defense. ABA has a positive relationship with these phytohormones and ABA is important for the biosynthesis of JA and the gene expression of JA-dependent defense genes (León *et al*., 2001). Inter-phytohormonal interactions are only partially understood. The relationship between ABA and the phytohormones SA, ET, and JA and their role in plant defense need further instigation.

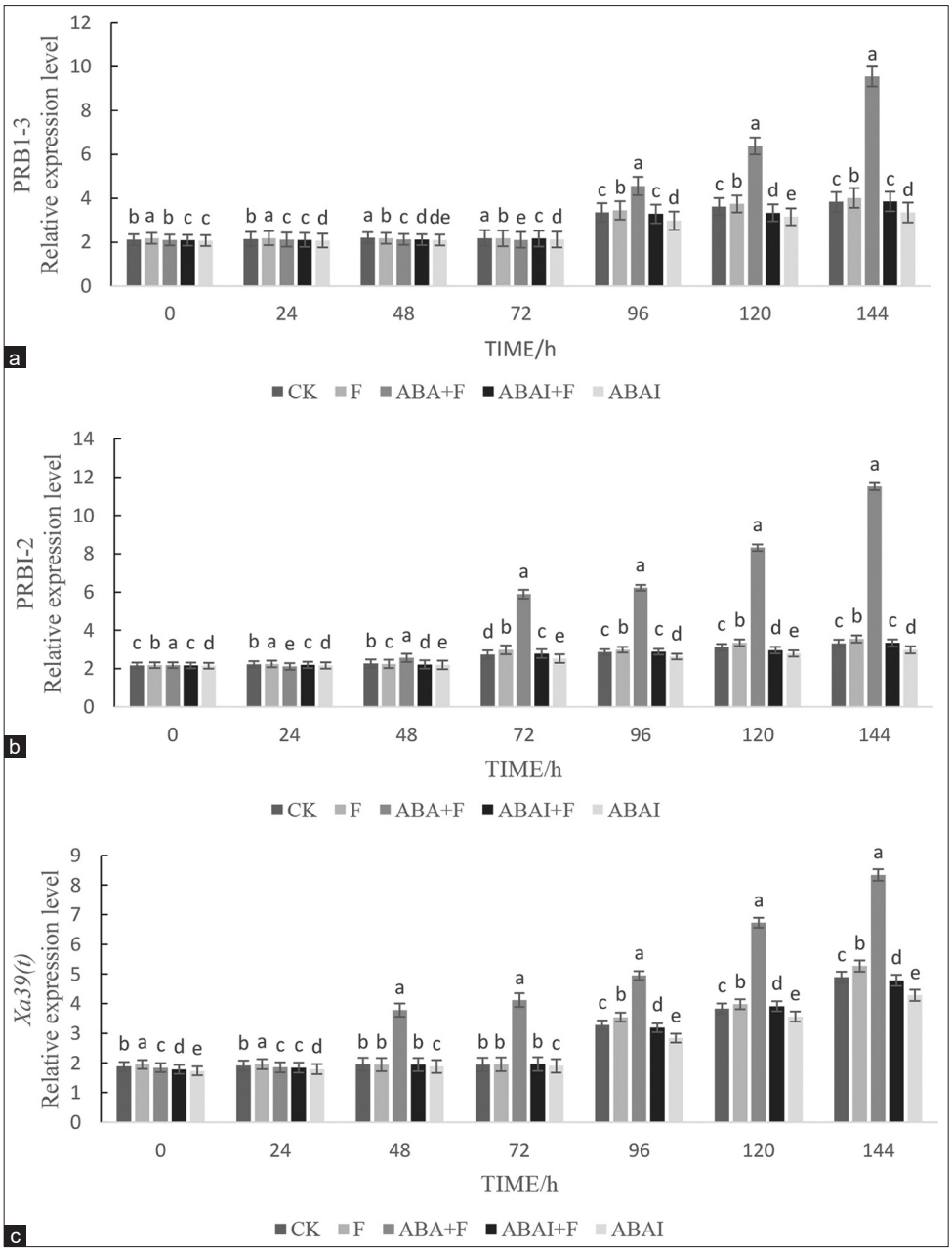

Figure 5: Effect of ABA inhibitor FLD and ABA on the expression of a) PRB1-3, b) PRBI-2, and c) *Xa39(t)* under *F. oxysporum* stress

Our results showed that when the plants come under pathogenic stress, their expression of defense-related genes starts to change. Plants treated with exogenous ABA exhibit an increased level of gene expression in comparison to the control group. These results are obvious because a meta-analysis of transcriptomic data revealed that approximately one-third of the plant genes are up-regulated by ABA (Adie *et al.*, 2007) indicating its importance in plant defense. In flax plants, application of exogenous ABA increased the expression level of the chitinase gene. These genes also possess ABA-responsive cis-regulatory elements. These flax plants with higher ABA levels showed increased resistance (Boba *et al.*, 2018).

Recent studies about ABA have suggested that the role of ABA depends on the stage of disease, type of tissue infected, pathogen infection strategy, or the pathogen (Maksimov, 2009). Our experiment preliminary proved that exogenous ABA was able to control the damage caused by *F. oxysporum* in rice by enhancing the disease resistance in rice. Exogenous ABA effectively decreases the disease incidence rate and disease index also they improve the quality of rice seedlings. Plants treated with ABA show better growth. Moreover, ABA effectively increases the activity of defense-related enzymes and also lowers down the MDA content, which proves its effectiveness. ABA was also able to slow down the spread of *F. oxysporum* when plants were treated with ABA before the infection. These results were confirmed by expression of defense-related genes these gene expressions indicate the involvement of ABA. ABA is a slightly toxic phytohormone, so optimization of ABA concentration is important. Tested ABA concentrations are 0.05 mmol/L,

0.1 mmol/L, 0.2 mmol/L and 0.25 mmol/L, among them plants treated with 0.2 mmol/L exogenous ABA show the best results. At present, spraying ABA on rice plants is rare, so we recommend the ABA application for better growth and a preventive strategy against *F. oxysporum*.

CONCLUSIONS

Our preliminary results showed that exogenous ABA could control the damage caused by *F. oxysporum* in rice by increasing disease resistance. Exogenous ABA effectively reduces disease incidence and index, while also improving rice seedling quality. ABA effectively increased the activity of the defense-related enzyme while also lowering the MDA content, demonstrating its effectiveness. ABA can also slow down the spread of *F. oxysporum*. The expression levels of three defense-related genes, PRBA1-3, PRBAI-2, and *Xa39(t)*, confirm the positive role of ABA; additionally, an ABA concentration of 0.2 mmol/L proves to be optimal. Based on these findings, we can recommend ABA to improve the rice defense mechanism.

AUTHORS' CONTRIBUTION

GP and HY plan and design this study. YMX, LYP and NZ executed the research experiment. XXF, LQR and XTL conducted the laboratory experiments GP organize and analyze the research data ZJH supervise the research.

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REFERENCES

Adie, B. A. T., Pérez-Pérez, J., Pérez-Pérez, M. M., Godoy, M., Sanchez-Serrano, J.-J., Schmelz, E. A., & Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in *Arabidopsis*. *The Plant Cell, 19*(5), 1665-1681. https://doi.org/10.1105/tpc.106.048041

Agarwal, P. K., & Jha, B. (2010). Transcription factors in plants and ABA dependent and independent abiotic stress signaling. *Biologia Plantarum, 54*, 201-212. https://doi.org/10.1007/s10535-010-0038-7

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R., & Kazan, K. (2004). Antagonistic Interaction between Abscisic Acid and Jasmonate-Ethylene Signaling Pathways Modulates Defense Gene Expression and Disease Resistance in *Arabidopsis*. *The Plant Cell, 16*(12), 3460-3479. https://doi.org/10.1105/tpc.104.025833

Aoki, T., O'Donnell, K., & Geiser, D. M. (2014). Systematics of key phytopathogenic *Fusarium* species: Current status and future challenges. *Journal of General Plant Pathology, 80*(3), 189-201. https://doi.org/10.1007/s10327-014-0509-3

Boba, A., Kostyn, K., Kozak, B., Wojtasik, W., Preisner, M., Prescha, A., Gola, E. M., Lysh, D., Dudek, B., Szopa, J., & Kulma, A. (2020). *Fusarium oxysporum* infection activates the plastidial branch of the terpenoid biosynthesis pathway in flax, leading to increased ABA synthesis. *Planta,* 251, 50. https://doi:10.1007/s00425-020-03339-9

Boba, A., Kostyn, K., Preisner, M., Wojtasik, W., Szopa, J., & Kulma, A. (2018). Expression of heterologous lycopene β-cyclase gene in flax can cause silencing of its endogenous counterpart by changes in gene-body methylation and in ABA homeostasis mechanism. *Plant Physiology and Biochemistry, 127*, 143-151. https://doi.org/10.1016/j.plaphy.2018.03.023

Chandrakar, V., Dubey, A., & Keshavkant, S. (2018). Modulation of arsenic-induced oxidative stress and protein metabolism by diphenyleneiodonium, 24-epibrassinolide and proline in *Glycine max* L. *Acta Botanica Croatica, 77*(1), 51-61. https://doi.org/10.2478/botcro-2018-0004

Das, K. K., Panda, D., Nagaraju, M., Sharma, S. G., & Sarkar, R. K. (2004). Antioxidant enzymes and aldehyde releasing capacity of rice cultivars (*Oryza sativa* L.) as determinants of anaerobic seedling establishment capacity. *Bulgarian Journal of Plant Physiology, 30*(1-2), 34-44.

Di, X., Takken, F. L. W., & Tintor, N. (2016). How phytohormones shape interactions between plants and the soil-borne fungus *Fusarium oxysporum*. *Frontiers in Plant Science, 7,* 170. https://doi.org/10.3389/fpls.2016.00170

García-Andrade, J., Ramírez, V., Flors, V., & Vera, P. (2011). Arabidopsis ocp3 mutant reveals a mechanism linking ABA and JA to pathogen-induced callose deposition. *The Plant Journal, 67*(5), 783-794. https://doi.org/10.1111/j.1365-313X.2011.04633.x

Geetha, N. P., Amruthesh, K. N., Sharathchandra, R. G., & Shetty, H. S. (2005). Resistance to downy mildew in pearl millet is associated with increased phenylalanine ammonia lyase activity. *Functional Plant Biology, 32*(3), 267-275. https://doi.org/10.1071/FP04068

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. *Plant physiology, 59*(2), 315-318. https://doi.org/10.1104/pp.59.2.315

Hauser, F., Waadt, R., & Schroeder, J. I. (2011). Evolution of abscisic acid synthesis and signaling mechanisms. *Current Biology, 21*(9), 346-355. https://doi.org/10.1016/j.cub.2011.03.015

Jiang, M., & Zhang, J. (2001). Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant and Cell Physiology, 42*(11), 1265-1273. https://doi.org/10.1093/pcp/pce162

Kim, T.-H., Hauser, F., Ha, T., Xue, S., Böhmer, M., Nishimura, N., Munemasa, S., Hubbard, K., Peine, N., Lee, B., Lee, S., Robert, N., & Parker, J. E., & Schroeder, J. I. (2011). Chemical genetics reveals negative regulation of abscisic acid signaling by a plant immune response pathway. *Current Biology, 21*(11), 990-997. https://doi.org/10.1016/j.cub.2011.04.045

León, J., Rojo, E., & Sánchez-Serrano, J. J. (2001). Wound signalling in plants. *Journal of Experimental Botany, 52*(354), 1-9. https://doi.org/10.1093/jexbot/52.354.1

Liao, C. M., Li, J., Liu, X. H., & Zhang, Y. S. (2014). An effective method for extracting total RNA from *Dioscorea opposite* Thunb. *Genetics and Molecular Research, 13*(1), 462-468. https://doi.org/10.4238/2014.January.21.15

Lievens, L., Pollier, J., Goossens, A., Beyaert, R., & Staal, J. (2017). Abscisic acid as pathogen effector and immune regulator. *Frontiers in Plant Science, 8*, 587. https://doi.org/10.3389/fpls.2017.00587

Lim, C. W., & Lee, S. C. (2015). Arabidopsis abscisic acid receptors play an important role in disease resistance. *Plant Molecular Biology, 88*, 313-324. https://doi.org/10.1007/s11103-015-0330-1

Liu, Z., Li, X., Sun, F., Zhou, T., & Zhou, Y. (2017). Overexpression of OsCIPK30 enhances plant tolerance to Rice stripe virus. *Frontiers in Microbiology,* 8, 2322. https://doi.org/10.3389/fmicb.2017.02322

Maksimov, I. V. (2009). Abscisic acid in the plants-pathogen interaction. *Russian Journal of Plant Physiology, 56*, 742-752. https://doi.org/10.1134/S102144370906003X

Peleg, Z., & Blumwald, E. (2001). Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology, 14*(3), 290-295. https://doi.org/10.1016/j.pbi.2011.02.001

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B-and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology, 110*(1), 125-136. https://doi.org/10.1104/pp.110.1.125

Robert-Seilaniantz, A., Grant, M., & Jones, J. D. G. (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology, 49*, 317-343. https://doi.org/10.1146/annurev-phyto-073009-114447

Ryerson, E., Li, A., Young, J. P., & Heath, M. C. (1993). Changes in abscisic acid levels in bean leaves during the initial stages of host and nonhost

reactions to rust fungi. *Physiological and Molecular Plant Pathology, 43*(4), 265-273. https://doi.org/10.1006/pmpp.1993.1056

Şirin, S., & Aslım, B. (2019). Determination of antioxidant capacity, phenolic acid composition and antiproliferative effect associated with phenylalanine ammonia lyase (PAL) activity in some plants naturally growing under salt stress. *Medicinal Chemistry Research, 28*, 229-238. https://doi.org/10.1007/s00044-018-2278-6

Soliva, R. C., Elez, P., Sebastián, M., & Martín, O. (2000). Evaluation of browning effect on avocado purée preserved by combined methods. *Innovative Food Science and Emerging Technologies, 1*(4), 261-268. https://doi.org/10.1016/S1466-8564(00)00033-3

Spence, C. A., Lakshmanan, V., Donofrio, N., & Bais, H. P. (2015). Crucial roles of abscisic acid biogenesis in virulence of rice blast fungus *Magnaporthe oryzae*. *Frontiers in Plant Science, 6*, 1082. https://doi.org/10.3389/fpls.2015.01082

Swarupa, V., Ravishankar, K. V., & Rekha, A. (2014). Plant defense response against *Fusarium oxysporum* and strategies to develop tolerant genotypes in banana. *Planta, 239*, 735-751. https://doi.org/10.1007/s00425-013-2024-8

Tjamos, E. C. (1989). Problems and Prospects in Controlling *Verticillium* Wilt. *Vascular Wilt Diseases of Plants, 28*, 441-456. https://doi.org/10.1007/978-3-642-73166-2_33

Ton, J., Flors, V., & Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. *Trends in Plant Science, 14*(6), 310-317. https://doi.org/10.1016/j.tplants.2009.03.006

Vleesschauwer, D. D., Yang, Y., Cruz, C. V., & Höfte, M. (2010). Abscisic acid-induced resistance against the brown spot pathogen *Cochliobolus miyabeanus* in rice involves MAP kinase-mediated repression of ethylene signaling. *Plant Physiology, 152*(4), 2036-2052. https://doi.org/10.1104/pp.109.152702

Voigt, C. A. (2014). Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Frontiers in Plant Science, 5*, 168. https://doi.org/10.3389/fpls.2014.00168

Wang, F., Cui, X., Sun, Y., & Dong, C.-H. (2013). Ethylene signaling and regulation in plant growth and stress responses. *Plant Cell Reports, 32*, 1099-1109. https://doi.org/10.1007/s00299-013-1421-6

Wang, S., Zhang, G., Zhang, Y., Song, Q., Chen, Z., Wang, J., Guo, J., Niu, N., Wang, J., & Ma, S. (2015). Comparative studies of mitochondrial proteomics reveal an intimate protein network of male sterility in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany, 20*, 6191-6203. https://doi.org/10.1093/jxb/erv322

Xu, G., Yang, S., Meng, L., & Wang, B. G. (2018). The plant hormone abscisic acid regulates the growth and metabolism of endophytic fungus *Aspergillus nidulans*. *Scientific Reports, 8*, 6504. https://doi.org/10.1038/s41598-018-24770-9

Xu, M.-R., Cruz, C. M. V., Fu, B.-Y., Zhu, L.-H., Zhou, Y.-L., & Li, Z.-K. (2011). Different patterns of gene expression in rice varieties undergoing a resistant or susceptible interaction with the bacterial leaf streak pathogen. *African Journal of Biotechnology, 10*(65), 14419-14438. https://doi.org/10.5897/AJB11.1317

Zeyen, R. J., Kruger, W. M., Lyngkjær, M. F., & Carver, T. L. (2002). Differential effects of D-mannose and 2-deoxy-D-glucose on attempted powdery mildew fungal infection of inappropriate and appropriate G ramineae. *Physiological and Molecular Plant Pathology, 61*(6), 315-323. https://doi.org/10.1006/pmpp.2003.0444

Zhang, F., Zhuo, D.-L., Zhang, F., Huang, L.-Y., Wang, W.-S., Xu, J.-L., Cruz, C. V., Li, Z.-K., & Zhou, Y.-L. (2015). *Xa39*, a novel dominant gene conferring broad-spectrum resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. *Plant Pathology, 64*(3), 568-575. https://doi.org/10.1111/ppa.12283