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Fungal Development, Respiration and Activity of Oxidative Enzymes in Rice Plants Inoculated with *Pyricularia oryzae* in Both Compatible and Incompatible Combinations

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벼 稻熱病菌에 感染된 親和 및 非親和 組合 벼에서의 菌生長, 呼吸 및 酸化酵素 活性

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ABSTRACT

Appressorial formation of *Pyricularia oryzae* on leaves showed no marked difference between highly resistant Tongil and susceptible Norin No. 6. Race N-2 of the blast fungus penetrated directly into motor cells of susceptible cultivar Norin No. 6, later extensively spreading hyphae were developed, while in the cultivar Tongil, after penetration, no further hyphal extension resulted. In discoloration of infected tissues, the highly resistant cultivar Tongil not only discolored rapidly, but also the percentage of discolored cells was higher than the susceptible cultivars, Jinheung and Norin No. 6.

The respiratory rate, was generally higher in infected tissue than in healthy tissue. No significant difference in the respiration rate of resistant Suwon No. 180 was not found between the infected and healthy leaf tissue, whereas, in susceptible Jinheung, a marked increase in respiratory rate was caused by blast infection. The respiratory rate increased at the appearance of the first visible symptom in all cultivars resistant or susceptible.

Higher peroxidase activity was found in infected tissues as compared with healthy tissue. Peroxidase activity increased in resistant and susceptible reactions. Particularly, in resistant reaction, the increase of the activity was more pronounced. In highly resistant reaction, there was no difference in peroxidase activity between healthy and infected tissues. Ascorbic acid oxidase, hydroquinone oxidase and catechol oxidase had the same trend as the peroxidase. In contrast, activity of catalase rather decreased in leaf tissues infected with compatible races of the fungus.

INTRODUCTION

The rice blast disease incited by *Pyricularia oryzae* Cavara has long been known as one of the most important causes of severe damage to rice in the rice growing countries of the world. Although there are many methods for controlling the disease, the

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development of resistant varieties through breeding has been given much attention.

In most previous studies, the nature of varietal resistance to the blast fungus was concerned with mechanical barriers, such as thickness of cuticle layer[2], amount of silicate[2,25,23] or with correlations between resistance and some antifungal compounds of rice plants, such as phenolic compounds[5,6,8,11,13,16,20,21,22,29] flavonoid content[10] and fluorescent substances[15].

As a prepenetration structure, Chung[3], Abe and Yasuda[1] showed the significance of the contact stimulus for appressorial formation by the blast fungus. There were great differences in hyphal penetration and hyphal extension between resistant and susceptible cultivars with some isolates, according to the report by Yoshino[31]. In responses of living cells infected by *P. oryzae*, Takahashi[23] and Ohata[15] summarized that in a highly resistant variety, infec ted cells responded rapidly with a fine granulation of the cytoplasm, while in susceptible tissues, infected cells responded slowly with a necrotic response after permitting full growth of the fungus within the cells.

With regard to host respiration by fungal infection, Toyoda et al.[24] reported that in the case of resistant tissue, respiration in infected tissue was stimulated to some extent, while in susceptible tissues, respiration in infectd tissue was stimulated up to four five fold more than that healthy tissue.

It was generally known that blast resistant cultivars contained more polyphenols than susceptible cultivars[11,22], but presence of polyphenol oxidase in rice leaves was still not completely investigated[17,25]. Toyoda and Suzuki[25] showed that the enhancement of peroxidase was exhibited in resistant rice cultivars, and the oxidase activity was greatly influenced by the race of the pathogen to which the host was exposed. In the case of incompatible and compatible combinations, Sridhar[20] reported that a higher increase of the phenolic compounds with peroxidase activity occurred in the compatible cultvar. In addition, ascorbic acid level was connected with varietal resistance.

The cultivar Tongil was bred from a cross between IR 8(Yukara× Taichung Native 1) in 1970, and that cultivar was known to be high yielding, high tolerance to fertilizer and resistant to existing races o the fungus at that time in Korea. However, Chung[4] reported that these IR lines were moderately susce tible to the new race IA 65. Recently, since 1976, Tongil, Tongilchal, Yushin and related IR lines ar being attacked by new virulent races of the fungus

The main purpose of this study is, therefore, t examine the nature of blast resistance of the cultiva Tongil and others to several races. Both compatibl and incompatible combinations will be used i studying the host-parasite interactions. The invest gation is extended to the histopathological an metabolic changes, including oxidases and respiratio changes in the host when several resistant an susceptible cultivars and specific race combinatio are used.

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MATERIALS and METHODS

Rice cultivars and races of *Pyricularia oryzae*

Two Indica-Japonica hybrids, Tongil(Suweon 213-1 and Yushin, and four Japonicas Suwoen No. 180, Kanto No. 51, Kimmazae, and Jinheung showing specific varietal reactions to a given race were used

Ten seeds of each cultivar were sown in plasti pots(15×10×5cm). One per cent of ammoniun sulphate was applied on the leaves of seedlings 1 days after seeding. The others were followed b standard cultural practices.

The races of *P. oryzae* used were obtained fron the Institute of Agricultural Sciences, Office of Rura Development(N-2('75-70), C-8('75-5), T-2('76-104) and T-2^{+1}('76-105)). The fungus was grow on oatmeal-sucrose agar medium at 27°C for on week.

Inoculation: The surface mycelia grown on oat meal sucrose agar was eliminated by a camel's hai brush in order to have good sporulation, and the the culture was kept in an incubator in whic fluorescent light was provided for 48 hours at 26~

28°C[9].

The spores were harvested by pouring 5m*l* of sterile water into the culture plates and gently scraping the mycelial growth with a camel's hair brush. The spore suspension was filtered through cheese cloth. The concentration of the spore suspen sion was adjusted to the required concentration(15 conidia per eye field 150X) using sterile water 1 m*l* of 2% Tween 20(polyoxyethylene sorbitan monolaurate) were added into the 100m*l* spore suspension. The suspension was sprayed on the leaves of 20 day old seedlings and the inoculated plants were placed in a moist chamber at 26°C. After 24 hour's incubation, the seedlings were placed on a bench in the greenhouse. Readings on lesion development were made 7 days after inoculation and lesion types were determined as the standard scale.

To measure respiration rate and oxidase activity, 3mm width lesions without necrosis were sampled. Discs of healthy tissue were used as checks.

Examination of fungal development

Appressorial formation and hyphal penetration on leaves: Appressorial formation of the blast fungus was observed in the 8 hours after treatment by using a whole leaf clearing method[19]. Rice leaves after inoculation were fixed and then stained using a safranin fast green staining schedule. Leaf samples were sectioned to 15 segments. Appressorial formations were microscopically observed. More than 500 conidia of the fungus was examined with 300 fields of the microscopic observations from 30 inoculated leaves.

Fungal invasion on leaf sheath cells: Test plants grown in Wagner pots for leaf sheath inoculation at heading stage. A main culm surrounding with leaf sheath was taken from the rice plant and cut into a piece about 10cm long. The culm of the 3rd leaf sheath was usually used. The culm in the center was removed carefully, and the empty leaf sheath was inoculated with a spore suspension of 4 to 5 conidia per field of 150 magnification. The culms inoculated were placed horizontally in a petri dish moist chamber and then incubated at 25°C for 48 hours. The petri dish was kept moist with wet filter paper, and the inoculated leaf sheath was held, midrib down on two pieces of a pillow placed parallel to each other on the filter paper.

After incubation, the outer green part of the leaf sheath was peeled off, and the hyaline epidermal tissue was soaked in 0.8M KNO_3 solution for 3 minutes. The leaf sheath tissues were examined about 500 conidia with 300 fields of 150 magnification for 30 sheath stems with a microscope. Readings on germination, appressorial formation, hyphal extension and response in host cells were observed by the Takahashi method[26].

Metabolic changes in infected tissues

Respiration: Respiratory rates quotients(RQ) were determined with a Warburg manometer as described in detail by Umbreit, Burris, and Stauffer[27]. Sliced marginal lesion tissues(5~10mm long) of rice plants were used.

The main compartment of each respiration flask contained 0.5g of fresh tissue discs in 1.0m*l* of distilled water. The central well of the flasks in which oxygen uptake determinations were made, a piece of folded filter paper and 0.2m*l* of 20% KOH, and the side arm contained 0.5m*l* of 10% HCl. The flasks were shaken(about 120 oscillations/min/2.5cm width) in water bath at 25°C. O_2 uptake by rice tissue was determined by Warburg's direct method. The first manometer readings were recorded after equilibration for 15 minutes and subsequent readings were taken at intervals of 10, 20 or 30 minutes. The changes in the manometer levels were then converted to microliters of gas per gram of fresh weight of lesion tissues. The experiment was repeated twice for all test samples.

Activity of Oxidative enzymes: The 50mg of tissue discs were homogenized in a glass homogenizer, containing 5g of sea sand under cool conditions with 20m*l* of 0.1M sodium phosphate buffer. The homogenate was filtered through cheese cloth and then centrifuged for 10 minutes at 9,000 r.p.m., and the supernatant fluids were used as the crude enzyme preparation[8].

Peroxidase and polyphenol oxidase activities were measured by the manometric methods as described by Umbreit and Toyoda, et al[25,27]. The experiment was repeated twice for the all test samples prepared

from 10 diseased seedlings.

RESULTS

Fungal development

Appressorial formation and hyphal penetration: Differences in appressorial formation of *P. oryzae* between resistant cultivar Tongil and susceptible cultivar Norin No. 6 were not found throughout the incubation period(Fig. 1). It was observed that the race N-2 of the fungus penetrated directly to the motor cells of susceptible cultivar Norin No. 6(Fig. 2) in contrast to the lack of penetration to that of the highly resistant cultivar Tongil.

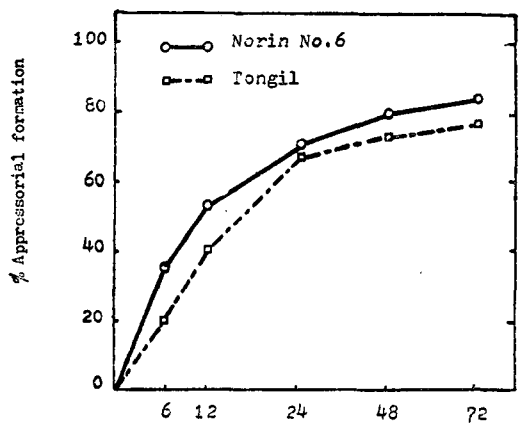

Fig. 1. Comparison of appressorical formation on seedling leaves between Tongil resistant and Norin No. 6 susceptible to race C-8 of *Pyricularia oryzae.*

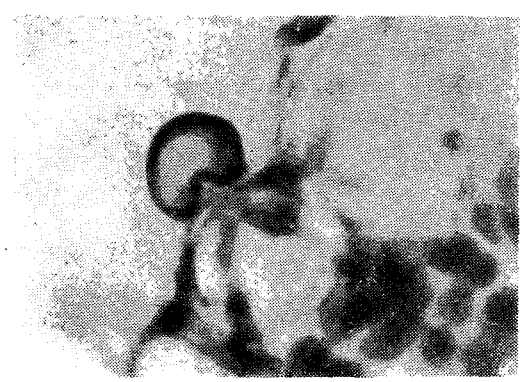

Fig. 2. Hyphal penetration on leaf motive cells of the susceptible cultivar Norin No. 6 inoculated by race N-2 of *Pyricularia oryzae.*

Fungal invasion into leaf sheath cells: A marked difference in the hyphal penetration and extension appeared between the resistant cultivar Tongi and the susceptible cultivar Jinheung(Table 1). The degree of fungal extension by the three races ranged from 1.6 to 1.8 in the resistant cultivar Tongil while susceptible Jinheung showed 4.3 to 6.6. The percentage of hyphal penetration was 24.0% by T-2 to the resistant cultivar, while that of susceptible cultivar Norin No. 6 was 36.3%. The other cultivar-race combinations showed nearly the same trend as those of Tongil and Norin No. 6 inoculated with race T-2.

Tongil highly resistant to the race T-2 was rather lower in the percentage of hyphal penetration than Norin No. 6 susceptible to race T-2.

Discoloration of the infected cells occurred rapidly within 18 hours in the resistant cultivar Tongil with the all three races, while in the susceptible cultivars Norin No. 6 and Jinheung, the response appeared slowly up to 48 hours(Table 2). The percentage of discolored leaf sheath cells in Tongil for the three races was almost twice than that of Norin No. 6 or Jinheung.

Metabolic changes in infected tissue

Respiration: Increase of respiration rate was found in the diseased tissues compared to that of

Table 1. Fungal development of races T,C and N of *Pyricularia oryzae* inoculated on the inner surfaces of detached leaf sheaths of resistant cultivar Tongil and susceptible Cultivar Norin No. 6 after 48 hour's incubation at 25°C

Reaction or Fungus penetration	Cultivars with races					
	Tongil			Norin No. 6		
	T-2	C-8	N-2	T-2	C-8	N-2
Reaction	HB[b]	HR	HR	S[c]	S	S
Hyphal penetration (%)	24.0	10.5	12.2	36.3	22.9	17.8
Degree fungal extension a	1.8	1.8	1.6	6.6	4.4	4.3

a=Refers to Takashashi's scale(23)
b=Highly resistant
c=Susceptible

able 2. Percentages of discolored leaf sheath cells of resistant cultivar Tongil and susceptible cultivars Jinheung and Norin No. 6 inoculated with races T-1, C-8, and N-2 of *Pyricularia oryzae*

Reaction, % discolored cells	Cultivars with races								
	Tongil			Jinheung			Norin No. 6		
	T-2	C-8	N-2	T-2	C-8	N-2	T-2	C-8	N-2
Reaction	HR[a]	HR	HR	S	S	S	S	S	S
discolored leaf sheath cells	13.0	10.3	13.9	7.3	3.9	6.9	7.6	2.0	6.2

=Highly resistant

=Discolored cells were obtained after inoculated with the different races of *P. oryzae* and soaked in 0.8M potassium nitrate solution

ealthy counterparts(Table 3). In the resistant ultivar Suweon No. 180, oxygen uptake was not ifferent between the infected and healthy tissues. n the susceptible cultivars respiration was markedly igher as compared with that of healthy tissue. Even n healthy tissue, the amount of oxygen uptake aried with the cultivars, i.e., Suweon No. 180 was wo times lower than that of Jinheung.

The initial increase in respiration occurred in the ppearance of the first visible symptom at 5th day fter inoculation regardless of resistant or susceptible ultivars(Fig. 3). In the resistant cultivar Suweon lo. 180, the respiration increased slightly at the rst day, even on 8th and 12th days. In the suscep ible cultivar Jinheung, on the other hand, the espiratory rate was markedly increased after the rst day, climbing steadily to the eighth day, and hen abruptly to about 80 percent on the 12th day.

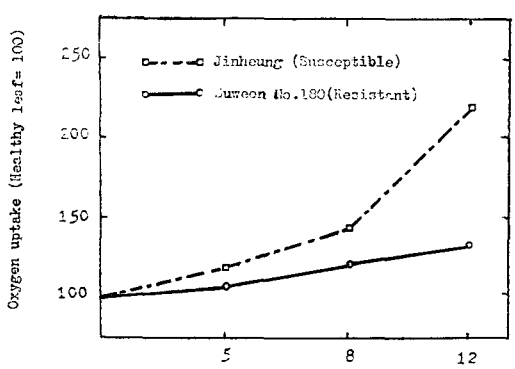

Fig. 3. Changes in respiration in seedling leaves of resistant rice cultivar Suweon No. 180 and susceptible rice cultivar Jinheung following inoculation with race N-2 of *Pyricularia oryzae*

Table 3. Comparision of respiration rate in seedling leaves between resistant cultivar Suweon No. 180 and susceptible cultivar Jinheung at the seventh day after inoculation with race N-2 of *Pyricularia oryzae*

Cultivar	O_2 Uptake[a]		
	Reaction with N-2	Marginal infected lesion	Healthy tissue
Suweon No. 180	R	1.9	1.9
Jinheung	S	6.0	3.9

[a]Unit: μl/30 min/0.5g fresh weight.
R···Resistant
S···Susceptible

Activity of oxidative enzymes: The peroxidase activity in infected tissues was higher than that of healthy tissue regardless of the cultivars tested (Table 4). In rsistant reaction, the level of peroxidase activity was about twice than that in susceptible reaction, when the less virulent race N-2 was inoculated on Suweon No. 180 and Kanto No. 51. The level of peroxidase activity was higher in the inoculated leaves with other races than that in leaves inoculated with the virulent race C-8. In addition, the peroxidase activity in the cultivar Tongil and Yushin, was remarkably different, even though the two cultivars showed highly resistant reaction to both C-8 and N-2 races. In combination of Yushin and race T-2^{+t}, infected leaves showed a high activity of this enzyme than the healthy ones.

The infected tissue exhibited an increase in ascorbic acid oxidase activity over that of healthy tissue (Table 5). In the case of cultivars with incompatible races, the level of ascorbic acid oxidase was almost the same as both cultivars with compatible races.

Table 4. Changes in peroxidase activity in the infected leaf tissues of six rice cultivars after inoculatio with different races of *Pyricularia oryzae*

Cultivar	Peroxidase activity[a] (μl) with races								
	C-8			N-2			T-2+		
	Reaction	Infected	Healthy	Reaction	Infected	Healthy	Reaction	Infected	Health
Tongil	HR[b]	10.1	9.9	HR	9.8	9.7	HR	10.1	10.0
Yushin	HR	5.1	5.0	HR	9.5	5.3	S	6.9	5.2
Suweon No. 180	S	6.6	4.5	R	9.9	4.9	R	10.3	5.0
Kanto No. 51	S	6.8	4.3	R	9.0	4.7	R	10.1	4.9
Jinheung	S	5.7	4.2	S	6.6	4.3	S	7.0	4.2
Kimmazae	S	6.2	4.4	S	5.8	3.9	S	6.9	4.0

[a] Unit: μl/15 min/50mg fresh weight
[b] HR=Highly resistant, R=Resistant, S=Susceptible

The oxidase activity was increased markedly in some cases.

Polyphenol oxidase activities i.e. hydroquinone and catechol were markedly higher activity in th infected tissues than that in healthy tissue(Table and 7). In cultivar-incompatible race combinations

Table 5. Changes in ascorbic acid oxidase in infected leaf tissues of six rice cultivars after inoculatio with different races of *Pyricularia oryzae*

Cultivar	Ascorbic acid oxidase activity (μl) with races[a]								
	C-8			N-2			T-2+		
	Reaction	Infected	Healthy	Reaction	Infected	Healthy	Reaction	Infected	Healthy
Tongil	HR[b]	23.1	23.0	HR	23.0	22.8	HR	22.9	22.5
Yushin	HR	14.3	14.0	R	20.3	14.5	S	21.1	13.8
Suweon No. 180	S	14.9	14.2	HR	14.3	14.0	R	15.9	13.4
Kanto. No. 51	S	16.6	14.4	R	20.6	14.7	R	21.9	14.9
Jinheung	S	13.4	11.2	S	13.8	11.5	S	14.2	11.2
Kimmazae	S	17.1	12.4	S	15.2	12.6	S	14.5	12.4

[a] Unit: μl/20 min/30mg fresh weight
[b] HR=Highly resistant, R=Resistant, S=Susceptible

Table 6. Changes in hydroquinone oxidase activities in the infected leaf tissues of six rice cultivars afte inoculation with different races of *Pyricularia oryzae*

Cultivars	Hydroquinone oxidase activity (μl) with races[a]								
	C-8			N-2			T-2+		
	Reaction	Infected	Healthy	Reaction	Infected	Healthy	Reaction	Infected	Healthy
Tongil	HR[b]	17.5	17.5	HR	17.8	17.6	HR	17.9	17.8
Yushin	HR	16.0	15.9	HR	16.3	16.1	S	16.9	15.2
Suweon No. 180	S	16.9	14.9	R	23.9	14.1	R	22.6	14.9
Kanto No. 51	S	17.4	15.7	R	20.3	14.8	R	22.1	15.4
Jinheung	S	12.6	10.3	S	11.1	10.9	S	12.9	10.6
Kimmazae	S	18.2	15.4	S	18.7	15.0	S	16.5	14.5

[a] Unit: μl/10min/50mg fresh weight
[b] HR=Highly resistant, R=Resistant, S=Susceptible

Table 7. Changes in catechol oxidase activity in the infected leaf tissues of six rice cultivars after inoculation with different races of *Pyricularia oryzae*

Cultivars	Catechol oxidase activity (μl)with races[a]								
	C-8			N-2			T-2+·		
	Reaction	Infected	Healthy	Reaction	Infected	Healthy	Reaction	Infected	Healthy
Tongil	HR[b]	17.5	17.4	HR	17.2	16.9	HR	17.8	17.8
Yushin	HR	7.7	7.4	HR	8.0	7.9	S	9.4	7.6
Suweon No. 180	S	11.9	6.8	R	14.6	7.8	R	14.7	7.6
Kanto No. 51	S	12.2	7.1	R	15.4	7.2	R	15.7	8.0
Jinheung	S	11.1	6.6	S	9.4	6.5	S	9.1	6.5
Kimmazae	S	12.2	6.1	S	7.1	6.3	S	7.7	6.0

[a] Unit: μl/10min/50mg of leaf fresh weight
[b] HR=Highly resistant, R=Resistant, S=Susceptible

Table 8. Changes in catalase activity in the infected leaf tissues of six rice cultivars after inoculation with different races of *Pyricularia oryzae*

Cultivars	Catechol oxidase activity (μl) with reaces[a]								
	C-8			N-2			T-2+·		
	Reaction	Infected	Healthy	Reaction	Infected	Healthy	Reaction	Infected	Healthy
Tongil	HR[b]	23.5	23.3	HR	23.7	23.5	HR	23.4	23.5
Yushin	HR	24.5	24.7	HR	24.4	24.7	S	15.0	25.0
Suweon No. 180	S	13.5	24.6	R	12.5	24.1	R	11.9	24.0
Kanto No. 51	S	13.4	23.9	R	12.3	23.8	R	11.7	25.9
Jinheung	S	15.7	25.5	S	15.5	25.3	S	14.1	25.2
Kimmazae	S	14.4	25.2	S	14.6	25.1	S	13.8	24.9

[a] Unit: μl/10min/50mg fresh weight
[b] HR=Highly resistant, R=Resistant, S=Susceptible

the oxidase activities of hyproquinone and catechol in the infected tissue were found to be slightly higher than that in compatible combinations. The polyphenols were almost 2-fold that of the tissues in many instances.

In contrast, catalase activity generally decreased in leaf tissues infected with compatible races, but remained nearly the same with incompatible races (Table 8). In cultivar Yushin inoculated with incom patible races N-2 and C-8, the catalase activity of the infected tissues of the cultivars were almost the same as the healthy one, but in compatible combination, the activity remarkably decreased in comparison with healthy tissue.

DISCUSSION

The data indicate that there was no marked difference in appressorial formation between the cultivars whether susceptible or resistant. Appressorial formation is known to be only related to the contact stimulus of the cuticle layer rather than host responses[1,3]. Since no definite theory as to the mechanism of appressorial formation by the blast fungus has so far been made[2], the percentage of appressoria formed can not be applied as a criiterion to determine varietal resistance.

As noted in previous studies[22,30,31] the percentage of hyphal penetration and the degree of fungal invasion is markedly lower in the resistant cultivar Tongil than in the susceptible cultivar Jinheung. The degree of fungal invasion may be a more reliable criterion of varietal resistance than that of hyphal penetration, because the further development of hyphal penetration is the same as the extension of invading hyphae. Only the highly resistant Tongil

cultivar exhibits tiny, brown, pinpoint necrosis as a hypersensitive reaction and also shows a lower degree of invading hyphal extension in the leaf sheath cells. This implies that no further hyphal extension occurred even after penetration is made into the epidermal cells. The responses of infected cells to the balst fungus in either resistant or susceptible cultivars may be in terms of the hypersensitive reaction as pointed out by Ohata[15].

Our findings show that respiration rate in the infected tissue was markedly higher than that in healthy tissues are in agreement with that reported by Toyoda et al.[24]. In the case of compatible culti var and race association, oxygen uptake was higher in the infected plants than the plants in incompatible association. The values of oxygen uptake were about twice as high as compared with that of healthy tissues. Although the respiratory increase was closely related to the hypersensitivity reaction, it is pointed out by Maine[12] that respiratory increases do not always accompany hypersensitivity. Even in healthy tissue, the respiratory rate is markedly different depending upon cultivars, whether resistant or susceptible to blast. With regard to daily respiratory alteration, there was the slight respiratory increase occurred in Suweon No. 180 while in susceptible cultivar Jinseung, the rate was continuously increased. These data suggest that in infected tissues of rice plants, the synthesis of energy for fungal development may be stimulated by increasing respiration especially the effect being more pronounced in suscept ible than in resistant reaction. However, further intensive studies on the alteration of respiration in infected tissue of rice should be carried out because the precise mechanism of the main causes of respiratory alteration in the infected tissue is still un known. Furthermore, differences in oxygen uptake in infected and healthy tissues in compatible and incompatible associations are still variable and unpredictable.

Sridhar[20] pointed out that the enhancement of peroxidase activity was greatly influenced by the pathogen to which the host was exposed. The present investigation indicates that the peroxidase activity in infected tissues was higher than that of healthy tissue in the compatible combinations. In the case of an incompatible combination, the level of peroxidase activity is markedly higher than that of a compatible one(Table 4). Differences in activities of peroxidase and ascorbic acid oxidase between two cultivars Tongil and Yushin to incompatible races of *P. oryzae* are suggested to be due to the cultivar's specific association. In addition, the reaction of oxidases, including the peroxidase, in Yushin which is susceptible to the race T-2^{+t}, was regarded as a typical varietal response. Therefore, this can not be adoptable to blast resistance on the basis of a single race tested against specific one cultivar.

Toyoda[24], pointed out that infected tissue exhibited higher activity in ascorbic acid oxidase, our results were also in agreement with the report that there were differences in ascorbic acid oxidase activities in leaves of cultivar infected with compatible races of *P. oryzae*. This means that the activities of polyphenols such as ascorbic acid oxidase react differently to races of the fungus. In cultivar-race combinations, the oxidase activity was higher in incompatible associations than compatible ones. In Yushin with race T-2^{+t}, the differences in ascorbic acid oxidase activity was slight even though this was a compatible associations. Here again, it is insufficient to generalize varietal resistance based upon one race and a cultivar.

Hydroquinone and catechol oxidase activities show the same trend to peroxidases except catalase, although one exception, the reaction of Yushin with the race T-2^{+t} was obtained.

On the contrary, catalase activity usually decreased to about one-half in the infected tissues of the compatible combinations(Table 8). This suggests that the effect of blast infection may result in reduction of catalase activity from the infection site(Table 8). In addition, there are several inconsistant difference in the catalase activities between incompatible and compatible cultivars regardless of races used. In this connection, no precise matabolic role of the oxidase has far been worked out except for the detoxifying agent for H_2O_2 with peroxidase[7,18].

The presence of polyphenol oxidase in rice leave is still unknown. Toyoda et al. [25] showed that th oxidase was absent in rice by showing no differenc in activity between unboiled and boiled homogenate

of infected tissue.

All matabolic processes in the incompatible or compatible cultivars may depend on their host-parasite interaction, it seems likely that several major metabolisms for energy synthesis may be differently altered for fungal development in infected tissues rice plants susceptible or resistant to all of the races of blast fungus.

摘 要

벼 잎위에서 稻熱病菌의 附着器形成은 高度抵抗性인 統一과 感受性인 農林 6號間에 큰 差異가 없었다. 感受性인 農林 6號에는 直接 機動細胞에 侵入 伸展되었으나 高度抵抗性인 統一品種에서는 그와 反對로 菌糸가 伸展되지 않았다. 高度抵抗性인 統一은 生理型에 關係없이 感受性인 振與, 農林 6號보다 急速히 寄生細胞가 變質되는 同時에 그 比率도 높았다.

呼吸率을 酸素吸收率로 보면 健全組織보다 病斑組織에서 높은 傾向이었다. 抵抗性品種인 水原 180號에서 健全組織과 呼吸率에 差異가 없었으나 感受性인 振與의 傾遇에는 健全組織보다 呼吸率이 훨씬 높았다. 그리고 品種間에도 健全部位의 呼吸率의 差異가 甚하였다. 呼吸率은 病斑이 最初로 形成되는 時期에 抵抗性 및 感受性을 막론하고 上昇하였는데 感受性組織은 健全組織에 比하여 急速히 增加하는 反面 抵抗性病斑에 있어서는 緩慢하게 增加하였다.

퍼옥시다제의 活性은 健全組織에 比하여 感受性組織에서 增加하였고 生理型과 品種間에 非親和性인 抵抗性病斑에 있어서 그 活性이 感受性病斑에 比하여 顯著히 增加하였다. 그러나 高度抵抗性인 反應에 있어서는 健全 및 羅病組織間에 퍼옥시다제의 活性差異가 거의 없었다.

아스코빅酸, 하이드로퀴논 및 카테콜 酸化酵素 活性도 퍼옥시다제와 같은 傾向이었다. 反對로 카탈라제의 活性은 親和性인 病斑組織에서 오히려 減少하였다.

LITERATURE CITED

1. Abe, T. and Y. Yasuda. 1961. On the formation of appressorium due to germination of conidia of *Piricularia oryzae* and it's meaning. Kyoto *Fudai Gakujitsu Hokoku* 13 : 36-44.
2. Akai, S. 1973. Recent advance in studying the mechanism of fungal infection in plants. Shokubutsu Byogai Kenkyu 8 : 1-43.
3. Chung, B.K. and K.S. Kim. 1977. A histological investigation on the resistant Tongil cultivar by inoculation rice blast fungus, *Pyricularia oryzae* Cav. Kor. J. Mycol. 5(1) : 13-19(In Korean with English summary)
4. Chung, H.S. 1974. New races of *Pyricularia oryzae* in Korea. Korean J. Plant Prot. 13 : 19-23.
5. Farkae, G.L. and Z. Kiraly. 1962. Role of phenolic compounds in the physiology of plant disease resistance. Phytopath. Z. 44 : 105-150.
6. Flood, A.E. and D.S. Kirkham. 1960. The effect of some phenolic compound on the growth and sporulation of two *Venturia* species. Pages 81-85 in Phenolics in Health and Disease, Pregamon Press, Oxford.
7. Goodman, R.N., Z. Kiraly and N. Zaitlin. 1967. The biochemistry and physiology of infectious plant desease. D. Van Nostrand Co., New Jersey.
8. Gopal S.R. and Merle, G. 1970. The relationship of peroxidase and ortho-diphenol oxidase to resistance of sugar beets to *Cercospora* leap spot. Phytopathology 60 : 238-245.
9. Furuda, C. and J. Shikuji. 1967. New method for conidial sporulation of the blast fungus. Shokubutsu-Boyoki 21(4) : 30-32(Japanese).
10. Kwon, S.H., J.H. Oh and H.S. Song. 1974. Studies on the relationship between chemical contents of rice plants and resistance to rice blast disease. Korean J. Plant Prot. 13(1) : 33-39(In Korean with English summary).
11. Kuwatsuka, S. and Y. Oshima. 1962. Studies on polyphenols in rice plants. I. Separation and identification of phenol carboxylic acids. II. Separation and identification of tricin. (In Japanese): J. Agr. Chem. Soci., Japan 35(1) : 67-71.
12. Maine, E.C. 1960. Physiological responses in the tabacco plant to pathogenesis by *Pseudomonas solanacearum*, causal agent of bacterial wilt. Doctoral thesis, North Carolina State College, Durtham, N.C., U.S.A.
13. Müller, K.O. 1956. Einige einfache Versuche zum Nachweis von Phytoalexinen. Phytopath. z. 27 : 237-254.
14. Ohata, K. 1967. Resistance reaction between

variety and races of the blast fungus with regard to the resistance mechanism. Shokubutsu-Boyoke 21 : 141-146. (In Japanese)

15. Ohata, K. 1972. Infection mechanism in fungal diseases (Discoloration of host cells of rice in response to the blast infection) Shokubutsu-Boyeke 26(10) : 1-36.
16. Park, J.S. 1958. Studies on highly resistant varieties to the blast fungus. Daeji. Choongnam. Nat' l Univ. College of Agr. 3 : 27-33.
17. Ramarkrishnan, L. 1966. Studies in the host-parasite relations of blast disease of rice. 2. Changes in the carbohydrates and phenolics. Phytopath. Z. 56 : 279-287.
18. Rudolph, K. and M.A. Stahmann. 1964. Inter action of peroxidases and catalases between *Phaseolus vulgaris* and *Pseudomonas phaseolicola* (Halo blight of bean). Nature 204 : 474-475.
19. Shipton, W.A. and J.F. Brown. 1962. A whole leaf clearing and staining technique to demon strate host-pathogen relationships of wheat stem rust. Phytopathology 52 : 1313-1315.
20. Sridhar, R. and S.H. Ou. 1974. Biochemical changes associated with the development of resistant and susceptible types of rice blast lesions. Phytopath. Z. 79 : 222-230.
21. Sridhar, R. and S.H. Ou. 1974. Extracellular enzymes produced by *Pyricularia oryzae* CAV., Philippine Phytopathology 8 : 52-56.
22. Suzuki, N. 1963. Nature of resistance to blast. Pages 277-301 in the Rice Blast Disease, the International Rice Research Institute, Johns Hopkins Press, Baltimore, Md., U.S.A.
23. Takahashi, Y. 1956. Studies on the mechanism of the resistance of rice plants to *P. oryzae*, II. Pathological changes microscopically observed in host cells in which fungus hyphae donot grow well. Bull. Yamagata Univ. (Agr. Sci.) 2 : 37-51. (In Japanese with English summary)
24. Toyoda, S. and N. Suzuki. 1957. Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae* Cav. IV. Changes in the activity of oxidases in infected tissue. Ann. Phytopathol. Soc. Japan 25(4) : 172-177. (In Japanese with English summary)
25. Toyoda, S. and N. Suzuki. 1960. Histochemical studies on rice blast lesions caused *Piricularia oryzae* IV. Changes in the activity of oxidases in infected tissue. Ann. Phytopathol. Soc. Japan 25(4) : 172~177. (In Japanese with English summary)
26. Tomiyama, K. 1956. Cell physiological studies on the resistance of potato plant to *Phytophthora infestans*. (IV) Ann. Phyto-pathol. Soc. Japan 21 : 54-62.
27. Umbreit, W.W., R.H. Burris and J.F. Stauffer. 1957. Manometric techniques, Burgess Publishing Co., Minneapolis, Minn., U.S.A.: 338pp.
28. Volk, R.J., R.P. Kahn and R.L. Weintraub. 19 58. Silicon content of the rice plant as a factor influencing its resistance to infection by the blast fungus *P. oryzae*. Phytopathology 48 : 179-184.
29. Wakimoto, S. and H. Yoshii. 1958. Relation between polyphenols contained in plants and phytopathogenic fungi. 1. Polyphenols contained in rice plant. Ann. Phytopathol. Soc. Japan 23 : 79-84. (In Japanese with English summary).
30. Yoshii, H. 1936. Pathological studies on rice blast caused by *Pyricularia oryzae*. II. On the mode of infection of the pathogen. Ann. Phyto pathol. Soc. Japan 13 : 14-18(In Japanese with English summary).
31. Yoshino, R. 1971. Detection method of mycelial infection of the rice blast fungus inside leaf tissue. Hokuriku Byokai Kenkyu 10 : 14-17. (In Japanese with English summary).