Exploration of Induced Resistance in Rice Plants by Buff Pigment Mutants of *Pyricularia oryzae*

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稻瘟病菌浅黄色素突变体对稻株诱导抗性的探讨

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提 要：经用紫外光诱变菲律宾的一个具有广谱毒性的菌株PO6-6中获得2个浅黄色素突变株A和B以及从浙江省分离的弱致病性菌株C，在25/16℃、RH70%的人工气候箱内，先在4个感病品种上接种48小时后再接种强致病性菌株D和E，浅黄色素突变株和弱菌株对强菌株在水稻感病植株上呈现出交叉保护现象。6批重复试验一致表明，浅黄色素突变株和弱致病性菌株均可明显地诱导水稻植株对稻瘟病的抗病性。本文并就利用稻瘟病菌的交叉保护作用，为开辟防治新途径进行了讨论。

关键词：稻瘟病菌；浅黄色素突变体；诱导抗性；交叉保护

Key words: *Pyricularia oryzae;* Buff pigment mutants; Induced resistance; Cross protection

Introduction

Rice blast is one of the most widely distributed diseases in different rice-growing regions of the world. the fungus, *Magnaporthe grisea* (Hebert) Barr comb nov. (anamorph *Pyricularia oryzae* Cavara) is highly variable with many races[6, 7] which can rapidly spread to epidemic proportions. Varietal resistance usually breaks down within a few cycles of commercial planting or under epidemic conditions[6]. According to the reports of recent years from the United States, Japan, Brazil and South America[1, 2], deliberate inoculation of greenhouse tomato crops with a 'mild isolate of tobacco mosaic virus (TMV) was widely used to cross-protect the plants against losses caused by severe TMV isolates. The most dramatic commercial success with the cross-protection phenomenon is with citrus tristeza virus[8]. Some mild isolates of the virus were found and shown to cross-protect against severe isolates, and symptomless-carrier isolates of avocado sunblotch viroid were shown to completely protect against virulent isolates of the same viroid. Therefore, the purpose of this study is to generate nonpathogenic isolates of the blast fungus by UV mutagenesis and to explore buff mutants for inducing resistance in rice plants to blast by cross protection.

Materials and Methods

Fungal isolates selected

Isolates PO-6-6, a Philippines field

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isolate with a broad spectrum of virulence, was used as the parental isolate to generate mutants, PO-6-6 has wild type pigment and sporulates profusely on culture media.

UV mutagenesis

Conidia of PO-6-6 were harvested from cultures on prune juice agar (PJA) slants (20g of prune, 5g of lactose, 1g of yeast and 17g of agar per liter of water). UV-mutagenesis as described by Leung *et al.*[10] was follow.

Pathogenicity test

Preparation of tested isolates. Two nonpathogenic buff mutants M13 (A) and M15 (B) were induced at the Genetics Lab., IRRI. Mild isolate 82-030G1 (C) and virulent isolate 85-14B1 (D) were isolated in the local field, Zhejiang Province, China and the field rice isolate PO-6-6 (E) was obtained from the Plant Pathology Lab., IRRI. A small piece of mycelium of each isolate was maintained on yeast extract starch agar (10 g of starch, 1 g of yeast extract and 20 g of agar per liter of distilled water) for one week. Actively growing mycelial pieces were suspended in distilled water and evenly spread on oatmeal agar in Petri dishes at 24-26℃ for 10 days. The mycelial surface was then scraped and incubated under fluorescent light at 26-28℃ for 3 more days. Conidia were washed off from the surface of OA with 0.1% tween 20 solution. The inoculum density was adjusted to 1×10^5 conidia per milliliter.

Preparation of plant materials. Five isolates were tested on four susceptible cultivars depending on the objective of the experiment. Seeds were soaked in water for one day and pregerminated in Petri dish with moistened filter paper for two days, then transplanted to field soil in plastic pots 4 cm in diameter. Five plants per pot were planted in plum blossom form with 3 replicates. All plants were grown in phytotron until 21-day-old for inoculation.

Co-inoculation experiments

Co-inoculation experiments were conducted to determine whether non-pathogenic buff mutants or mild isolates of *Pyricularia oryzae* can induce resistance to blast.

Fourteen treatments were used in this study as shown in Table 1. Four susceptible cultvars were first inoculated with the buff mutants or mild isolates. Inocula were produced as previously described and adjusted to 1×10^5 spores per ml. Plants were sprayed with 30 ml of inoculum of each culture and incubated in a dark chamber for 24 hr at 24℃, followed by inocubation in a lighted phytotron at 25/16℃, RH 70% for 1 day at 24-26℃, and then reinoculated with a wild type isolate. Disease reactions were scored 6 days after the last individual plants were rated on a 0-9 scale and lesion types and numbers were recorded for the two youngest leaves at the time of inoculation.

Disease evaluation

Disease reaction was scored based on the standard evaluation system of 0-9 scale with 6 interaction phenotypes[5]. Plants were scored 5 days after inoculation with the second isolate.

Results

Exposure of spore of isolate PO-6-6 to different UV dosages

The minimal time for obtaining buff mutants was 1 min and almost five fold more buff mutants were recovered with

increased exposure (4 to 4.5 min). In addition to the buff mutants, colonies with white surface mycelia appeared frequently, reaching as high as 15% at 4 min exposure. No apparent loss of fitness was observed among the buff and white mutants. The buff and white mutants appeared to have the same growth rate and *in vitro* sporulation capacity as the parental strain PO−6−6.

The longer the exposure time to UV, the lower (higher) the survivor (mobility) of isolate PO−6−6. The occurence of white colonies of isolate PO−6−6 increased with increasing exposure time to UV light.

Pathogenicity and interaction phenotypes

Six replicates of the experiment indicate that both nonpathogenic isolates and mildly virulent isolates of the blast fungus can induce evidently the resistance of rice plants to blast (Table 1).

1. The preinoculation of nonpathogenic strain M13 (A) or M15 (B) 48 hrs before the inoculation of mild strain 82−030G1 (C) led to a decrease of blast severity from scale 3 (MR) to 1 (R) in cultivars Mokoto and Guang−Lu−Ai 4,and 1(R) to 0 (HR) in cultivars Zhen−Shan 97 and IR50 in comparison with check treatment.

2. The preinoculation of nonpathogenic strains A and B 2 days before the inoculation of strong isolate 85−14B (D) or PO−6−6 (E), made the symptom scale drop from 9 (HS) to 5 (MS) in cvs. Mokoto, Zhen−Shan 97 and Guang−Lu−Ai 4, and from 7 (S) to 5 (MS) in cv. IR50. The urgent lesions become chronic ones, and their expansions are restricted while margins turn brown; the formation of spores diminishes, and lesion number drops.

3. The preinoculation of mild isolate (C) before the inoculation of strong isolate D or E also resulted in the reduction of disease scale from 9 (HS) to 3 (MR) in cvs. Mokoto and Guang-Lu-Ai 4,9 (HS) to 5(MS) in cv. Zhen-Shan 97 and 7(S) to 5(MS) in cv. IR50. The size and number of lesions decreased to some extent in comparison with checks.

Table 1. The pathogenic reactions of 4 susceptible rice cultivars to various inoculation treatment

Treatment	Inoculum no.		Pathogenic reaction			
	1st inocul.	2nd inocul.	Zhen−Shan 97	Mokoto	Guang−Lu−Ai 4	IR50
F × A	Mater	M13	0	0	0	0
F × B	Water	M15	0	0	0	0
F × C	Water	82−030G1	1,+(R)	3,+(MR)	3,+(MR)	1,+(R)
F × D	Water	85−14B1	9,+++(HS)	9,+++(HS)	9,+++(HS)	7,+++(S)
F × E	Water	PO−6−6	9,+++(HS)	9,+++(HS)	9,+++(HS)	7,+++(S)
A × C	M13	82−030G1	0(HR)	1,+(R)	1,+(R)	0(HR)
A × D	M13	85−14B1	5,++(MS)	5,++(MS)	5,++(MS)	5,++(MS)
A × E	M13	PO−6−6	5,++(MS)	5,++(MS)	5,++(MS)	5,++(MS)
B × C	M15	82−030G1	0(HR)	1,+(R)	1,+(R)	0(HR)
B × D	M15	85−14B1	5,++(MS)	5,++(MS)	5,++(MS)	5,++(MS)
B × E	M15	PO−6−6	5,++(MS)	5,++(MS)	5,++(MS)	5,++(MS)
C × D	82−030G1	85−14B1	5,++(MS)	3,+(MR)	3,+(MR)	5,++(MS)
C × E	82−030G1	PO−6−6	5,++(MS)	3,+(MR)	3,+(MR)	5,++(MS)
F × F	Water	Water	0	0	0	0

Note: +, 1−4 lesions per leaf; ++, 5−9 lesions per leaf; +++, over 10 lesions per leaf

In a word, both buff mutants and mildly virulent isolates can apparently induce resistance in four susceptible varieties to a subsequent challenge with virulent isolates of rice blast fungus.

Discussion

The results show that an earlier infection with an avirulent or mildly virulent isolate of a pathogen can protect susceptible hosts against a virulent isolate.

The phenomenon of such cross protection suggests that the existence of mild isolates may hinder the extension of germ tubers of compatible isolates, and protect plants from external interference by inducing antigerm substances—phytoalexins[3, 9, 11]. The other possible reason for this phenomenon is that the existence of the avirulent buff mutants may inhibit the synthesis of melanin in appresoria and the penetration of appresoria into the epidermis of hosts[4, 12]. However, the effect of resistance induction diminishes as the time passes, and sometimes even got the opposite results.

Although the promising phenomenon has not been tested yet in the field to confirm the feasibility of its commercial applications, our present experiment results suggest that the utilization of the cross protection of blast fungus isolates may provide a way for the control of the epidemic of rice blast as the deepening of the study on the trend.

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