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Induced Disease Resistance of Endophytic Bacteria REB01 to Bacterial Blight of Rice

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Abstract. Endophytic bacteria strain REB01 was isolated from the seeds of rice. The induced resistance of strain REB01 to rice sheath blight was studied by measuring the change of the activities of defense enzymes, peroxidase (POD) and polyphenol oxidase (PPO) and the contents of pathogenesis-related biochemicals, malondialdehyde (MDA) in rice leaves inoculation with REB01, Rhizoctonia solan (pathogen of rice sheath blight) and the control in pot tests. The treatments included inoculated sterile NB broth only (CK), inoculated REB01 only (REB01), inoculated sterile NB broth and R. solan (RK), inoculated REB01 and R. solan (REB01-RK). The results indicated the activity of POD and PPO and the Content of MDA in rice leaves was very significantly affected by endophytic bacteria strain REB01 inoculation. effectively affected activities of POD and PPO, also reduce the content of MDA. Systemic resistance in rice against to R. solan could be induced by the strain REB01.

INTRODUCTION

Induced systemic resistance (ISR) [1-2], termed as which results from the perception of rhizobacteria by plant roots giving rise to an increased level of resistance that is expressed upon subsequent infection by a pathogen, is a state of enhanced defensive capacity developed by a plant when appropriately stimulated [3].

All plants have active defense mechanisms against pathogen attacks. The most important physiological and biochemical defense mechanisms maintained plants normal growth and development after being pathogens and the role of stress factors is the changes or induce the production of plant disease-related defense enzymes, such as peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and superoxide dismutase (SOD) [4-7]. Malondialdehyde (MDA) is a end product of membrane lipid peroxidation decomposition. MDA affected activities of the mitochondrial respiratory chain complexes in vitro and activities of key mitochondria enzymes in vivo. The content of MDA in plant tissue can reflect the extent of the plant suffered injury by pathogens or stress factor [8].

Endophytic bacteria widely exists in the healthy plant tissue [9]. There are a variety of groups endophytic bacteria in Rice [10], in which some strains have better effect of prevention and control plant diseases [11-14].

There was 45 strains of endophytic bacteria recently isolated from the stems and seeds of rice grown in Jiajiang county and Muchuan county, Sichuan province, China. Relative inhibition ratio (RIR) of strain REB01 was 219.2 percent and RIR of the primary extracellular product of REB01 was 204.0 percent. In this experiment, an investigation about the changes of activity of POD and PPO and content of MDA in rice leaves was carried out. The goal of the research outlined in this paper was to explore the systemic resistance induction in rice by REB01.

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MATERIALS AND METHODS

Biomaterials in this Experiment

Rice seeds were purchased from the market. Endophytic bacteria strain REB01 used in this experiments was isolated from the seeds of rice and Rhizoctonia solan (pathogen of rice sheath blight) were isolated from diseased rice plants.

Mediums in this Experiment

Medium for bacterium development was nutrient agar (NA) medium and TY broth medium (peptone 5 g, yeast extract 3 g, $CaCl_2$ or NaCl 0.7 g, distilled water 1.0 L). Medium for fungi culture was potato dextrose (PD) broth. Medium formulation of NA, TY and PD refered to the method described by Fang (2001).

Preparation for Strains

Endophytic bacteria strain REB01 was inoculated and cultured in NA plates or TY broth for 48 h growth condition at 30°C, 200 r/min. Concentration of fermentation broth was 108 CFU/mL of antagonistic bacteria diluted with sterile water. R. solan were inoculated and cultured in PD broth for 72 h growth condition at 25°C, 200 r/min. Spore suspension concentration of R. solan was 106 CFU/mL diluted with sterile water.

Experimental Treatment

Fermentation broths of strain REB01 108 CFU/mL were inoculated in rice root region by root pouring when rice grew to three leaves. Sterile NB broth instead of REB01 were inoculated by root pouring as controls. Pathogenic fungi R. solan with 106 CFU/mL concentrations of spore suspension were inoculated in rice leaves by spraying after 2 d inoculated REB01 or sterile NB broth. Activity of POD and PPO and content of MDA and Vc in rice leaves were determinated after inoculated with pathogenic fungi 0, 5, 10, 15, 20 days. The treatments included inoculated sterile NB broth only (CK), inoculated REB01 only (REB01), inoculated sterile NB broth and R. solan (RK), inoculated REB01 and R. solan (REB01-RK). Each treatment was repeated three times with 100 seedlings one time.

Determination of Activity of POD and PPO and Content of MDA

Determination of activity of POD and PPO and content of MDA refered to the method described by Xiao and Wang (2005). Definition of a POD activity units was OD470 value increased 0.01 per minute per gram of fresh weight. Definition of a PPO activity units was OD525 value increased 0.01 per minute per gram of fresh weight.

RESULTS

POD Activity in Rice Leaves Induced by Endophytic Bacteria Strain REB01

POD activity in rice leaves was very significantly affected by endophytic bacteria strain REB01 inoculation (Figure 1). POD activity in each treatment was very close on the pathogenic fungi inoculation day. POD activity in treatment RK and REB01-RK was gradually increased in 10 days after pathogenic fungi inoculation, but it was gradually decreased after 10 days. Change speed increased and decreased processing of POD activity of REB01-RK was faster than of RK. POD activity of treatment REB01 was its highest on the fifth day, then gradually reduced, was same level as CK and REB01-RK on 15 days. POD activity of CK was maintained stability.

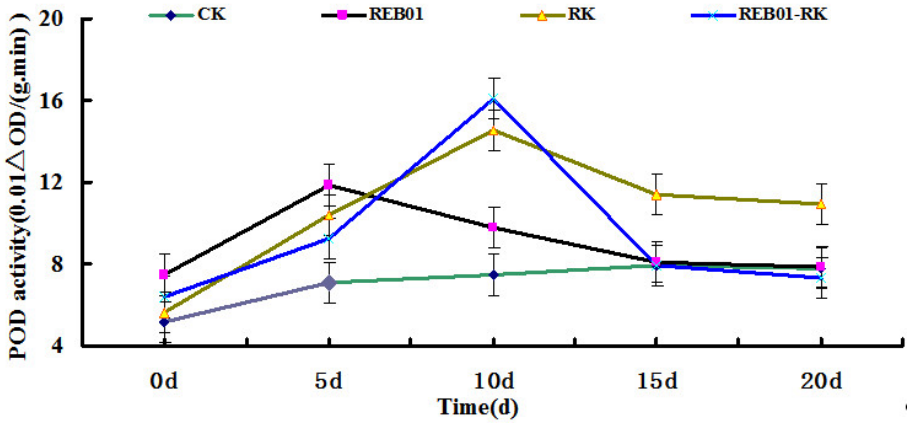

FIGURE 1. Activity of POD in rice leaves induced by endophytic bacteria strain REB01.

PPO Activity in Leaves of Rice Induced by Endophytic Bacteria Strain REB01

PPO activity in rice leaves was very significantly affected by endophytic bacteria strain REB01 inoculation (Figure 2). PPO activity in each treatment was very close on the pathogenic fungi inoculation day. PPO activity treatment RK and REB01-RK was gradually increased in 10 days after pathogenic fungi inoculation, but it was gradually decreased after 10 days. Change speed increased and decreased processing of POD activity of REB01-RK was relatively faster than of RK. PPO activity of treatment REB01 was its highest on the fifth day, then gradually reduced, was same level as CK and REB01-RK on 15 days. PPO activity of CK was maintained stability.

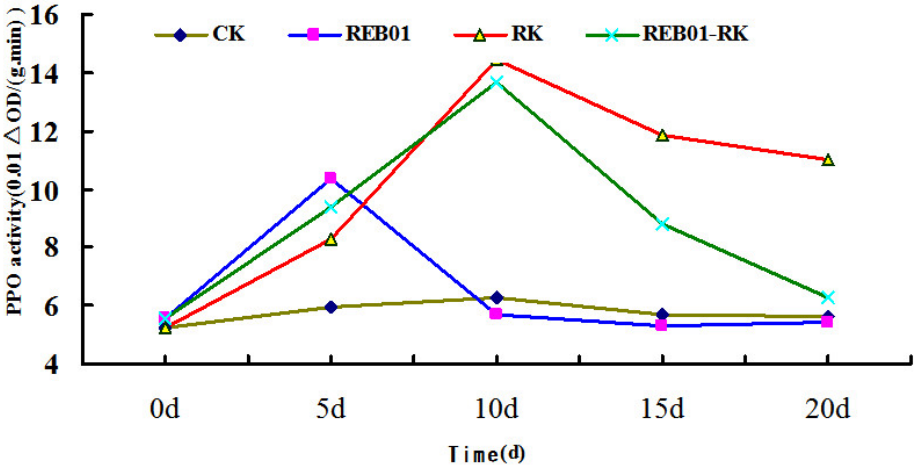

FIGURE 2. Activity of PPO in rice leaves induced by endophytic bacteria strain REB01.

Content of MDA in Rice Leaves Induced by Endophytic Bacteria Strain REB01

Content of MDA in rice leaves was very significantly affected by endophytic bacteria strain REB01 inoculation (Figure 3). Concentration of MDA in each treatment was same on the pathogenic fungi inoculation day. Content of MDA in rice leaves in all treatments only inoculated pathogenic fungi, MK and MH was gradually increased in 10 days after pathogenic fungi inoculation, reached the highest on 10th day, then gradually decreased in 10 to 15 day, reached to a minimum on 15th day. Content of MDA in rice leaves in all treatments inoculated strain REB01 and pathogenic fungi, REB01-MK, and REB01-MH was gradually increased in 5 days, reached the highest on 5th day, then gradually decreased in 5 to 20 day, reached to a minimum on 20th day. The content of MDA in rice leaves in all treatments inoculated strain REB01 was no significant difference to treatment CK after 15 day.

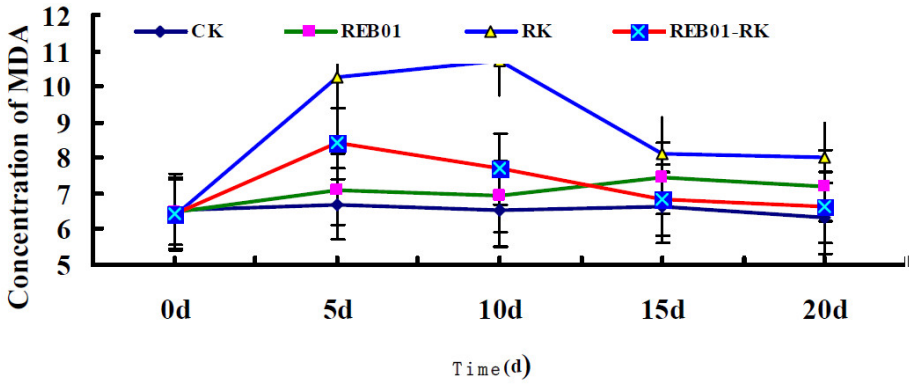

FIGURE 3. Content of MDA in rice leaves induced by endophytic bacteria strain REB01.

DISCUSSIONS AND CONCLUSION

Activity of POD and PPO in rice leaves was very significantly affected by endophytic bacteria strain REB01 inoculation. Activity of POD and PPO in rice leaves in all treatments inoculated pathogenic fungi, RK and REB01-RK was gradually increased from 0 day to 10 day, reached the highest on 10th day, then gradually decreased in 10 to 15 day, reached to a minimum on 15th day. Change speed increased and decreased processing of POD and PPO activity of REB01-RK was faster than of RK. The result indicated that endophytic bacteria strain REB01 effectively affected activities of POD and PPO.

Membrane lipid tend to occur peroxidation in plant organ suffered injuries from senescence or under stress. MDA is a final decomposition products of membrane lipid peroxidation, and its content can reflect the degree of plant injury suffered from adversity. After released from the position of produced in film, MDA can react with protein and nucleic acid, thus the loss of their function, but also relax the bridge between the cellulose molecules or inhibit the synthesis of protein. Therefore, the accumulation of MDA may cause some damage of cell and membrane. The content of MDA in rice leaves in treatments only inoculated strain REB01 was reached the highest on 5th day and no significant difference to treatment CK after 10 day. The content of MDA in rice leaves in treatments inoculated the pathogenic fungi, RK and REB01-RK, was reached the highest on 10th day, far higher than of treatment CK and REB01. The result indicated that endophytic bacteria strain REB01 effectively reduce the content of MDA.

Change speed of activity of POD and PPO of REB01-RK increased and decreased processing was faster than of RK, while the change trend of MDA content was on the contrary. The results indicated that endophytic bacteria strain REB01 played an important role in inducing resistance, greatly improving resistance ability of the rice to pathogen. The experimental results revealed the probable influence of induced systemic resistance by endophytic bacteria strain REB01.

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REFERENCES

1. J.W. Kloepper, S. Tuzun and J.A. Kuc. Biocontrol Sci.Technol. **2**, 349 (1992).
2. V.Ramamoorthy, R. Viswanathan, T. Raguchander, V. Prakasam and R. Samiyappan. Crop Prot. **20**, 1 (2001).
3. L.C.V. Loon. Eur. J. Plant Pathol. **103**, 753 (1997).
4. A.L. John. Pestic. Sci. **55**, 193 (1999).
5. Y.F. Liu, M.R. Li, Z.Y. Chen, J.J. Yu, Y.Z. Liu, Y.F. Nie and C.P. Luo. Microbiology **37**, 1753 (2010).
6. L. Sticher, B. Mauchmani, J.P. Betraux. Annu.Rev,Phytopathol. **35**, 235 (1997).
7. N.E. Trobel, C. Ji, S. Gopalan, J.A. Kuc and S.Y. He. Plant J. **9**, 432 (1996).
8. Y.S. Xiao, Z.Z. Wang and B.C. Guo. Agricultural Sciences **24**, 118 (1998).

9. D. Wilson. Microbiology **73**, 274 (1995).
10. L. Sun, F.B. Qiu, X.X. Zhang, X. Dai, X.Z. Dong, W. Song. Microbial Ecology **55**, 415 (2008).
11. F. Zhu, X.J. Chen, Y.H. Tong, Z.L. Ji, J.Y. Xu. Chinese Journal of Biological Control **23**, 68 (2007).
12. L. Lin, Y.S. Qiao, Z.Y. Ju, C.W. Ma, Y.H. Liu , Y.J. Zhou, H.S. Dong. Biosci. Biotechnol. Biochem. **73**, 1489 (2009).
13. R.P. Ryan, K. Germaine, A. Franks, D.J. Ryan, D.N. Dowling. FEMS Microbiol. Lett. **278**, 1 (2008).
14. Q. Nong, X.F. Chen, Q.Q. Li, G.Q. Yuan, W. Lin and Y.L. Huang. Chinese Journal of Biological Control **27**, 99 (2011).