Journal of Oceanology and Limnology
Vol. 38 No. 2, P. 509-516, 2020
https://doi.org/10.1007/s00343-019-9144-3

Defensive physiological characters of *Pyropia yezoensis* resistant lines to the red rot disease*

ZOU Dandan[1, 2, #], TANG Xianghai[1, 2, #], QIU Liping[1, 2], MAO Yunxiang[1, 2, **]

[1] *College of Marine Life Sciences*, *Ocean University of China*, *Qingdao 266003*, *China*

[2] *Key Laboratory of Marine Genetics and Breeding of Ministry of Education*, *Ocean University of China*, *Qingdao 266003*, *China*

Received May 23, 2019; accepted in principle Jul. 11, 2019; accepted for publication Aug. 5, 2019

Abstract To explore effective physiological indexes to distinguish the resistant lines and susceptible lines of *Pythium porphyrae*, the causal agent of red rot disease of *Pyropia yezoensis*, and establish the disease-resistance breeding strategy, we obtained and analyzed the candidate resistant and susceptible lines by population selection. Gametophytes of the candidate lines were cultured in seawater containing *Pyt. porphyrae* zoospores. Antioxidase activities, including superoxide dismutase (SOD), peroxidase (POD) and polyphenol oxidase (PPO), were measured and compared between the two lines before and after infection. In the resistant lines, SOD and POD activities increased and then decreased, but PPO activity rose with the prolongation of the infection time. The phenylalanine ammonia lyase (PAL) activities also increased and then decreased after infection, but it had significantly different expression in the two lines without pathogen attack. The synthesis rates of β-1,3-glucanase, and cell-wall degrading enzyme were different from each other between the two lines after infection of *Pyt. porphyrae*. Comparison in the contents of malondialdehyde (MDA) and reactive oxygen species (ROS) in the two lines showed that, the two contents varied synchronously in response to the pathogen attack. Changes of these enzymes activities or contents demonstrated that *Pyr. yezoensis* could resist against the pathogen of *Pyt. porphyrae* with the antioxidant defense capacity. In addition, β-1.3-glucanase content showed extremely significant difference between the two lines, and the PAL had consistent expression difference. Therefore, phenylalanine ammonia lyase (PAL) and β-1,3-glucanase can be considered as an effective index to distinguish susceptible line and resistant line, with which the workload of the resistant breeding could be reduced in the future.

Keyword: population selection; resistant lines; susceptible lines; *Pyropia yezoensis*; *Pythium porphyrae*

1 INTRODUCTION

Red rot disease of *Pyropia yezoensis* caused by *Pythium porphyrae* is one of the most serious diseases in the nori culture industry. As the main nori production area in Japan, red rot disease is the most common and serious problem. The disease occurs throughout the farming period from October to March, and the yield reduction was estimated to reach 20% in the Ariake Sea (Kawamura et al., 2005). The pathogen severely infects nori thalli and causes death of the host within a few days. At present, we know that *Pyt. porphyrae* oospores are produced in nori thalli throughout the farming period and remain in seawater after it decays. However, we know nothing about its pathogenesis. Although we can detect the pathogen by direct PCR using a specific primer to amplify internal transcribed spacer (ITS) regions of rDNA, the density of *Pyt. porphyrae* propagules is very low in naturally infested areas, its detection has been unsuccessful by direct assays using these techniques (Park et al., 2001).

Woo et al. (2002) found a novel antifungal protein (e.g. SAP) in the culture supernatant of a marine bacterium, *Streptomyces* sp. strain AP77, but this protein cannot be directly applied for the production.

* Supported by the National Natural Science Foundation of China (Nos. 31372517, 41006082)

** Corresponding author: yxmao@ouc.edu.cn

# ZOU Dandan and TANG Xianghai contributed equally to this work and should be regarded as co-first authors.

Other methods of controlling this disease have been developed, e.g., exposing nets to air at -20°C for several days, or immersing the nets into an organic acid-seawater mixture (pH 2) for 5–10 min to inactivate the pathogen (Park and Hwang, 2014). These traditional treatments have a positive effect on the infections in early stages, but not on those in later stages.

Plants have developed a variety of inducible defense mechanisms against diverse biotic stress, such as the necrotic hypersensitive response, biosynthesis of phytoalexins, and the production of pathogenesis-related proteins. An apparently ubiquitous feature of the plant response to pathogen attack is the induction of defense-related enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (POD), and β-1,3-glucanase. A range of phenylpropanoids in defensive roles has been produced in the pathways that begin with PAL. Therefore, PAL is considered the key enzyme in phenylpropanoid metabolism (Koukol and Conn, 1961). Enzyme PPO is widely distributed in plants, and it catalyzes the oxidation of monophenols, diphenols, and trihydric compounds into their corresponding quinines that are more toxic than phenolic substances to pathogens (Baucher et al., 1999). Superoxide dismutase (SOD) and POD participate in scavenging excessive reactive oxygen species (ROS), including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH) in plant cells. These enzymes contribute greatly to the health of plant growth (Mittler, 2002; Koca et al., 2007). Excessive ROS lead to membrane lipid peroxidation, which can be measured via its byproduct malondialdehyde (MDA). Therefore, the MDA content is considered a useful index of membrane lipid peroxidation and membrane system damage.

At present, knowledge of defensive physiological characters of *Pyr*. *yezoensis* that infected by *Pyt*. *porphyrae* remains blank. In this study, we screened resistant lines and susceptible lines of *Pyr*. *yezoensis* by population selection and evaluated their disease resistance. In addition, we compared the activities of various defensive enzymes between resistant and susceptible lines to identify the indexes of disease resistance that can be used for screening resistant lines in future work.

2 MATERIAL AND METHOD

2.1 Culture and zoospore induction

The *Pyt*. *porphyrae* NBRC33253 strain was purchased from the Biological Resource Center of Japan and maintained on corn-meal seawater agar (CMSA). To obtain sufficient material for experiments, mycelia were transferred from agar to liquid culture medium and cultured under axenic conditions for 7 days. The production of zoospores was induced using 10 mmol/L $CaCl_2$ in seawater (Zhang et al., 2015).

2.2 Screening of disease-resistant and susceptible lines

Wild *Pyr*. *yezoensis* gametophytes were collected from sea areas around Shandong Peninsula (including Qingdao: 36.04°N, 120.37°E; Weihai: 37.49°N, 122.14°E; Yantai: 37.47°N, 121.14°E; and Rizhao: 35.38°N, 119.56°E) and cultivated in the laboratory for 7 days at 15°C in 12 h:12 h light/dark scheme with light at 80 μmol/(s·m^2). Healthy and intact gametophytes were washed with sterile seawater, soaked in 0.7% potassium iodide (KI) solution for 10 min, and finally washed with sterile seawater. Spores of *Prt*. *porphyrae* were added into seawater at a rate of 10^3/L. After 15 days, the symptom-free area of each *Pyr*. *yezoensis* gametophytes that infected by the pathogen were digested with enzymes. Single cells were differentially cultured to get resistant lines. Some gametophytes were infected quickly (generally in 2 days) and the disease spots spread quickly. The healthy area of gametophytes, which was infected but the spot did not spread, was digested with enzymes to get susceptible lines (Aoki and Kamei, 2006). The resistant and susceptible lines will be used in the following works.

2.3 Evaluation of disease resistance

The resistant and susceptible lines were cultured in seawater containing *Pyt*. *porphyrae* zoospores (10^3/L) for 15 days, from which 30 gametophytes of each line were randomly selected to calculate the degree of disease infection and the disease resistance level of each gametophyte. We used the spot rate to grade the status of health of gametophytes. The spot rate is the percentage of the disease spots area in the whole area of gametophyte. The status of health of gametophytes was divided into 5 grades as following. 0: whole gametophyte healthy without disease symptoms; 1: gametophyte with few or very small disease spots, and the spot rate ≤20%; 2: gametophyte with more but small disease spots, and the spot rate 20%–40%; 3: gametophyte with more and larger disease spots, and with disease spot rate between 40% and 60%; 4: gametophyte with large number of disease spots

and disease spot rate 60%–80%; and Score 5: multiple disease spots joined together and the disease spot rate >80%. The disease index (DI) was calculated using the following formula: DI=(Σ(v×n)/I×N)×100 (where v is the value of each grade, n is the gametophytes number of each grade, I is the value of the highest grade, and N is the total number of gametophytes evaluated) (Taylor et al., 1994). The lines were then classified based on their DI, as follows: immune (I): DI=0; highly resistant (HR): 0<DI≤10; resistant (R): 10<DI≤20; moderately resistant (MR): 20<DI≤40; and susceptible (S): DI>40 (Veitía et al., 2014).

One HR line and one S line were selected to re-evaluate resisted level to the pathogen. After culturing, we randomly took out 30 gametophytes from HR line and S line respectively and calculated the disease incidence rate and disease spots diffusion rate according to the number of infected gametophyte and the area of disease spots.

2.4 Collection and processing of materials

One HR line and one S line were selected to analyze changes in the activities of enzymes related to pathogen resistance. Gametophytes of resistant and susceptible lines were collected at 0, 1, 3, 5, 7, and 9 days after infection. Samples were powdered after freezing in liquid nitrogen quickly, and then split into 0.1-g per unit and stored in liquid nitrogen for follow-up analysis.

2.5 Assay of enzyme activity

All extraction procedures were conducted at 4°C. For the SOD activity assay, 0.1-g tissue powder was homogenized in 5-mL ice-cold 50 mmol/L sodium phosphate buffer (pH 7.8) and then centrifuged at 4°C for 20 min at 10 000×g. The supernatant was immediately assayed for SOD activity that expressed as enzyme units per gram fresh weight per min. The amount of enzyme corresponding to 50% inhibition of nitroblue tetrazolium reduction was defined as one enzyme unit.

To analyze POD activity, 0.1-g tissue was homogenized in 1 mL extraction buffer (pH 7.0, 50 mmol/L phosphate buffer); the mixture was centrifuged at 10 000×g at 4°C for 20 min, and the supernatant was used in the POD activity assay. The reaction mixture contained 100-μL extract, 1 mL, 50-mmol/L guaiacol, and 2.9-mL extraction buffer, and was incubated at 34°C for 3 min. The increase in absorbance at 460 nm was spectrophotometrically assayed after adding 1 mL H_2O_2 (2%). The POD activity was expressed as units per gram fresh weight per min. One unit was defined as an increase in A_{460} of 0.01/min.

To determine PAL activity, 0.1-g tissue was homogenized in 50 mmol/L sodium borate buffer (pH 7.8) containing 5-mmol/L β-mercaptoethanol, 1-mmol/L EDTA, glycerin, and polyvinylpyrrolidone (PVP) at 5% (w/v). The extract was centrifuged at 10 000×g at 4°C for 20 min and the supernatant was used in the PAL assay. The PAL activity was determined by monitoring the production of cinnamate during 30 min at 30°C, as indicated by the change in absorbance at 290 nm. The PAL activity was expressed as units per gram fresh weight per min. A unit was defined as the amount of enzyme required to change the A_{290} in 0.01 min (Cheng and Asada, 1989).

For analyses MDA contents, β-1,3-glucanase activities, and ROS contents, 0.1-g samples were extracted with 5 mL PBS in different pH values depending on the analysis. The mixture was centrifuged as described above and then the supernatant was used to determine MDA content (Liang et al., 2003), β-1,3-glucanase activity (Wu and Bradford, 2003), and ROS content (Foyer et al., 1994).

To determine PPO activity, 0.1-g sample was homogenized in 5 mL chilled phosphate buffer (0.1 mol/L, pH 6.8) and the mixture was centrifuged at 10 000×g at 4°C for 20 min. The supernatant was used in the PPO assay. The reaction mixture contained enzyme extract, 2.5 mL 0.05-mol/L phosphate buffer (pH 6.8), and 0.5 mL 0.1-mol/L catechol, and was incubated at 37°C for 10 min. The absorbance was measured at 420 nm with an ultraviolet spectrophotometer (Cheng and Asada, 1989).

2.6 Statistical analysis

Statistical analyses were carried out using the statistical package SPSS v.23 for Windows. The statistical significance of the data was assessed by independent samples t-test. The results shown in figures and tables are mean±SEM (standard error of the mean) and P<0.01 was considered statistically significant.

3 RESULT

3.1 Evaluation of disease resistance

By screening the population, we obtained 16 disease-resistant lines and two susceptible lines. Among them, three were classified as HR, seven as

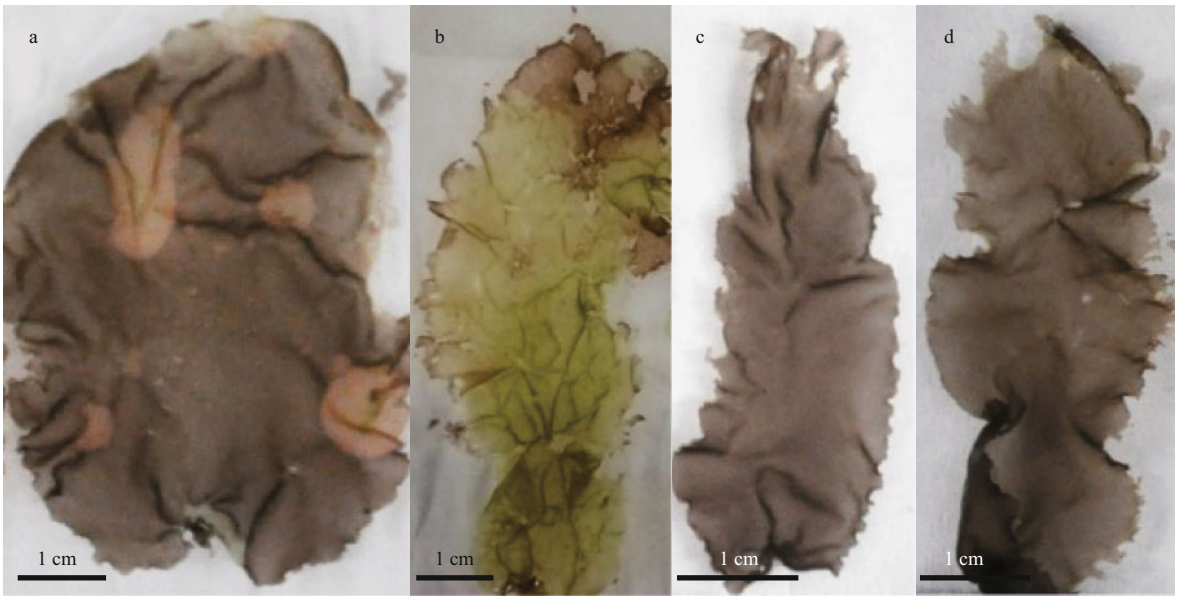

Fig.1 Evaluation of disease resistance

The susceptible line on Day 3 (a) and Day 7 (b). and the resistant line on Day 3 (c) and Day 15 (d) after infection by *Pyt. Porphyrae*. Scale bar: 1 cm.

Table 1 Number of lines in different disease degrees, the disease index after 15 days of infection, and resistance classification

Strain No.	Disease index (DI)	Resistance type
3	2–5.3	HR
7	20.67–31.33	MR
4	17.3–19.33	R
2	98.6–100	S

HR: highly resistant; R: resistant; MR: moderately resistant; S: susceptible.

MR, four as R, and two as S (Table 1). Some resistant lines were completely resistant to the disease. The susceptible lines developed spots by Day 3 after inoculation and the spots spread quickly to cover the whole blade by Day 5.

Pythium porphyreae spores (concentration reached 10^3/L) are inoculated in the culturing system, and HR line and S line were cultured in this system for 15 days. The S line showed apparent spots on Day 3 (Fig.1a & b), but there was no spot in the HR line gametophytes (Fig.1c & d).

3.2 Effects of *Pyt. porphyrae* infection on activities of defense-related enzymes

In plants, PAL is considered as a key enzyme in pathogen defense (Koukol and Conn, 1961). The PAL activity was significantly higher in the resistant line than in the susceptible line (P<0.01). After infection by *Pyt. porphyrae*, the PAL activity in the susceptible line increased gradually to the peak on Day 7, but peaked on Day 3 in the resistant line. The maximum activity of PAL in the resistant line was 2.87 times of that in the susceptible line, and its activity peaked sooner than that in the susceptible line after infection (Fig.2a).

As shown in Fig.2b, the PPO activities fluctuated on a small scale and remained at low levels in healthy resistant and susceptible lines. After infection, the PPO activities rose significantly in both lines, but to a higher level in the resistant line than in the susceptible line (P<0.01). As more infection time, the enzyme activity increased significantly, showing that *Pyt. porphyrae* infection induced a striking response in the algal cells, and that PPO activity was closely related to disease resistance.

The superoxide produced under stress conditions is detoxified by SOD. The activity of SOD was low and stable in healthy resistant and susceptible lines. After infection, the SOD activity increased immediately and peaked on Day 3, then returned to low levels similar to the control in both lines (Fig.2c). The maximum SOD activity was much higher in the resistant line than in the susceptible line, indicating that increased SOD activity was characteristic of the defense response against *Pyt. porphyrae*.

There was no significant difference in the constitutive activity of POD between the resistant and susceptible line before infection. After infection, the POD activities increased quickly and sharply, and then decreased rapidly on Day 3 after infection in both lines. However, the POD activity remained at higher levels in the susceptible line than in the

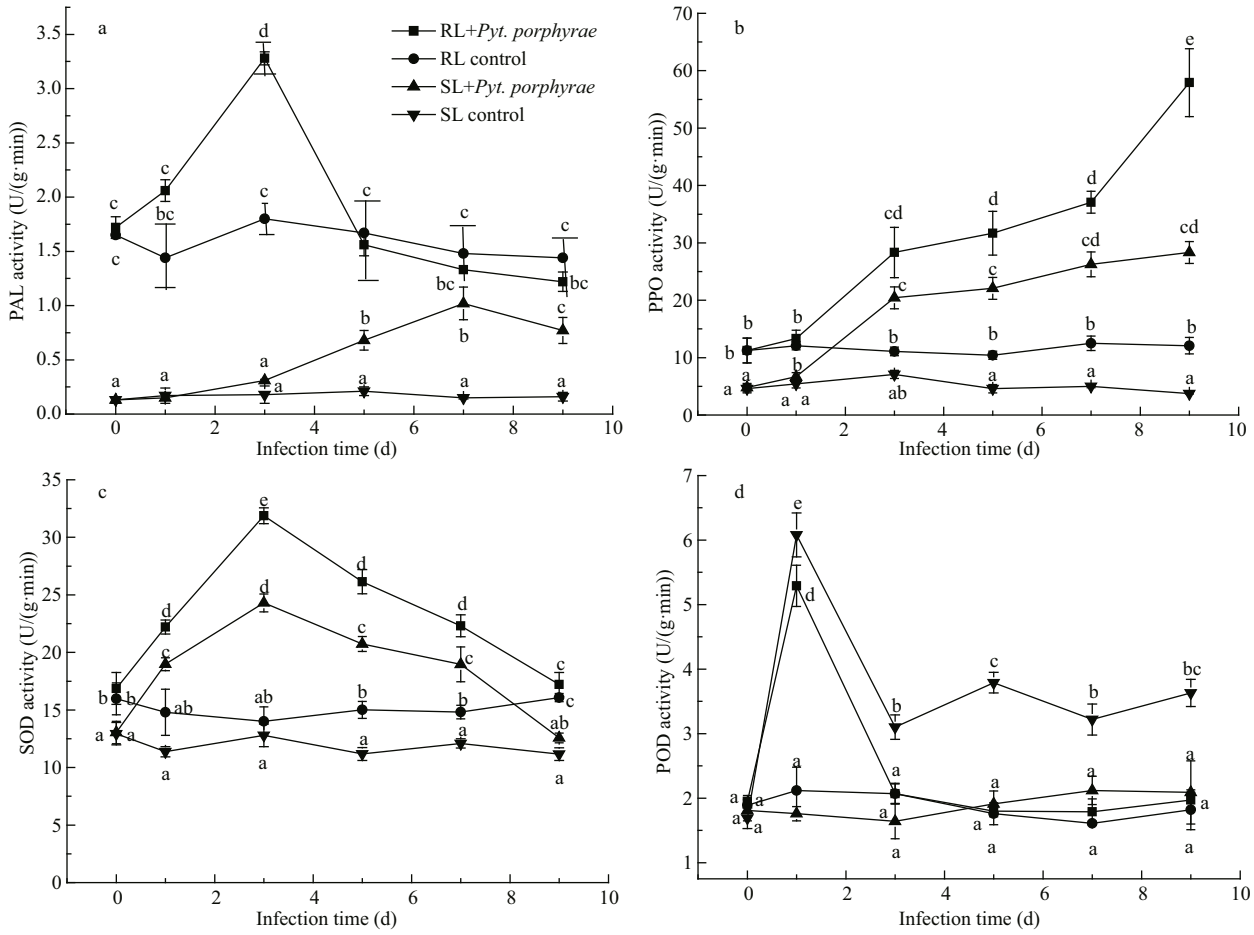

Fig.2 Activities of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), superoxide dismutase (SOD), and peroxidase (POD) in *Pyr. yezoensis* gametophytes infected by *Pyt. porphyrae*

RL: resistant line; SL: susceptible line; RL+*Pyt. porphyrae*: resistant line infected with *Pyt. porphyrae*; SL+*Pyt. porphyrae*: susceptible line infected with *Pyt. porphyrae*; RL control: uninfected resistant line; SL control: uninfected susceptible line. Different letters show a significant difference (P<0.05).

resistant line (Fig.2d), indicating that POD eliminated peroxide continuously in the susceptible line.

3.3 Changes in ROS and MDA contents in *Pyr. yezoensis* gametophytes infected with *Pyt. porphyrae*

When *Pyr. yezoensis* gametophytes were infected with the pathogen, the ROS contents increased rapidly in the susceptible and resistant lines, but the peak occurred earlier in the resistant line (on Day 1) than in the susceptible line (on Day 3). The peak ROS level was 1.75 times higher in the susceptible line than in the resistant line (P<0.01) (Fig.3a), indicating that the susceptible line was severely infected. Both the resistant and susceptible lines had higher ROS contents during infection than in control conditions, indicating that ROS was continuously produced in response to the pathogen.

A product of lipid peroxidation is MDA, and so a higher MDA content is indicative of serious cell damage. The MDA content was low in both lines before infection. After infection, the MDA content increased sharply and peaked on Day 3 in the susceptible line before decreasing again the following day (Fig.3b). Although the MDA content also increased in the resistant line, reached much lower levels than that in the susceptible line (P<0.01). The results indicate that the cell membrane lipid peroxidation was stronger in the susceptible line than in the resistant line.

3.4 Effect of *Pyt. porphyrae* infection on β-1,3-glucanase activity

As shown in Fig.4, there was no significant difference in β-1,3-glucanse activity between the disease resistant and susceptible lines before infection. After infection, the β-1,3-glucanse activity increased significantly (P<0.01) in the resistant line, but not in the susceptible line (P>0.05). It remained at a high

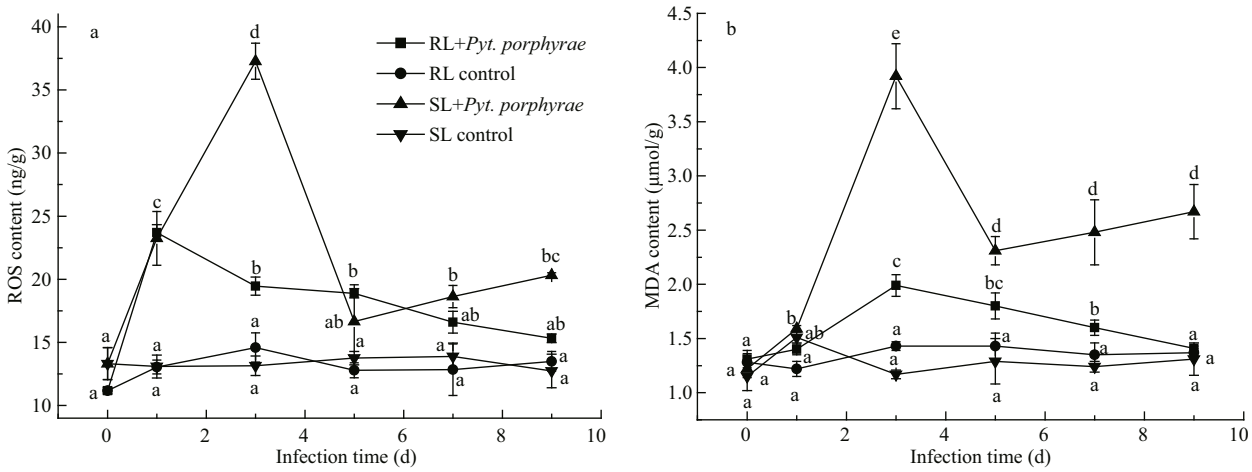

Fig.3 Contents of reactive oxygen species (ROS) (a) and malondialdehyde (MDA) (b) in *Pyr. yezoensis* gametophytes infected by *Pyt. porphyrae*

RL: resistant line; SL: susceptible line; RL+*Pyt. Porphyrae*: resistant line infected with *Pyt. porphyrae*; SL+*Pyt. Porphyrae*: susceptible line infected with *Pyt. porphyrae*; RL control: uninfected resistant line; SL control: uninfected susceptible line. Different letters show a significant difference (P<0.05).

level from Day 1 to Day 3 after infection, and then decreased to a level similar to that in the control. This pattern of activity suggested that β-1,3-glucanase played an important role in defense during the early stage of infection with *Pyt. porphyrae*.

4 DISCUSSION

4.1 Membrane system in *Pyr. yezoensis* gametophyte affected by *Pyt. porphyrae*

Generally, ROS maintains a dynamic balance in plant cells. The ROS level increases as a first response to pathogen attack, and has strong chemical activity to destroy pathogens directly. They also function as signaling molecules to induce defense responses; for example, the synthesis of phytoalexins (Peng and Kuc, 1992) and the expression of genes related to defense (Foyer et al., 1994). In kelps, ROS can move to adjacent healthy cells to resist pathogens (Weinberger et al., 2005). In red algae, constitutive oxylipin signals increase the expression of stress-related genes (Collén et al., 2006). However, a rapid accumulation of ROS can disrupt the balance, and excess ROS can then oxidize nucleic acids, proteins, carbohydrates, and lipids, resulting in cellular damage (Shao et al., 2007). In this study, ROS accumulated in the two lines after infection with *Pyt. porphyrae*, but to much higher levels in the susceptible line than in the resistant one. This result shows that *Pyr. yezoensis* accumulates ROS under pathogen attack, as do land plants and other algae, also that the susceptible line was damaged more

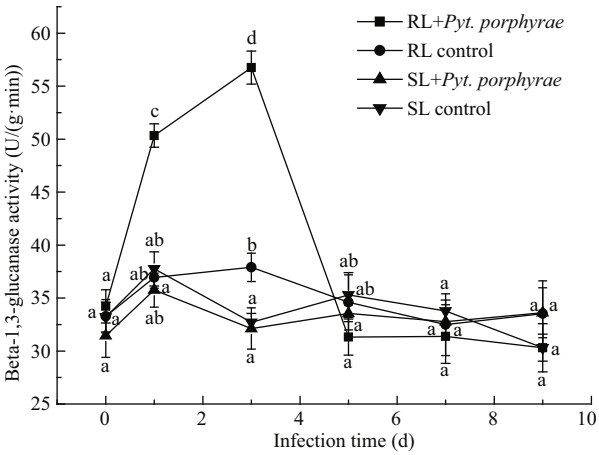

Fig.4 Activity β-1,3-glucanase in *Pyr. yezoensis* gametophytes infected by *Pyt. porphyrae*

RL: resistant line; SL: susceptible line; RL+*Pyt. porphyrae*: resistant line infected with *Pyt. porphyrae*; SL+*Pyt. porphyrae*: susceptible line infected with *Pyt. porphyrae*; RL control: uninfected resistant line; SL control: uninfected susceptible line. Different letters show a significant difference (P<0.05).

severely by pathogen infection. When ROS are transmitted to adjacent healthy cells, they accumulate more ROS to protect themselves, even though the ROS can harm the cells.

The MDA content reflects the degree of membrane lipid peroxidation, and thus the extent of membrane damage. The MDA content has been showed to be negatively correlated with disease resistance (Küpper et al., 2001). In this study, the ROS and MDA contents increased synchronously in both lines after infection. The susceptible line accumulated more ROS and MDA, and the MDA content remained high throughout infection, even at the late stages, in the susceptible

line. The results show that the susceptible line was more severely infected than the resistant line. Similar trends in MDA content (first increase and then decrease) were observed in disease resistant and susceptible potato varieties infected with *Fusarium trichothecioides* (Fenglan et al., 2015).

4.2 Changes in defensive enzyme activity in *Pyr. yezoensis* gametophytes infected with *Pyt. porphyrae*

As ROS accumulates in cells, they function as messaging molecules to stimulate the expression of defense-related genes (Apostol et al., 1989). We found that the activities of protective enzymes such as PAL and PPO were positively correlated with disease resistance. The activities of PAL and PPO increased in potato after infection with potato blight disease (Friend et al., 1973). In this research, the PAL and PPO activities increased in both disease-resistant and susceptible lines after infection, but to higher levels in the resistant line. The activities of these two enzymes were also found increased in *Chondrus crispus* gametophytes infected by an endophytic pathogen (Bouarab et al., 2004). Our results suggest that PAL and PPO play very important roles in disease resistance. In addition, the constitutive activity of PAL differed significantly between the resistant and susceptible line ($P<0.01$). Therefore, PAL was identified as a candidate index to screen for disease resistant lines.

β-1,3-glucans are important structural elements of the cell walls of many fungi and oomycetes (Werner et al., 2002). β-1,3-glucanase has long been suggested to play a role in the plant defense against fungi (Stefani et al., 2010). However, β-1,3-glucans are also present in plants as a minor component (Abeles et al., 1971). The bean pathogen *Colletotrichum lindemuthianum* produces proteins that inhibit the β-1,3-glucanase activity of bean (Albersheim and Valent, 1974). When the gene encoding β-1,3-glucanase was overexpressed in pea, its disease resistance increased and the β-1,3-glucanase enzyme activity boosted to 40-fold of that the control (Mauch et al., 1984). In this study, the β-1.3-glucanase activity increased instantly in both lines upon infection, but to higher levels in the resistant line than in the susceptible line, suggesting that β-1,3-glucanase activity plays a very important role in disease resistance. Therefore, high β-1.3-glucanase activity immediately after infection was identified as another effective index to screen for resistant lines.

5 CONCLUSION

Defensive enzymes played crucial roles in the resistance of nori to red rot disease. The antioxidase activities (SOD, POD, and PPO) appeared increased after infection to resist pathogen. With the infection time delayed, the defensive enzymes activities decreased as cell were destroyed. Therefore, we believe that SOD, POD, and PPO had certain effects on the resistance to pathogen. In addition, we selected other resistant and susceptible lines to repeat the experiment, and found the same trends in enzyme activities after pathogen infection. In this study, the constitutive expression level of PAL differed significantly between resistant and susceptible lines, making it an effective index to select disease resistant lines. The activity of β-1.3-glucanase also increased to greater levels in the resistant line than in the susceptible line at the start of infection. The activities of these two enzymes can be considered as effective indexes to screen for disease-resistant lines. The results of this study provide information about the disease responses of nori, and provide a theoretical basis for screening for disease-resistant lines. Moreover, in the future works, we will analyze the expression differences of related regulatory genes in PAL and β-1.3-glucanase synthesis pathways at the molecular level, to prepare for the development of molecular markers and marker-assisted breeding.

6 DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Abeles F B, Bosshart R P, Forrence L E. 1971. Preparation and purification of glucanase and chitinase from bean leaves. *Plant Physiology*, **47**(1): 129-134.

Albersheim P, Valent B S. 1974. Host-pathogen interactions VII. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. *Plant Physiology*, **53**(5): 684-687.

Aoki Y, Kamei Y. 2006. Preparation of recombinant polysaccharide-degrading enzymes from the marine bacterium, *Pseudomonas* sp. ND137 for the production of protoplasts of *Porphyra yezoensis*. *European Journal of Phycology*, **41**(3): 321-328.

Apostol I, Heinstein P F, Low P S. 1989. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells role in defense and signal transduction. *Plant physiology*, **90**(1): 109-116.

Baucher M, Bernard-Vailhé M A, Chabbert B et al. 1999. Down-regulation of cinnamyl alcohol dehydrogenase in transgenic alfalfa (*Medicago sativa* L.) and the effect on lignin composition and digestibility. *Plant Molecular Biology*, **39**(3): 437-447.

Bouarab K, Adas F, Gaquerel E et al. 2004. The innate immunity of a marine red alga involves oxylipins from both the eicosanoid and octadecanoid pathways. *Plant Physiology*, **135**(3): 1 838-1 848.

Chen, G X, Asada K. 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant and Cell Physiology*, **30**(7): 987-998.

Collén J, Hervé C, Guisle-Marsollier I et al. 2006. Expression profiling of *Chondrus crispus* (Rhodophyta) after exposure to methyl jasmonate. *Journal of Experimental Botany*, **57**(14): 3 869-3 881.

Foyer C H, Descourvieres P, Kunert K J. 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant Cell and Environment*, **17**(5): 507-523.

Friend J, Reynolds S B, Aveyard M A. 1973. Phenylalanine ammonia lyase, chlorogenic acid and lignin in potato tuber tissue inoculated with *Phytophthora infestans*. *Physiological Plant Pathology*, **3**(4): 495-507.

Kawamura Y, Yokoo K, Tojo M et al. 2005. Distribution of *Pythium porphyrae*, the causal agent of red rot disease of *Porphyrae* spp., in the Ariake Sea, *Japan*. *Plant Disease*, **89**(10): 1 041-1 047.

Koca H, Bor M, Özdemir F et al. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and Experimental Botany*, **60**(3): 344-351.

Koukol J, Conn E E. 1961. The metabolism of aromatic compounds in higher plants IV. Purification and properties of the phenylalanine deaminase of *Hordeum vulgare*. *Journal of Biological Chemistry*, **236**(10): 2 692-2 698.

Küpper F C, Kloareg B, Guern J et al. 2001. Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiology*, **125**(1): 278-291.

Li F L, Fu Y, Yuan Q, Lü W H, Xu Y Q, Liu R M, Hu B Z, Chen F W, Xu Y Y, Feng Y Z. 2015. Variations in antioxidase activities and MDA content in potato tubers infected by *Fusarium trichothecioides*. *Agricultural Science and Technology*, **16**(11): 2 433-2 436.

Liang Y, Chen Q, Liu Q et al. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *Journal of Plant Physiology*, **160**(10): 1 157-1 164.

Mauch F, Hadwiger L A, Boller T. 1984. Ethylene: symptom, not signal for the induction of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. *Plant Physiology*, **76**(3): 607-611.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, **7**(9): 405-410.

Park C S, Hwang E K. 2014. Isolation and evaluation of a strain of *Pyropia yezoensis* (Bangiales, Rhodophyta) resistant to red rot disease. *Journal of Applied Phycology*, **26**(2): 811-817.

Park C S, Kakinuma M, Amano H. 2001. Detection and quantitative analysis of zoospores of *Pythium porphyrae*, causative organism of red rot disease in *Porphyra*, by competitive PCR. *Journal* of *Applied Phycology*, **13**: 433-441.

Peng M, Kuc J. 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. *Phytopathology*, **82**(6): 696-699.

Shao H B, Jiang S Y, Li F M et al. 2007. Some advances in plant stress physiology and their implications in the systems biology era. *Colloids and Surfaces B*: *Biointerfaces*, **54**(1): 33-36.

Stefani F O, Tanguay P, Pelletier G et al. 2010. Impact of endochitinase-transformed white spruce on soil fungal biomass and ectendomycorrhizal symbiosis. *Applied and Environmental Microbiology*, **76**(8): 2 607-2 614.

Veitía N, García L R, Bermúdez-Caraballoso I et al. 2014. Field resistance of selected potato clones to early blight. *Biotecnología Vegetal*, **14**(4): 215-221.

Weinberger F, Leonardi P, Miravalles A et al. 2005. Dissection of two distinct defense-related responses to agar oligosaccharides in *Gracilaria chilensis* (Rhodophyta) and *Gracilaria conferta* (Rhodophyta). *Journal of Phycology*, **41**(4): 863-873.

Werner S, Steiner U, Becher R et al. 2002. Chitin synthesis during in planta growth and asexual propagation of the cellulosic oomycete and obligate biotrophic grapevine pathogen *Plasmopara viticola*. *FEMS Microbiology Letters*, **208**(2): 169-173.

Woo J H, Kitamura E, Myouga H et al. 2002. An antifungal protein from the marine bacterium *Streptomyces* sp. strain AP77 is specific for *Pythium porphyrae*, a causative agent of red rot disease in *Porphyra* spp. *Applied and Environmental Microbiology*, **68**(6): 2 666-2 675.

Wu C T, Bradford K J. 2003. Class I chitinase and β-1,3-glucanase are differentially regulated by wounding, methyl jasmonate, ethylene, and gibberellin in tomato seeds and leaves. *Plant Physiology*, **133**(1): 263-273.

Zhang X N, Sun P P, Mao Y X et al. 2015. The variation of defensive compounds content and protective enzymes activity of *Pyropia yezoensis* gametophyte infected by oomycete *Pythium porphyrae*. *Journal of Ocean University of China* (*Natural Science*), **45**(12): 57-64. (in Chinese with English abstract)