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# Increased Peroxidase Activity and Ethylene Production in Association with the Resistance of Tai-Sen-Waxy-Yu 7 rice (*Oryza sativa*) to Bacterial Leaf Blight Disease

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## ABSTRACT

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Resistance of 6 rice cultivars or breeding lines namely Taichung Native 1 (TN 1), Tai-Sen-Waxy-Yu 7 (TSWY 7), Tainung 67, TKM 6, Tetep, and Nigeria 5 at their 45-day-old seedling stages was evaluated by clipping inoculation method in a greenhouse using XF13 isolate of *Xanthomonas campestris pv. oryzae* as a challenging inoculum. The lesion development indicated that TSWY 7, TKM 6 and Nigeria 5 were resistant, whereas TN 1, TNG 67 and tetep were susceptible. The resistance performance was in accordance to that observed in a previous field trial. In order to explore the physiological characteristics associated with bacterial leaf blight resistance, seedlings of both TSWY 7 and TN 1 plants were grown by hydroponic culture in a growth chamber as a model system for the resistance evaluation. Artificial inoculation of TSWY 7 plants with XF13 isolate generally led to an enhanced rate of ethylene production throughout a four days experimental period. Whereas for TN 1 plants, this pathogenesis related ethylene production enhancement was not observed. In accompany to the increased ethylene production, continued increase of peroxidase activity was detected from the soluble protein contents of XF13 inoculated TSWY 7 plants for 4 days after inoculation. And likewise, the enhanced peroxidase activity was not observed in the compared TN 1 plants. Examination by tissue isolation indicated that in TSWY 7 plants, the internal spread and multiplication of test bacteria were both greatly inhibited. The inhibitory effect was due at least in part to the increased peroxidase activity during the disease progress. This view was further supported by the observation that the detected increase of peroxidase activity was mainly located in the vascular bundle sheath cells. An isoelectrofocusing gel electrophoresis analysis revealed the association of two novel cationic peroxidase isozymes, with pIs at 8.91 and 9.23 respectively, in the resistant plant-pathogen interaction. Although the role of these pathogenesis related peroxidase isozymes in disease resistance remained to be elucidated, the results obtained in this study suggest the possibility of ethylene production and peroxidase activation as physiological characteristics closely related to the bacterial blight resistance.

Key words: Bacterial leaf blight of rice; Cationic peroxidase; Disease resistance; Ethylene production; *Oryza sativa*; TN 1; TSWY 7; *Xanthomonas campestris pv. oryzae*.

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, especially among Asian countries. In Taiwan, bacterial leaf blight caused by *Xanthomonas campestris pv. oryzae* has become one of the most serious problems in rice cultivation recently due to the introduction of certain susceptible breeding

lines (3). Disease management for preventing the bacterial infection is one of the routine cultural practices for safeguarding the yield and grain quality in rice production in the majority of rice growing countries (19). For the control of this disease, application of certain bactericidal agents was often a recommended measure. However, the effectiveness of available chemicals was in general quite limited (18); and the deleterious effect on the living environment due to the pesticide application is now of great concern worldwide. Breeding and application of disease resistance remain to be a prime strategy persued by most related workers for the disease control. Already some efforts have been devoted to elucidate the mechanism(s) contributing to the disease resistance. The known defensing mechanisms of rice to this bacterial pathogen include the development in vessel tissue a fibrillar material capable of enveloping and eventually killing the invading bacteria and the production in infected tissues various antibacterial components (10). A notable example of the involvement of antibacterial component appears to be the bacterial leaf blight resistance conferred by the gene *Xa*-10, in which the rapid accumulation of lignin-like material and the accompanied increases of cationic peroxidase activities were found to be determinative characteristics closely associated with the disease resistance (20,21). For plant resistance, the antimicrobial activity of lignin has been well recognized (26). The activity of peroxidase (EC 1.11.1.7, donor: $H_2O_2$ oxidoreductase) was an essential factor for the formation of lignin (9). It was also known that biochemical processes catalyzed by this enzyme were generally accompanied with the production of microbicidal oxygen species (7).

In Taiwan, to cope with the increasing importance of bacterial leaf blight disease, screening and breeding for disease resistance have become a routine program in Taichung District Agricultural Improvement Station (DAIS) by field trials since 1975. One of the main difficulties encountered in the proceeded traditional disease resistance breeding and screening works, however, was the requirement of enormous man power and the inconsistency of the symptom development due to the biological as well as environmental variations. The frustration prompted us attempts to explore (if any) additional dependable physiological or biochemical traits that maybe helpful for disease resistance screening. A model system for disease resistance evaluation was established with rice plants grown in greenhouse or growth chamber as test material. The resistance performance of 6 test rice plants in the model system was found to be consistent to that observed in resistance screening tests performed in the field. As disease resistance of rice to bacterial leaf blight known so far suggested connections to the enhanced peroxidase and lignin biosysthetic activities typical of hypersensitive reaction, ethylene production and changes of peroxidase activities in typical resistant and susceptible plant hosts during the disease progression were compared. The results obtained strongly indicated the association of increased ethylene production and peroxidase activities with the resistant host pathogen interaction. The possibly involved resistance mechanism and the potential of their uses as an aid in disease resistance screening are herein discussed.

## MATERIALS AND METHODS

### Plant materials

Six rice cultivars or breeding lines including Taichung Native 1 (TN 1), Tai-Sen-Waxy-Yu 7 (TSWY 7), Tainung 67 (TNG 67), Tetep, Nigeria 5, and TKM 6 were used in the initial screening tests. In greenhouse, water-imbibed seeds were first treated with 0.1% Benlate overnight, and about 60 seeds were then sowed in each 60 (L) × 30 (W) × 7 (H) cm black plastic tray. The seedlings developed 45 days after sowing were used for resistance performance assay by clip inoculation method (15). For ethylene production and peroxidase assay, only TN 1 and TSWY 7 were used. And instead of grown in soil in greenhouse, the test plants were cultivated by the hydroponic culture system described by Kao and Yu (14) and kept in a growth chamber in which a 12 h day light cycle and a 28 (light) /23 (dark) C temperature fluctuation regime were provided. Relative humidity was maintained at 70–90% and light intensity was approximately 10,000 luxes. About 70–80 test rice seeds, after thorough water imbibition and Benlate pretreatment, were evenly distributed on a stainless steel screen (144 mesh) shelf at 8 cm in diameter and then placed in an aluminum foil wrapped 500 ml plastic beaker which contains Kimura's hydroponic culture solution barely enough to contact rice seeds. Throughout the experimental process, distilled water was added to each beaker daily to maintain the water level; and the culture solutions were replaced every 4 to 5 days. The seedlings developed 10–12 days after sowing were used for the artificial inoculation and the performed biochemical analysis.

### Bacterial isolate and the inoculum preparation

The XF13 isolate of *Xanthomonas campestris pv. oryzae* was kindly provided by Dr. Chien of Taiwan Agricultural Research Institute (TARI). The original bacterial culture was allowed to grow on Wakimoto agar slant (27) at 27 C for 2 days; and the bacterial cells harvested therein were submerged in 15% glycerol containing sterile distilled water and placed in -80 C deep freezer for long term preservation. In each performed test, the bacterial cells from long term

storage vials were first revived on Wakimoto medium, subcultured in 523 broth (13) at 27 C under continuous shaking at 100 rpm for 24 h, and then adjusted to approximately $10^9$ CFU/ml for the artificial inoculation.

### Determination of disease resistance and detection of bacterial colonization

The test plants were artificially inoculated with test bacterial suspension by clip-inoculation method as that described by Koch and Mew (15). The symptoms developed on the six test plant hosts were recorded by measurement of lesion extension on a daily basis after inoculation. For detection of the bacterial colonization, leaf samples were collected, surface sterilized by 1% chlorax, washed with sterile water, cut into 5 mm segments from the point of inoculation, plated out in order on 523 agar plate, and incubated at 27 C in the dark. The number of leaf segments that had bacterial colonies grew out from the cut end were then recorded. Whereas for the estimation of bacteria multiplication in test plants, the leaf samples collected were homogenized in sterile distilled water, diluted in 10 X series, and plated out on 523 agar plates. The number of colonies developed were determined for the approximation of total bacterial counts in the test plants.

### Detection of ethylene production during disease progression

The apical 6 cm leaf segments were collected from test plants each day after inoculation. A total of 12 leaf segments from each replicate (4 replicates for each treatment) were placed in a serum tube in which 20 $\mu$l distilled water were added and the interior space was flushed with fresh air right before sample collection. The serum tubes were then sealed immediately by serum caps. After 2 h incubation at room temperature, a 1 ml gas sample was withdrawn from the head space of each serum tube by a gas-tight syringe. The amount of ethylene produced was determined by a Shimadzu GC-14A gas chromatograph equipped with an alumina column and a flame ionization detector as that described by Tzeng *et al.* (24). Calibration gas at 1.006 ppm in concentration was obtained from Matherson Gas Products (New Jersey, U.S.A.) and used as the standard for the quantification of ethylene generated in test samples.

### Detection of peroxidase activity in diseased tissue

About 50 mg of the apical 4 cm leaf segments were harvested from the test plants daily after inouclation. The collected samples were ground into powder in the presence of liquid nitrogen with a motar and pestle. A 750 $\mu$l of ice-cold 1% (W/V) polyvinyl-polypyrrolidone containing phosphate buffer (0.2 M, pH 7.0) were then added to the homogenate; the plant debris in test samples were removed by centrifugation at 12,000 *g* for 30 min. The amount of soluble proteins obtained was determined by the BioRad protein assay kit according to the protocol provided by the manufacturer. Whereas the peroxidase activity present in these sample extracts was determined by the method described in Worthington Enzyme Manual (5). To a 100 $\mu$l sample extract, a 1.5 ml 0.0017 M $H_2O_2$ containing potassium phosphate buffer (0.2 M, pH 7.0) and 1.4 ml 0.17 M phenol amended 4-amino-antipyrine (0.0025 M) were added together in a quatz cuvette. The temperature of the reaction mixture was maintained at 25 C by a circulating water bath jacket; and rates of changes of $A_{510}$ during the first 3 minutes were monitored by a Shimadzu UV-160A spectrophotometer. The horseradish peroxidase with known amount of enzyme activitiy was obtained from Sigma Chemical Co. for the calibration of that detected from test samples. To further understand the varietal changes of peroxidases in the test samples, the enzyme extracts were purified by ammonia sulfate precipitation according to the method of Shannon *et al.* (22). The sample extracts were first clarified by 36% ammonia sulfate precipitation and a low speed centrifugation (2,000 *g*, 5 min). The remaining supernatants were then treated with 90% ammonia sulfate at 4 C overnight; and the protein precipitates were then spinned down (12,000 *g*, 15 min at 4 C), desalted by dialysis using a Pierce Microdialyzer System 500 setup, and lyophillized. For peroxidase isozyme analysis, these protein extracts were separated on 5% Servalyte Precote ultrathin polyacrylamide gel (125 × 0.15 mm, with pIs ranged from 3.0 to 10.0) using a BioRad Biophoresis Horizontal Isoelectrofocusing system. With the presence of cathode buffer (composed of 0.4% arginine, 0.06% L-lysine, and 12% ethylene diamine) and anode buffer (composed of 0.33% L-aspartic acid, and 0.37% L-glutamic acid), the gel was prefocused at 4 W constant power until voltage of the power supply reached 500 volts before sample application. The protein extracts were applied by a sample applicator provided in the Serva Precote package. Isoelectrofocusing separation was proceeded with the maximum voltage of power supply set at 1700 volts and the run was stopped after total power output have reached 2200 volthours (approximately 3 hours). Peroxidase activity of the resolved protein bands was revealed by staining the gel for 10 to 15 min in 50 ml phosphate buffered saline (PBS, 50 mM, pH 6.0) which contained 5% EtOH, 30 mg 4-chloro-1-naphthol and 0.26 ml 30% $H_2O_2$ (16).

### Histochemical detection of peroxidase activity in diseased tissue

Leaf segments about 1 cm from the tip were collected from inoculated plants and treated with fixative solution [a 1% formaldehyde containing PBS

(50 mM, pH 7.4)] for 2 h at 4 C as that described by Al-Azzawi and Hall (2). After a thorough washing by PBS, the sample tissues were embedded in IEC Cryoform (International Equipment Company, Needham Hts., Massachusetts) at −20 C. Thin-sectioning was performed by an IEC Microtome (Microtome Cryostat Model IM236) with the thickness adjusted to 12 μm. The thin sections obtained were stained with the above stated IEF Gel staining mixture for approximately 5 min. After a thorough washing by PBS (50 mM, pH 6.0), the thin sections were examined by an Olympus BH2 microscope for the presence of peroxidase activity. The pictures were taken with an Olympus Model PM-10AD photomicrographic system equipped with the microscope.

## RESULTS

### Determination of resistance against XF13 isolate in greenhouse

Among six test cultivars or breeding lines, TN 1, Tetep and TNG 67 appeared to be susceptible to XF13 isolate of *X. campestris pv. oryzae* (Table 1). In the greenhouse test, apparent dark-greenish water soaking symptoms, starting from the tips, were generally observed on these three test hosts 4 days after inoculation. The symptoms then extended rapidly downward evidentally through the vascular vessel against the water flow; the test plants developed brownish or whitish blight symptoms about 6–14 days after inoculation. In contrast to this, TSWY 7, TKM 6 and Nigeria 5 showed different level of disease resistance to the XF13 isolate of *X. campestris pv. oryzae.* Observable symptoms on these plant hosts were not detected until 9 days after inoculation; and extensive symptom progression thereafter remained quite slow and limited. As shown in Fig. 1, about 14 days after inoculation, the leaf blight symptoms on TN 1, Tetep, and TNG 67 have all extended to the whole leaf; whereas those of the three resistant breeding lines were still restricted only around the apical portion. TSWY 7 appeared to be, among the 6 test plant hosts, most resistant to the XF13 isolate.

### Detection of bacterial colonization in test plant hosts

To resistant TSWY 7, for 6 days after inoculation, the test bacterium was detected only from the apical 1 cm leaf segments (Fig. 2-a). Whereas for the comparable susceptible TN 1, the bacterial colonization in the plant host has extended to greater than 2 cm length on the 5th day; on the 6th day, the colonization even reached further downward. Accompanied with the extension of the bacterial colonization, a linear increase of the number of colony forming unit of test bacteria was observed in TN 1 plants 2 to 5 days after inoculation (Fig. 2-b). On the 6th day, the total count of the test bacteria in this plant host slightly declined. In TSWY 7, although a slight increase of the test bacteria, comparable to that in TN 1, was detected during the begining 3 days, it then declined steadily. The total count of test bacteria in TSWY 7 on the 6th day declined to less than 0.1% of that in TN 1 plants.

### Ethylene production from diseased plants after inoculation

Production of ethylene from XF-13 isolate inoculated TSWY 7 plants showed significant increases, although leveling off on the third day, for four days after inoculation (Fig. 3). In the compared TN 1 plants inoculated with test bacteria, on the contrary, the increase of ethylene production during the same period was not detected. Rate of ethylene production from these plants on the 4th day after inoculation was less than one fifth of that of TSWY 7 plants. Moreover, it

TABLE 1. The development of necrotic lesions (cm in length) on seedling of 6 test rice varieties or breeding lines after clip inoculation by XF-13 isolate of *Xanthomonas campestris pv. oryzae* (approximately $10^9$ cfu/ml) in greenhouse

| Test plants | Days after inoculation | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| TN 1 | 0 | +[1] | 1.27 ± 0.25 | 4.83 ± 0.29 | 5.67 ± 0.58 | 6.00 ± 0.00 |
| TNG 67 | 0 | + | 0.50 ± 0.00 | 1.67 ± 0.29 | 3.33 ± 0.58 | 4.17 ± 1.04 |
| Tetep | 0 | + | 0.50 ± 0.00 | 1.10 ± 0.17 | 2.33 ± 0.58 | 3.67 ± 0.58 |
| TKM 6 | 0 | 0 | 0 | 0 | 0.16 ± 0.29 | 0.33 ± 0.29 |
| Nigeria 5 | 0 | + | + | + | + | + |
| TSWY 7 | 0 | 0 | 0 | 0 | 0 | 0.17 ± 0.29 |

[1] +, diseased plants showed only water soaking symptom around the infected tissue; no necrotic lesion was observed at the indicated time. Data shown were average length (cm) of necrotic lesions developed on 10 surveyed plant leaves.

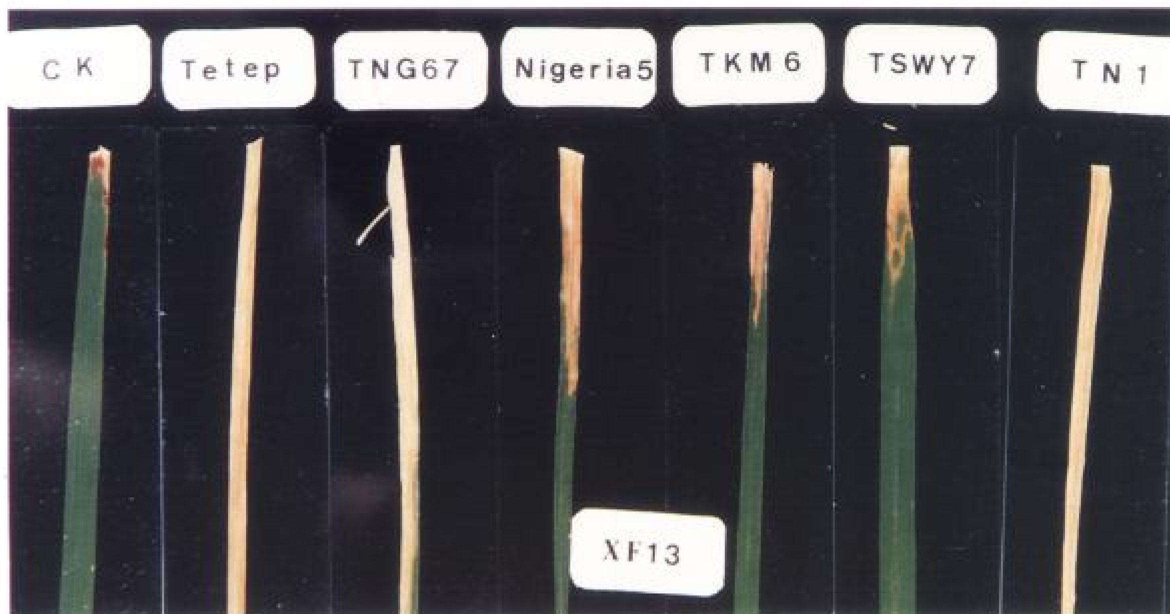


**Fig. 1.** Symptom development of six test rice varieties 14 days after clip inoculation with XF13 isolate of *Xanthomonas campestris pv. oryzae* ($10^9$ cfu/ml). The seedlings grown in a greenhouse for 35 days were used for the resistance performance assay.

was noted that mock-inoculation of TSWY 7 plants with sterile distilled water also led to increased rate of ethylene production in a followed 4 days after treatment. However, the production rate detected from these plants were generally significantly less than the pathogen treated ones although that detected on the third day appeared to be at the same level. And similar to that of pathogen treated plants, the rate of ethylene production detected from TN 1 plants mock-inoculated with sterile distilled water remained at the background level during the course of experiment.

### Changes of peroxidase activity during the disease progression

The amounts of proteins detected from leaves of the two test plant hosts both indicated a steady decline during the course of study (Fig. 4). For TN 1 plants, the protein contents of leaf tissues dropped from about 30 mg/gfw to about 10 mg/gfw during the 6-day experimental period. The protein contents detected from TSWY 7 plants were consistently about 10 mg higher than the compared TN 1 plants. However, the rate of decline of the protein contents in TSWY 7 leaves appeared to be the same as that of TN 1 plants. The protein contents of both test plants were not affected by the inoculation treatment. In contrast to the changes of protein contents, peroxidase activity of TSWY 7 plants showed a steady increase for four days after inoculation by test bacteria (Fig. 5). The activity of peroxidase detected at the 4th day after inoculation was approximately twice of that at day zero. Mock-inoculation of TSWY 7 plants with sterile distilled water also led to the increases of peroxidase activity; however, the level of enhancement appeared to be somewhat less as compared to that of pathogen treated ones. Peroxidase activities of test bacteria inoculated TN 1 plants seemed to remain at the same level during the 6-day experimental period. The protein contents extracted from leaf samples 4 days after inoculation were concentrated by ammonium sulfate precipitation and subjected to isoelectrofocusing gel electrophoresis. As shown in Fig. 6 and Table 2, the result of peroxidase activity staining revealed that the kinds of peroxidase detected from this two test plant hosts were very different. From TN 1 plant leaves, approximately 10 peroxidase isozymes were detected. The pIs of the major bands appeared to be around 10.65, 5.26, and 4.29. The inoculation by test bacteria and mock-inoculation by sterile distilled water both did not change the profile of these isoenzymes. Whereas from TSWY 7 leaves, about 18 peroxidase isozymes were identified; the pIs of the major bands appeared to be around 8.49, 5.48, 5.26 and 4.29. And in contrast to that of TN 1 plants, the inoculation by test bacteria led to the production of 2 unique peroxidase isozymes with pIs at 8.91 and 9.23 respectively. These two isozymes were not detected in the sterile distilled water mock-inoculated or the non-treated control plants.

### Histochemical detection of peroxidase activity in test plants

By histochemical staining, it was observed that peroxidase activity can be detected among epidermal cells, mesophyll cells, and vascular tissues of both test plant hosts (Fig. 7). The enzyme activity appeared to be most prominent around cell wall portion, also evident was in cytoplasms of mesophyll or paravascular parenchyma cells (Fig. 7,C&D). Proceeded time course

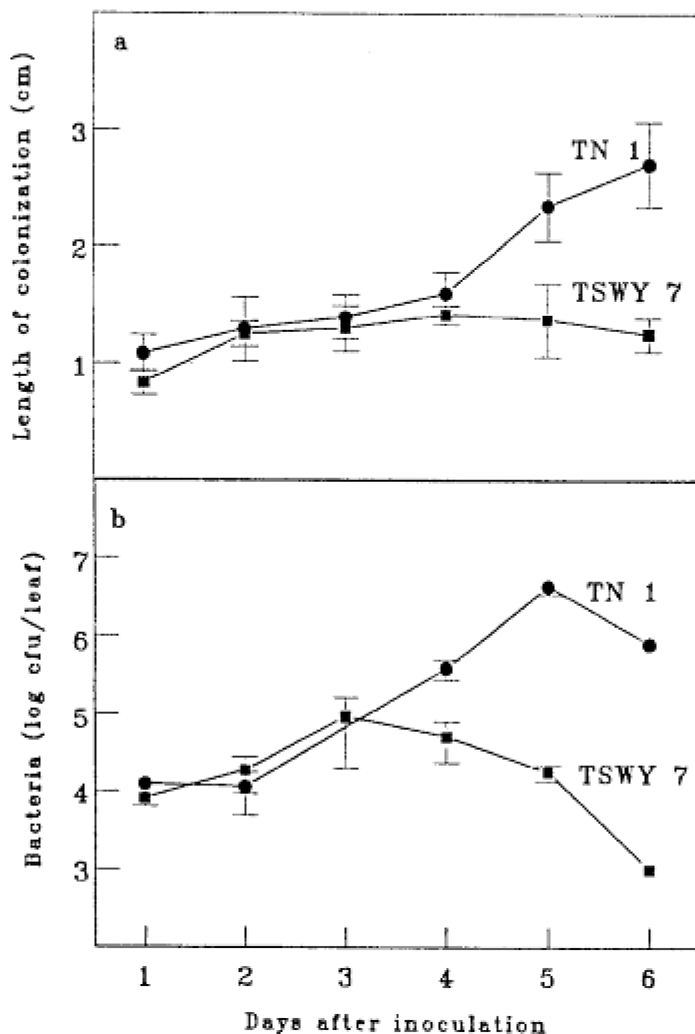


**Fig. 2.** Detection of internal migration (a) and multiplication (b) of *Xanthomonas campestris pv. oryzae* (XF13 isolate) in TSWY 7 (■) and TN 1 (●) plants during disease progression in a growth chamber. Details of the detection method were described in the text.

study on the changes of peroxidase activity further revealed that along with the pathogen ingression, there were significant tissue specific increases of the enzyme activities in test bacteria inoculated TSWY 7 plants. The peroxidase activity enhancement was detected mainly on the vascular bundle sheath cells which started about 3 days after inoculation and became most prominent on the 4th day (Fig. 7, F). In other portion of pathogen affected TSWY 7 leaf tissue, the enhanced peroxidase activity was not evident. The same situation seemed to hold for TSWY 7 plants mock-inoculated with sterile distilled water (Fig. 7, D) and TN 1 plants inoculated with the test pathogen (Fig. 7, E).

## DISCUSSION

Breeding for disease resistance is one of the most enchanting prime subjects in nowadays agricultural

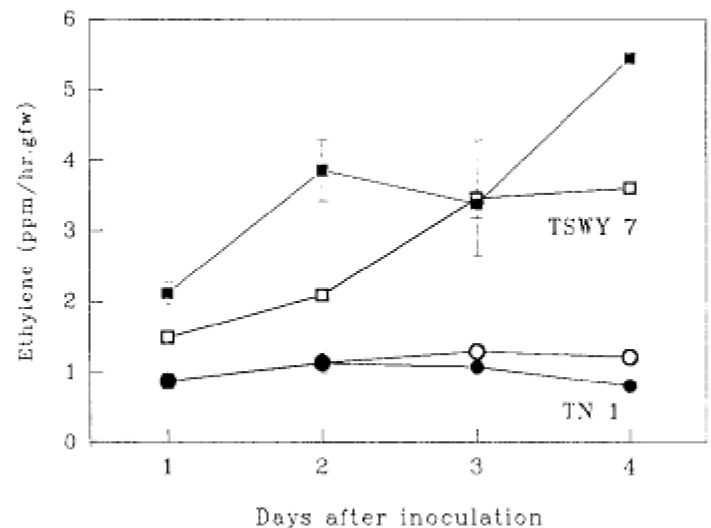


**Fig. 3.** Ethylene production from *Xanthomonas campestris pv. oryzae* (XF13 isolate) inoculated TSWY 7 (■) and TN 1 (●) rice seedlings during disease progression in a growth chamber. The plants were artificially inoculated with XF13 isolate at $10^9$ cfu/ml by clipping methods. The control plants (○、□) were mock-inoculated with sterile distilled water.

biotechnological researches worldwide. For bacterial leaf blight disease on rice, the resistance breeding works are of extreme significance and great rewarding since none of the available control strategies known so far works satisfactory. However, as above stated, the resistance breeding works were often discouraging and frustrating because of the complication of biological as well as the environmental factors. Success of this attempt thus depends greatly on the understanding of the molecular basis of the disease resistance. Unfortunately, the exploration on the resistance of this disease has been limited; and a lot more efforts are needed for finding a resolution for the disease problem.

Resistance of rice plants to the studied disease known so far includes the entrapment and eventually killing of the invading pathogen by the generation of certaiin fibrillar substance in the affected tissue, the generation of phenolics, lignin and/or phytoalexins, and the activation of certain cationic (or basic) peroxidase enzymes (10,17,20,21,25). Convincing evidences have been provided which showed that a great proportion of these known resistances were conferred by certain race specific major genes. In the present study, the six test plant hosts in the initial greenhouse screening test were selected from a three consecutive years field resistance screening works carried out in Taichung DAIS by the same clip inoculation technique. The observed disease responses of these test plant hosts at their seedling stage to challenging inoculation of XF13 isolate of *X. campestris pv. oryzae*, as shown in Table 1, were consistent to the resistance performance observed in previous field trials (data not shown). While studying

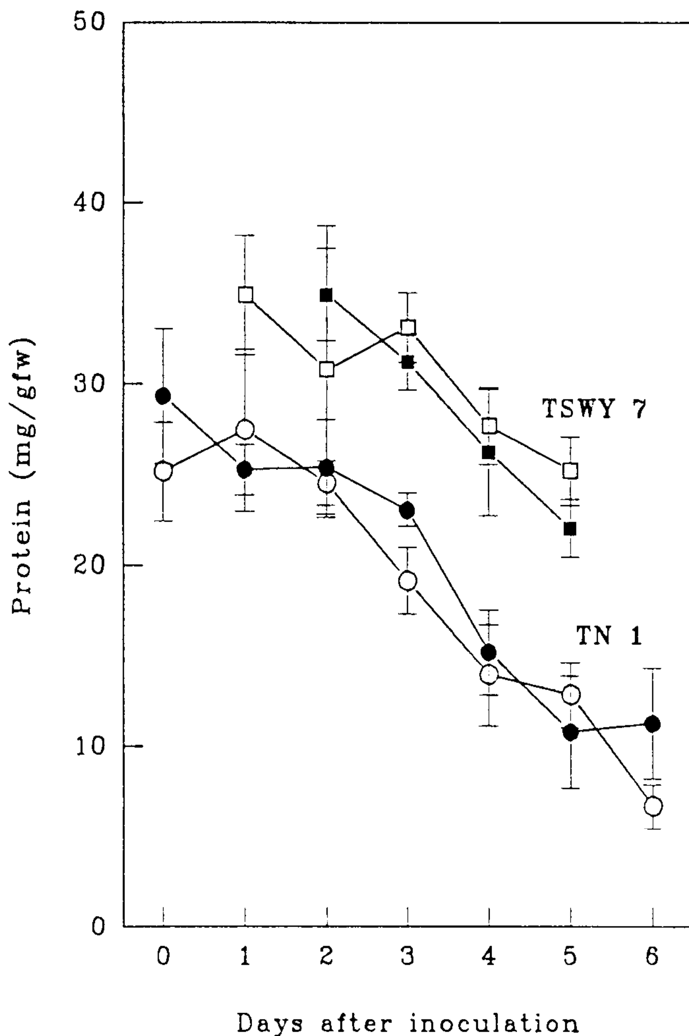


**Fig. 4.** Changes of soluble protein contents of *Xanthomonas campestris pv. oryzae* (XF13 isolate, $10^9$ cfu/ml) inoculated TSWY 7 (■) and TN 1 (●) rice leaves during disease progression in a growth chamber. The compared control plants (○ ˎ □) were mock-inoculated with sterile distilled water.

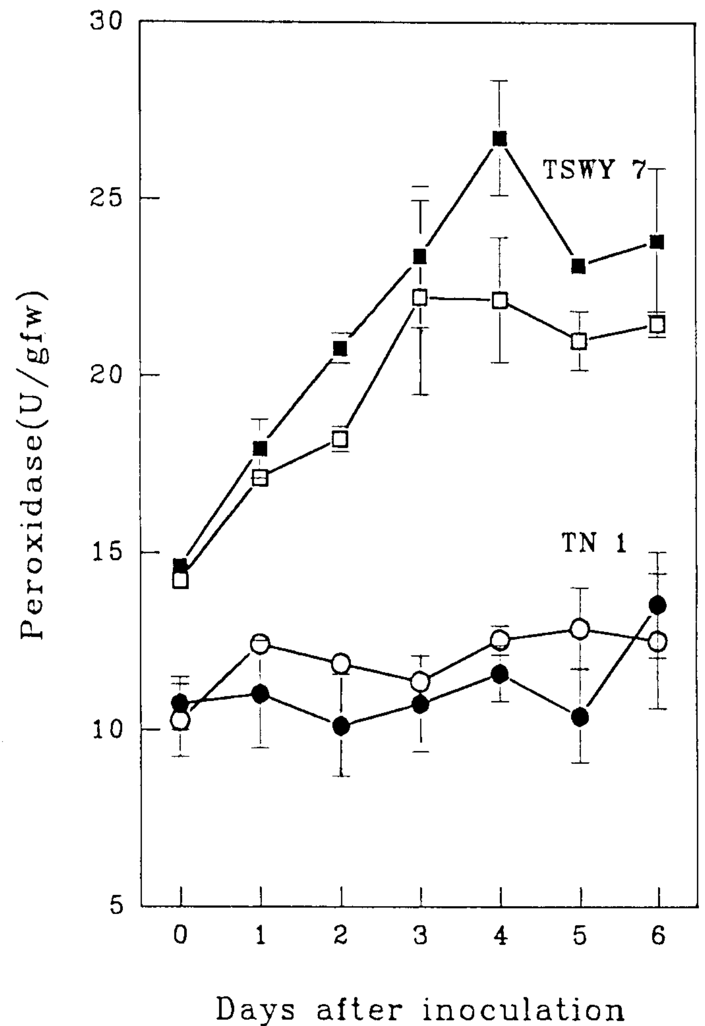


**Fig. 5.** Changes of peroxidase activities of *Xanthomonas campestris pv. oryzae* (XF13 isolate, $10^9$ cfu/ml) inoculated TSWY 7 (■) and TN 1 (●) rice leaves during disease progression in a growth chamber. The compared control plants (○ ˎ □) were mock-inoculated with sterile distilled water.

the bacterial leaf blight resistance of rice cultivar carrying *Xa*-10 gene, Reimers and Leach (21) noted that the resistance response expressed by rice seedlings infiltration-inoculated with the incompatible pathogen strain in a model greenhouse experiment were comparable to that by field grown plants. These authors also indicated that although the plant resistance may increase with the development of plant age, results shown by seedling assay can be dependable traits for accelerating resistance screening. The greenhouse results presented here echoed their view in regard to the usefulness of seedling assay as a model system for the screening of bacterial leaf blight resistance.

The strong resistance of TSWY 7 to XF13 isolate, manifested by the inhibition of the bacterial colonization and multiplication (Table 1 and Fig. 1), suggested clearly the involvement of certain major gene. For a major gene conferred plant resistance, including that against fungal, bacterial or even viral pathogens, rapid increases of rate of ethylene production and peroxidase activities were typical physiological characteristics associated with an avirulent pathogen elicited hypersensitive responses (4,6,11,21,23). The detection of increased ethylene production and peroxidase activities from XF13 isolalte inoculated TSWY 7 rice plants one day after inoculation (Figs. 3 and 5) indicated that changes of these biochemical activities an early feature associated with the resistance response of the studied disease. The activity enhancement at the early stages of pathogenesis implicated their correlations to the inhibited bacterial colonization and multiplication and thus the prohibited lesion development. In plant physiology, peroxidase is an essential factor known for ethylene biosynthesis and lignin formation. Moreover, the effectivity of ethylene to activate peroxidative enzymes and lignin biosynthetic process was well documented (6,12). It is generally believed that the inhibitory effect of peroxidase, as well

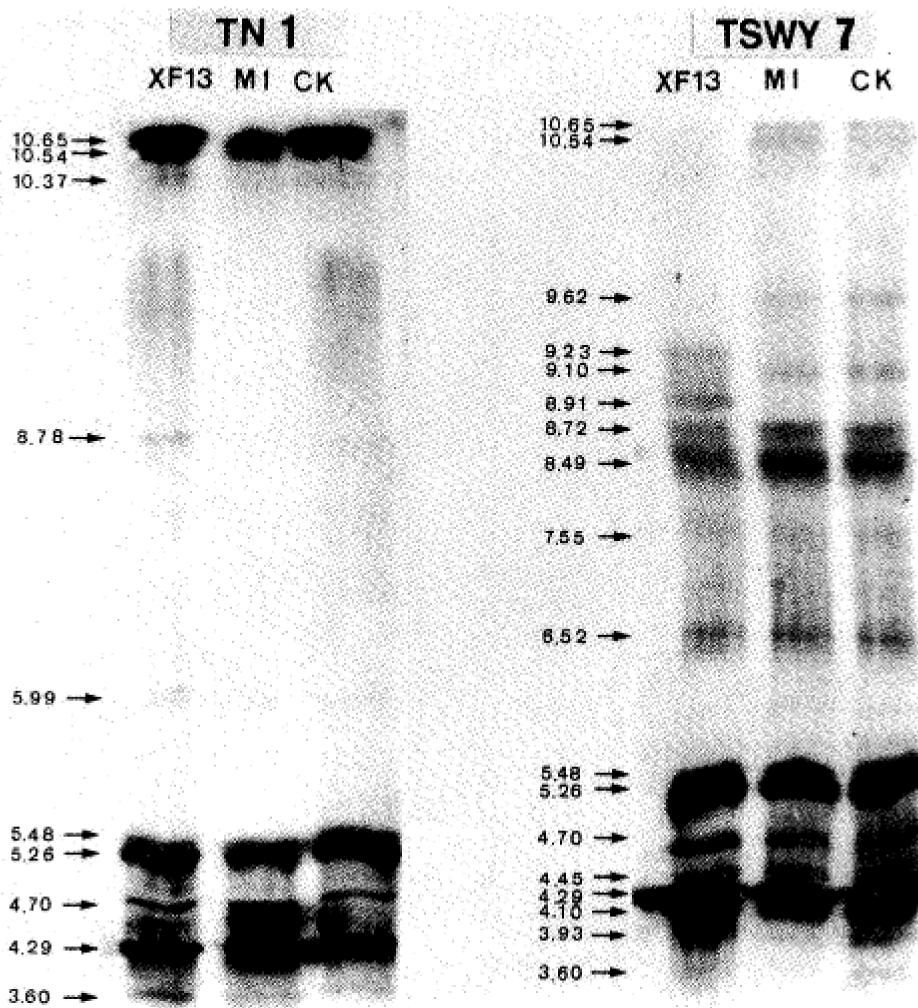


**Fig. 6.** Isoelectricfocusing gel electrophoresis of peroxidase preparations extracted from *Xanthomonas campestris pv. oryzae* (XF13 isolate) inoculated TSWY 7 and TN 1 rice leaves 4 days after artificial inoculation. The compared control plants were either mock-inoculated with sterile distilled water (MI) or remained untreated (CK). The peroxidase activities were revealed by 4-chloro-1-naphthol staining.

lignin, to a biological activity is due mainly to the generation of toxigenic peroxidants. For resistance of rice to bacterial leaf blight disease, Akutsu and Watanabe (1) reported that increases of peroxidase activity in test plants were correlated with the susceptibility rather than resistance. Reimers *et al.* (20) however, demonstrated recently the correlation of an approximately 3 folds increase of peroxidase activity with the leaf blight resistance of rice conferred by *Xa-10* gene. A plausible explanation for these contradictory results was the use of different plant materials and different methods for the enzyme assay. In Akutsu and Watanabe's works, the test enzyme samples were extracted from the plant tissue by homogenization with cold buffer at pH 5.9. While in that of later authors, the extracellular fluid of plant leaves obtained by centrifugation were used for the enzyme assay. In our experiment, the enzyme protein were extracted from plant tissue by liquid nitrogen homogenization and a followed concentration by lyophillization. The results obtianed supported the later author's view on the importance of peroxidase in bacterial leaf blight resistance.

In the test model system, soluble protein contents of test leaf samples, from either the resistant or the susceptible plant hosts, appeared to decline steadily during the experimental period (Fig. 4). The reduction of soluble protein content might simply reflect the fact that the test leaves have reached their maturity and have approached the point of undergoing the senescence process. The observed enhancement of both ethylene production and peroxidase activity in pathogen inoculated plants seemed to due in great part to the wounding effect caused by clip inoculation since similar responses, although at a somewhat lower extent, were also evident in those sterile distilled water mock-inoculated control plants. In the susceptible TN 1 plants, the same phenomenon was not observed indicating that

TABLE 2. Comparisons of peroxidase isozymes detected from *Xanthomonas campestris pv. oryzae* (XF13 isolate) inoculated TSWY 7 or TN 1 rice seedlings 4 days after inoculation. The compared control plants were either mock-inoculated with sterile distilled water (MI-SDW) or remained untreated (CK)

| pI | TSWY 7 | | | TN 1 | | |
|---|---|---|---|---|---|---|
| | XF13 | MI-SDW | CK | XF13 | MI-SDW | CK |
| 10.65 | +[1] | + | + | ++ | ++ | ++ |
| 10.54 | – | + | + | ++ | ++ | + |
| 10.37 | – | – | – | + | + | + |
| 9.62 | – | + | + | – | – | – |
| 9.23 | + | – | – | – | – | – |
| 9.11 | – | + | + | – | – | – |
| 8.91 | + | – | – | – | – | – |
| 8.78 | – | – | – | + | + | + |
| 8.72 | + | + | + | – | – | – |
| 8.49 | ++ | ++ | ++ | – | – | – |
| 7.55 | + | + | + | – | – | – |
| 6.52 | + | + | + | – | – | – |
| 5.99 | – | – | – | + | + | + |
| 5.48 | ++ | ++ | ++ | + | + | + |
| 5.26 | +++ | +++ | +++ | ++ | ++ | ++ |
| 4.70 | ++ | + | + | + | + | + |
| 4.45 | + | + | + | – | – | – |
| 4.29 | ++ | ++ | ++ | ++ | ++ | ++ |
| 4.1 | + | + | + | – | – | – |
| 3.93 | + | + | + | – | – | – |
| 3.6 | + | + | + | + | + | + |

[1] Data presented were relative activities of detected peroxidase isozyme bands at indicated pI value. "–", no activity; "+", observable activity; "++", strong activity; and "+++", very strong activity. Details of the performed isoelectric focusing gel electrophoresis and enzyme activity staining were described in the text.

the detected resistance related reaction machinery can be also turned on by simply wounding treatment.

The activation of peroxidase enzymes is a well recognized feature for higher plants subjecting to various stresses. In regard to its significance to plant growth and development, Gaspar *et al.* (8) indicated that the activation generally follow a two-step and interdependent control mechanisms. For most known examples, the activation and release of cationic isoperoxidases takes place first. The activation process appeared to be instant and it seemed to start from the membrane perturbation by the stress factors. It was also indicated by these authors that the rapid activation of cationic peroxidase may then bring about changes in auxin and ethylene metabolisms and thus further induce the *de novo* synthesis of anionic peroxidase. For stresses due to studied leaf blight pathogen, Reimers *et al.* (20) recently demonstrated that disease resistance of rice cultivar Cas 209 might have certain connections to the rapid accumulation of a cationic peroxidase during the pathogenesis. The detection of enhanced cationic peroxidase activity and two novel cationic peroxidase in TSWY 7 plants inoculated with XF13 isolate (Fig. 6) herein described was in agreement to the observation by these authors. The results obtained from the followed histochemical studies further indicated that the enzyme activation occurred mainly in the bundle sheath cells surrounding the vascular tissue. In regards to the molecular basis of leaf blight resistance, the role of these observed biochemical changes deserves great attention. While for disease resistance screening and breeding works, the determination of changes of ethylene production and peroxidase activity by the discussed model system may provide additional valuable phenotypic data for the judgement of resistance performance as well as the genetic inheritance of the involved gene(s).

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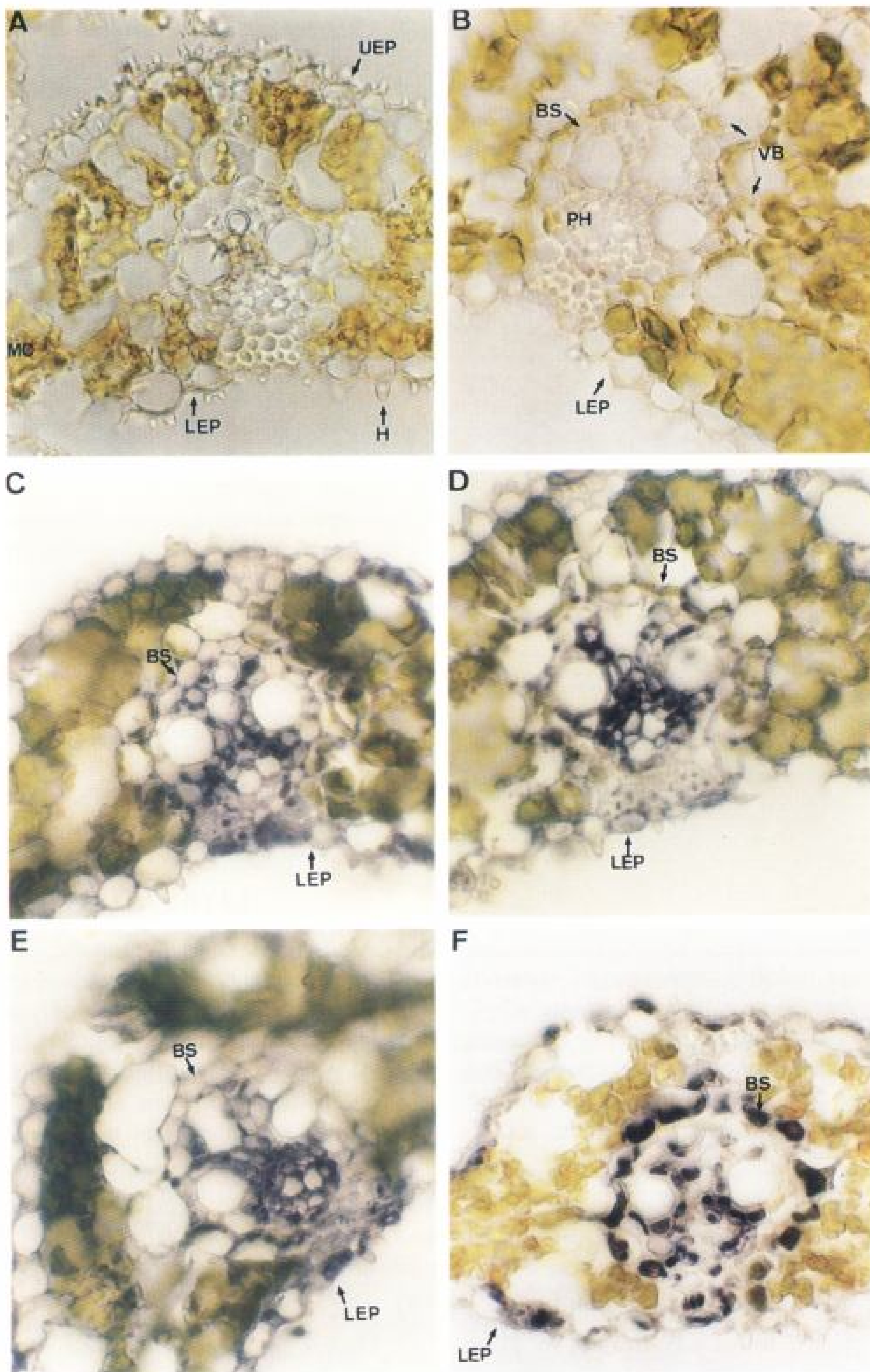


**Fig. 7.** Comparative histochemical detection of peroxidase (PO) activity in hydroponically cultured TN 1 and TSWY 7 rice leaves 4 days after inoculation with XF13 isolate of *Xanthomonas campestris pv. oryzae* in a growth chamber. The cross sections were obtained by IEC-Cryomicrotome as that described in the text. The peroxidase activity was revealed by 4-chloro-1-naphthol staining. All the pictures shown were taken approximately 5 min after adding PO staining reagent. A, TN 1, XF13 inoculated, no PO staining; B, TSWY 7, XF13 inoculated, no PO staining; C, TN 1, SDW mock-inoculated, PO stained; D, TSWY 7, SDW mock-inoculated, PO stained; E, TN 1, XF13 inoculated, PO stained; F, TSWY 7, XF13 inoculated, PO stained. (UEP, upper epidermis; LEP, lower epidermis; H, hair; MC, mesophyll cell; VB, vascular bundle; BS, bundle sheath; PH, phloem.).

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## 摘　要

李成章[1]、張素貞[2]、許富翔[3]、曾德賜[3]. 1994. 過氧化酵素活性及乙烯產生增加與台秈糯育七號水稻對白葉枯病抗病性之關係. 植病會刊3:107-118. (1. 台中市　國立中興大學農藝學系, 2. 彰化縣　台中區農業改良場作物生產課, 3. 台中市　國立中興大學植物病理學系)

利用剪葉接種法於溫室以台中在來一號(TN 1)、台秈糯育七號(TSWY 7)、台農67號(TNG 67)、TKM 6、Tetep及Nigeria 5等水稻品種或品系之盆栽幼苗，接種XF13白葉枯病菌(*Xanthomonas campestris pv. oryzae*)分離株，測試其抗病性，結果發現六個供試水稻品種(系)中，(TSWY 7)、TKM 6與Nigeria 5分別呈現抗病性，而TN1、TNG 67及Tetep則分別爲感病性，此以盆栽幼苗測試所見之抗病性表現，與先前利用田間材料之測試所得結果一致，

爲瞭解與白葉枯病抗病作用有關之生理特性，繼而以生長箱中水耕種植之TSWY 7與TN 1水稻幼苗爲抗病性測試模式系統，試驗結果發現TSWY 7水稻植株於接種XF13病原菌株後四天中，乙烯產生有明顯增加的現象，此一現象於對照組之TN 1植株則未見之，此外，伴隨乙烯產生之增加，XF13接種之TSWY 7水稻植株，葉片可溶性蛋白中所含過氧化酵素活性在接種後四天中明顯逐日提高，此現象於對照組之TN 1植株亦未見及，以組織分離方法測試，發現於抗病TSWY 7植株中，病原之向下蔓延移行與增殖均明顯受到抑制，此一抑制作用與病勢進展過程中寄主體內過氧化酵素活性的提高明顯有關，本研究中另以顯微鏡進行組織化學檢視，發現抗病寄主體內接種測試菌後，過氧化酵素活性之提高主要局限在維管束鞘細胞，此一試驗結果亦支持過氧化酵素活性提高與抗病性關係之觀點，以等電點焦集電泳分析此抗病寄主可溶性蛋白成份，結果顯示其抗病性寄主病原交互作用過程與等電點分別爲8.91及9.23兩個陽離子性過氧化酵素同功酵素之產生有明顯關連，雖則此過氧化酵素在抗病作用上所扮演之角色仍待究明，由本研究所獲得之各試驗結果似不難看出，乙烯產生以及特定過氧化酵素活性的提高，或可作爲水稻白葉枯病抗病性篩選應用之有用生化指標。

關鍵詞：水稻白葉枯病，陽離子性過氧化酵素，抗病性，乙烯產生，台中在來一號(TN 1)，台秈糯育七號(TSWY 7)。