Protocols for the sampling of diseased specimens and evaluation of blast disease in rice

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Introduction

This protocol was written for scientists who were facing the problems of blast disease and want to start the study. When we decide a pathogenic race of Pyricularia oryzae using the new differential lines, the observation of infection type formed by leaf blade of seedling and the conversion to the binarization in resistance (R) or susceptible (S) are required. However, actually, we come up against the case that hesitates to judge R or S due to various conditions. So it is necessary to make criteria of the judgment, a point to keep in mind clear during the spread of new differential system, so that lead us determination not depend on a researcher. In this protocol, from the sampling of the pathogenic fungus to evaluation of the infection type concretely is mentioned. We want in particular you put panel at hand and refer on the occasion of evaluation of the infection type. Though blast resistance also included another type called a partial resistance, this manual is limited to the evaluation method for complete resistance to participate in determination of the pathogenic race.

This protocol includes as following:

I. Medium preparation
II. Sampling of diseased specimens
III. Monoconidial isolation
IV. Preparation of long-term storage (Stock culture)
V. Preparation of plant materials for inoculation
VI. Inoculum production
VII. Counting of spores using hemacytometer
VIII. Coordinated inoculation procedure (Spraying method)
IX. Disease assessment
X. Designation of new international differential system
XI. List of International Rice Blast Line (LTH monogenic lines)

I. Medium preparation

a) Water agar

[Purposes] for monoconidial isolation
[Materials]

Bacto Agar	30 g
Distilled water	1,000 ml
Streptomycin	40 mg

[Procedure]

1. Weigh 30 g of Bacto agar.
2. Put it in a 2-liter capacity flask.
3. Add 1000 ml *double distilled water* ($D^2H_2O^1$) or deionized water.
4. Plug with cotton and autoclave for 15 min at 15 psi.
5. Before plating 3% water agar add 40 mg streptomycin per liter.

When you promote growth of mycerium, add 5g sugar and 2g yeast extract.

b) Prune agar

[Purposes] for preparation of long-term storage (Stock culture)
[Materials]

Prune agar (PA)	3 pcs
Lactose	5 g
Yeast extract	1 g
Agar	20 g (If stick bar, 17 g if bacto)
D^2H_2O	1000 ml

[Procedure]

1. Boil the prunes in 500 ml distilled deionized water (D^2H_2O) for one hour.

2. Dissolve the agar by heating in 500 ml distilled deionized water (D^2H_2O).
3. Mash the prunes thoroughly and strain or filter the prune juice through cheese cloth or nylon mesh.
4. Pour the strained prune juice into the agar.
5. Adjust the volume to 1000 ml by adding distilled deionized water (D^2H_2O).
6. Add the lactose and yeast extract and stir well.
7. Adjust the pH to 6.5
8. Before it cools, dispense the melted agar into tubes (for slants – 10 ml) or flasks (for plates – 250 ml).
9. Plug with cotton and autoclave for 15 min at 15 psi.
10. Before plating prune agar, add 10 mg of streptomycin for every 250 ml media.

Note: Gulaman bar is locally available and much cheaper. Please use this for most experiments.

c) Rice flour agar

[Purposes] for preparation of long-term storage (Stock culture)

[Materials]

Complete rice flour	15 g
Yeast extract	4 g
Agar	15 g
Distilled water	1,000 ml

[Procedure]

Same as the Prune agar

d) Oatmeal agar

[Purposes] for inoculum production

[Materials]

Rolled oats	30-50 g
Sucrose	5 g
Agar	16 g
Distilled water	1,000 ml

[Procedure]

1. Boil the rolled oats in 500 ml D^2H_2O for 15 min. This may need constant stirring.
2. Add the agar to 500 ml D^2H_2O and heat to dissolve.
3. Strain or filter the "juice" through a strainer.
4. Pour the strained rolled oats (oatmeal "juice") into the agar. Adjust the volume to 1 liter with D^2H_2O.
5. Add the 16g sucrose and stir well.
6. Dispense into tubes (for slants – 10 ml) or flasks (for plates – 250 ml).
7. Plug with cotton and autoclave TWICE for 40 min at 15 psi.
8. Before plating oatmeal agar, add 10 mg of streptomycin for every 250 ml oatmeal agar.

II. Sampling of diseased specimens

Rice growth stage suitable for a sampling: Leaf blast season or panicle blast season.

Suitable place for collection: less windy place such as hill side where dew stays longer time. Leaf color of rice plant is dark among rice field.

Point 1: Parts to observe: Specimens of neck, grain, branch diseased blast and young aged lesion in leaf are easy to isolate the spore than fully elongated lesion in leaf.

Point 2: Rapid dryness management:

[Procedure]

1. Put collected sample inside between coffee filter.
2. Write down the information such as the date, place (name of place, longitude, latitude, altitude), paddy field/upland field/other, rice (cultivar name)/wild rice/weed.
3. Dry it quickly in the room temperature.
4. For the most dried samples, put silica gel and seal the container (coffee filter), and keep in 4℃ refrigeration.

The diseased leaves unattended in room temperature for one month make few sporogenesis, and fail to separate into single spore in the next step procedure.

When you put diseased leaves in the moist chamber,

Fig. 1. Oatmeal agar medium poured in Petri dishes

Fig. 2. The coffee filter put the information of the specimen

Fig. 3. The diseased plants stored with silica gel in the plastic box

the sporogenesis is good if the intensity of green leaf is high. If it is dull, keeping state is not good, and sporogenesis is bad.

III. Monoconidial isolation

Method 1) without a microscope

[Procedure]

1. Cut infected leaf or panicle samples in 3-5 cm sections.
2. Place cut sections on moist filter paper in a Petri dish.
3. Incubate plate for 24 hr at room temperature.
4. Examine lesions under a dissecting microscope.
5. Using very fine tip Pasteur pipettes (tips melted by heating with a gas burner, pulled and then cooled), pick conidial masses and spread on plated 3% water agar containing streptomycin (10 mg/250 ml agar).
6. Incubate plates for 24 h at room temperature.
7. Pick germinated conidia and transfer to prune agar slants.
8. Incubate at room temperature for four days. The prune agar cultures will be used to inoculate liquid cultures to prepare stock cultures (See the protocol for Medium preparation).
9. Be sure you pick a single conidium.

If any collected leaf samples remain after the monoconidial isolation, put them properly in a plastic bag and keep them in the cold room at about 4℃ for future use.

Method 2) for single spore isolation using a microscope

[Procedure]

Same as above until 4 in Method 1)

5. Prepare a microscope
6. Set an insect pin (No.1) or a glass needle in the rubber stopper and align an axis.
7. Put 3% agar plate on the stage inside out.
8. Focus a spore on the agar plate.
9. Move an insect needle to the upper portion using condenser adjustment knob and focus the tip of needle on a spore.
10. Pick a single conidia and down some needles once, and then transfer it to the other place of agar plate.
11. Incubate the plates at room temperature for 1 day.
12. Confirm the germination of spore and no contamination of other fungus or bacteria.
13. Incubate more 2 or 3 days to see the mycerium, transfer to rice flour agar plates.

The rice flour agar cultures will be used to prepare stock cultures (See the protocol for Medium preparation.

Fig. 4. Cultivation of blast fungus on field infected stem or leaf in wet paper

Fig. 5. Selection of single spore

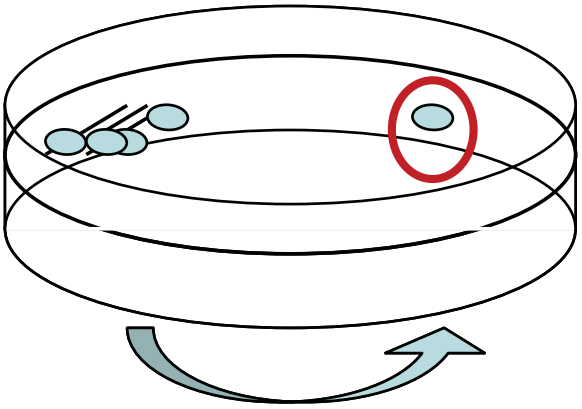


Fig. 6. Selection of single spore on water agar

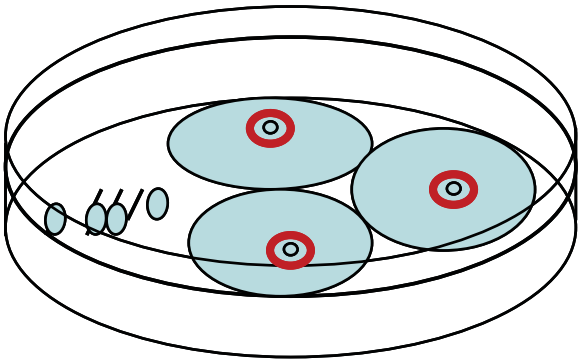


Fig. 7. Cultivation on water agar after 1 day at room temperature (ca. 25-28 ℃)

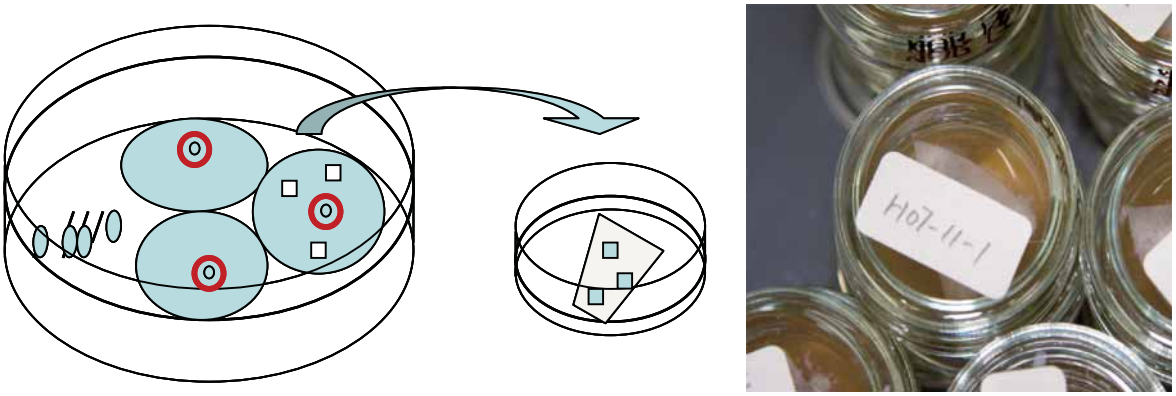


Fig. 8. Cultivation of blast fungus on rice flour agar medium for long-term storage of the blast isolate

IV. Preparation of long-term storage (Stock culture)

Method 1) IRRI method

The following steps describe the removal of paper disks from slants.

(If isolate is very important or will be used often, prepare more of this one.)

(If there appears to be contamination or no germination from the stock culture, report this to all concerned.)

[Procedure]

1. Cut the filter paper into small pieces about 3 mm disks.
2. Put about 750-1000 pieces of paper disks into a 50-ml flask.
3. Plug the flask containing paper disks with cotton, cover cotton plug with aluminum foil then sterilize 2x by autoclaving for 15 min at 15 psi twice.
4. Put sterile paper disks on the surface of 4-day-old prune agar slant growth of *P. oryzae* [see step 4 of previous protocol (Monoconidial Isolation).]
5. Incubate at room temperature (25-27 ℃) for 25 days.
6. After 25-day growth period, carefully remove colonized paper disks taking extra care not to include any agar, and place in 2x sterilized coin envelopes (#00 brown coin envelopes, Miracle Envelope & Specialty Papers, 4.3×7cm in size).
7. Keep the envelopes in a cool, safe, and dry place for 14 days.
8. Place the envelope in a plastic bag containing silica gel dessicant (indicating type, 12-28 mesh gr 408). The color of this gel is blue after dehydration in the oven – simultaneously done with the petri plates.
9. Seal the bag (use of 2 plastic bags is preferred) and store in cold storage -20℃ or -70 ℃. The silica gel should be changed whenever the color changes from blue to pink.

Method 2) NIAS method

[Procedure]

The following steps describe the removal of paper disks from plates and the preservation of collected samples.

1. Cut the filter paper (Advantec 101) into small pieces about 15 mm square.
2. Put them in a petri dish, cover with aluminum foil then sterilize by autoclaving for 15 min at 15 psi.
3. Put a sterile paper disk on the surface of rice flour agar plate, inoculate by a few fungal mycelium block of *P. oryzae* using transfer needle.

4. Incubate at room temperature (25-27 ℃) for 4-5 days until the filter paper is covered by fungal mycelium (Fig. 9).
5. Carefully remove colonized filter paper square, and place it in Petri dish laid a sterilized filter paper (Fig. 10).
6. Dry it in desiccator at room temperature for 3 days .
7. Cut the filter paper into small pieces about 3-5 mm square (Fig. 11).
8. Carefully remove cut filter papers, and place in sterilized coin envelopes.
9. Place the envelope in a plastic bag containing silica gel dessicant (indicating type, 12-28 mesh gr 408). The color of this gel is blue after dehydration in the oven – simultaneously done with the petri plates.
10. Seal the bag and store in cold storage -20 ℃ or -70 ℃. The silica gel should be changed whenever the color changes from blue to pink. Stocked isolates still alive over 10 years (Fig. 12).

Fig. 9. Mycerium grown on the filter paper in Petri dish with a diameter of 3cm

Fig.10. Dried filter paper with mycelium

Fig.11. Cut filter paper into small pieces before storing

Fig.12. Isolates stored in -20C freezer

V. Preparation of plant materials for inoculation

Method 1) IRRI protocol

[Procedure]

1. If it is necessary, break seed dormancy by placing seeds in incubator at 50° for 3 days.
2. Treat test materials with 2% Chlorox for 10 min, wash 4x with D^2H_2O.
3. To pre-germinate seeds, place the seeds in petri dishes on wet blotting paper for 4 days at about 30℃ (if it is necessary, rinse blotting paper and seeds with D^2H_2O).
4. Choose what kind of soil to use and the kind of container or pot that suits your experiment.
5. Transplant test materials in the greenhouse.
6. After 3 weeks, test plants will be ready for inoculation (5-6 leaf stage, including an imperfect leaf, 21 DAS).
7. In case of rare plant materials wherein seeds are very few (like *O. nivara*, *O. rufipogon*, *O. minuta* etc), dehull seeds after seed dormancy is broken, then proceed to the next step.

[The 4-5 leaf stages of wild species appear 34 DAS, while in *O. sativa*, it is about 21 DAS]

Chlorox: 5.25% solution of sodium hypochlorite

Method 2) NIAS method

[Procedure]

1. If necessary, break seed dormancy by placing seeds in incubator at 40° for 1 day and 50° for 4 days.
2. Treat test materials with seed disinfectant.
3. To pregerminate seeds, place the seeds in Petri dishes of water for 2-3 days at about 28 ℃. Change water every day to supply oxygen.
4. Choose what kind of soil to use and the kind of container or pot that suits your experiment.
5. Sow 5-10 seeds each line.
6. If it is possible, incubate 28℃ for 2day to emergence uniformly.
7. After growing 3 weeks in green house, plants will be ready for inoculation (5-6 leaf stage, including an imperfect leaf, actually changes by season from 14 to 21 days).

VI. Inoculum production

Method 1) IRRI protocol

1. To revive stored culture, place a colonized paper disk on a prune agar slant, and allow to grow at room temperature for about 7-10 days.
   1) Revive 3 disks (3 slants) per isolate. After 3-4 days, choose the best two of the three that were revived. Use these two best slants to multiply.
2. The number of slants needed depends on how many ml of inoculum you need. Two 10-day-old slants can be used for 10 ml of macerated mycelium and can be spread on 5-7 plates for sporulation, which can produce 100-150 ml of inoculum at 100,000 spores /ml depending on the isolate used.
   1) When the stock culture disks are few (less than 20-40 in the coin envelope), revive 1-2 disks, and add new sterile disks as specified below (See the Preparation of Stock Culture) to prepare more stock culture disks.
3. Using a transfer needle, slice off as much of the mycelium as possible from the slant, while taking the least amount of agar possible. Transfer the mycelium to a test tube containing 10 ml of sterile D^2H_2O. (This should be done in a clean transfer hood to avoid contamination.)
4. Macerate the mycelium by mashing with a transfer needle or spatula.
5. Pour the suspension of mycelial fragments and conidia onto the surface of prune agar plates and spread the inoculum (2 mls/plate that would be 5 mls per slant used).
6. Incubate the plates at about 27℃ for 7 days.
7. Scrape the fungal growth with a "rubber policeman" (a hard rubber spatula). Sterilized glass slides could be a substitute.
8. Leave the scraped plates OPEN under a fluorescent lights for 3-4 days to induce sporulation.
9. Before harvesting conidia, record the appearance of the scraped plates (of each isolate) and the number of plates to be used. When counting spores take note of the appearance of the conidia – Are they normal in color or shape? Is there a contaminant?
10. Pour 10-20 mls distilled water into the Petri dish and gently scrape the surface re-growth with a sterilized rubber policeman or glass slide.
11. Filter the conidial suspension through 4 layers of cheese cloth or through nylon mesh.
12. Determine the conidial concentration using a hemacytometer (see below).

13. Adjust the concentration to 10^5 (100,000) conidia per milliliter (dilute with distilled water if spore count is high). Scrape some more plates if count if low.
14. To aid in the adhesion of the inoculum to the leaves of the plants, add a tiny drop of Tween 20 (final concentration approximately 0.02%) and shake well before using. Alternatively, 0.5% gelatin can be added.

Method 2) NIAS method

1. To revive stored culture, place a colonized paper disk on a oatmeal agar plate, and allow to grow at about 25 ℃ for about 12-13 days. Use Petri dish 20mm in depth. The number of plates needed depends on how many ml of inoculum you need (Fig. 13).
2. One 12-day-old plate can produce 50-150 ml of inoculum at 100,000 spores /ml depending on the isolate used. Forty-five ml of inoculum is enough for spray inoculation to each 5 plants of 30 LTH monogenic lines.
3. Scrape the surface of the fungal growth with a toothbrush. The scraped plates opened in the tray, covered with wrapping film pitted several holes, and leave under a fluorescent lights for 3-4 days to induce sporulation (Fig. 14-19).
   1) Before scraping plates and harvesting conidia, record the appearance of the plates (of each isolate) and the number of plates to be used. When counting spores take note of the appearance of the conidia − Are they normal in color or shape? Is there a contaminant?
4. Pour 10-20 mls distilled water into the Petri dish and gently scrape the surface of sporulated plates with a paintbrush (Fig. 20).
5. Filter the conidial suspension through 4 layers of cheese cloth or through nylon mesh (Fig. 20).
6. Determine the conidial concentration using a hemacytometer (see below).
   1) Adjust the concentration to 10^5 (100,000) conidia per milliliter (dilute with distilled water if spore count is high). Scrape some more plates if count if low.
   2) To clear a infection type on differential variety, the concentration of spores needs to be changed to the susceptibility of rice plant through the season or by resistance gene harboring in each line. For example, in the line having *Pish* or *Pita*, inoculation of too high concentration of spores result in fused lesions, and difficult to determine whether the infection types of the line is susceptible or resistance reaction. In this case, the concentration from 2 to 5 $\times 10^4$ (20,000 to 50,000) conidia per milliliter is enough.
7. To aid in the adhesion of the inoculum to the leaves of the plants, add a tiny drop of Tween 20 (final concentration approximately 0.01%) and shake well before using. Alternatively, 0.5% gelatin can be added.

Fig.13. Multiplication of fungus with paper filter stock

Fig.14. Sporulation of blast fungus culture under fluorescent lamp

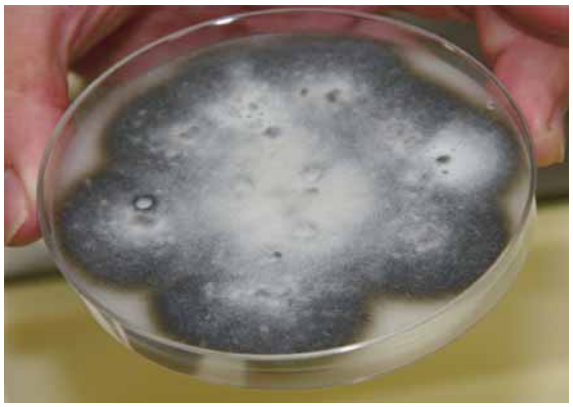


Fig.15. Cultivation of fungus after 12 days at 25 C

Fig.16. Clean the surface with toothbrush

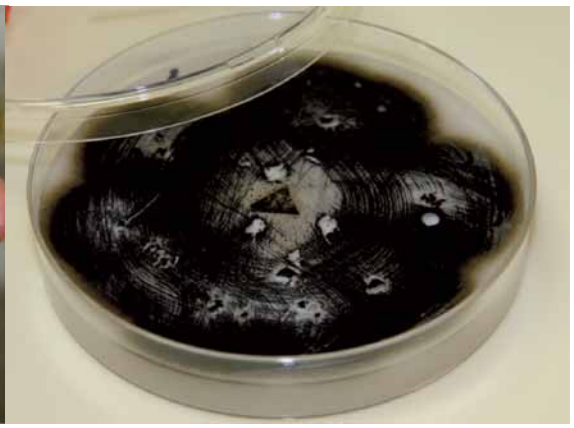


Fig.17. Enhance the spore appearance by the cleaning

Fig.18. Make the holes for the exchange the air in rapped box

Fig.19. Cultivation of balst spore under the fluorescent light
The spore covering the surface of the medium appeared after 3-4 days

Fig.20. Scraping plates and harvesting conidia

VII. Counting of spores using hemacytometer

1. There are 8-1 mm square squares on the hemacytometer
2. Place one drop of a spore suspension on the hemacytometer
3. Put a coverslip to expel some contents.
4. Count spores in ONE 1mm square surrounded in a red.
   (The volume in one square is approximately 10^{-4} ml.)
   So the number of cells per ml (cells/ml) equals:
   Average count per square x dilution factor x 10^4
   So: If there are 10 spores per mm square, then the spore count is 10 x 10^4 = 10^5
   Now: If there are 4 spores per square, then the spore count is 4 x 10^4 = 40,000
   Why? One square (16 little squares) = 1 mm^2
   WITH COVERSLIP (the volume becomes 10^{-4} ml = 1/10000 ml)
   If there are 4 spores in a square, 4 spores/0.00001 ml = 40,000 spores/ml

Fig.21. Counting area in hemacytometer

Count the spores on the line in two sides among four sides.

VIII. Coordinated inoculation procedure (Spraying method)

1. Prepare oatmeal agar or prune agar as described above.
2. Preparation of plant material as described above.
   1) Schedule the inoculum production to begin with step one on the same day seeds are sown for the plants which are going to be evaluated.
   2) Be sure to include susceptible and resistant checks in the planting.
3. Grow for three weeks (to 5-6 leaf stage) for inoculation.
4. Inoculum production

As described above

Measure the conidial concentration with a hemacytometer to obtain a final concentration of 10^5 (100,000) conidia per milliliter.

   1) If spore count is too high, dilute with sterile D^2H_2O
   2) If spore count is too low, scrape some more plates to obtain more spores, using the same D^2H_2O suspension until the desired concentration is obtained.

Add a few drops of Tween 20 to the suspension and shake well.

   1) Use approximately 10 µl per 100 ml of suspension (to 0.01%). Alternatively, use 0.5-1% gelatin instead of Tween 20.
   2) After inoculum preparation and spore counting are finished, proceed at once to inoculation. If two or more hours elapse before inoculation, spors may germinate in the suspension and this will adversely affect the pathogenicity test. If many isolates need to be used and the time to prepare the inoculum becomes longer, glass flasks containing the prepared suspensions may be put in a cool place (on ice) until ready to start spraying.

5. Inoculation

Method 1) IRRI procedure

Be sure that all equipment is sterile and properly labeled like D^2H^2O, tips, glassware, and sprayers. Dew chambers should be decontaminated.

1. Prepare about 10-20ml of inoculum per pot of five test plants.
2. Test plants are planted in 15-20cm diameter cups with 5 plants per cup. Plants are inoculated at approximately 21 days after sowing.
   1) Assemble sprayer and rinse with 95% EtOH, then sterile D^2H_2O.
3. To inoculate place pots on a rotating rack and spray all plants simultaneously while rotating the rack to ensure even coverage of the leaves.
4. Spray the conidial suspension onto the plants until runoff.
   1) When different plants are inoculated with different isolates, be sure that leaves of the plants do not touch or drip onto other inoculated plants inside the dew chamber.
5. Place the plants inside the dew chamber at 25 ℃ for 20h.
6. And then transfer them to a greenhouse high humidity room (mist room) at 25-30 ℃.
   1) Take care that the mist room spray nozzles do not drip into the pots or food containers, because excess moisture at this time can cause adverse physiological reactions leading to the death of otherwise healthy leaves and even whole plants.
   2) Observe time periods properly. Inoculated plants should be in the dew chamber for 24h and mist room for 6 days. Scoring should be no more than 8 days after inoculation.

Fig.22. Dew chamber at IRRI

Fig.23. Mist room at IRRI maintained at 26 ℃

Method 2) Using dew chamber by cooling the outer case (NIAS)

1. Prepare about 5-15 ml of inoculum per pot of thirty test plants.
   1) Test plants are planted in 20 x 5 x 10 height case with 30 plants per case. Plants are inoculated at approximately 21 days after sowing (leaf age ca.4.6).
   2) Assemble sprayer and rinse with 95% EtOH, then sterile D^2H_2O.
   3) To inoculate place pots on a rotating rack and spray all plants simultaneously while rotating the rack to ensure even coverage of the leaves.
2. Spray the conidial suspension onto the plants until visible.
   1) When different plants are inoculated with different isolates, be sure that leaves of the plants do not touch or drip onto other inoculated plants inside the dew chamber.
3. Place the plants inside the dew chamber at 25℃ for 20-24h, then transfer them to a green house (humidity approximately 60%) at 25-30 ℃.
4. Observe time periods properly. Scoring should be 7 to 8 days after inoculation.

Fig.24. Spray inoculation on turn table

Fig.25. Inoculated plant in dew chamber

IX. Disease assessment

1. Infection type on individual differential lines

Susceptibility of the test plants is assessed by examining the leaves for blast symptoms which appear on the latest expanding leaf. Five to seven days after inoculation, infection type is rated as the following (Goto and Yamanaka, 1968, Mackill and Bonman, 1992):

0 = No evidence of infection

1 = Brown specks smaller than 0.5mm in diameter, No sporulation.
Uniform or scattered brown specks, no sprulation

2 = Brown specks about 0.5-1.00mm in diameter, no sporulation.
Small lesions with distinct tan centers surrounded by a darker brown margin approximately 1mm in diameter, No sporulation.

3 = Roundish to elliptical lesion about 1-3 mm in diameter with gray center surrounded by brown margins, lesions capable of sporulation.
Small eyespot lesions less than one and a half times the interval between thin veins or less than 1.5mm in diameter surrounded by dark brown, lesions capable of sporulation.

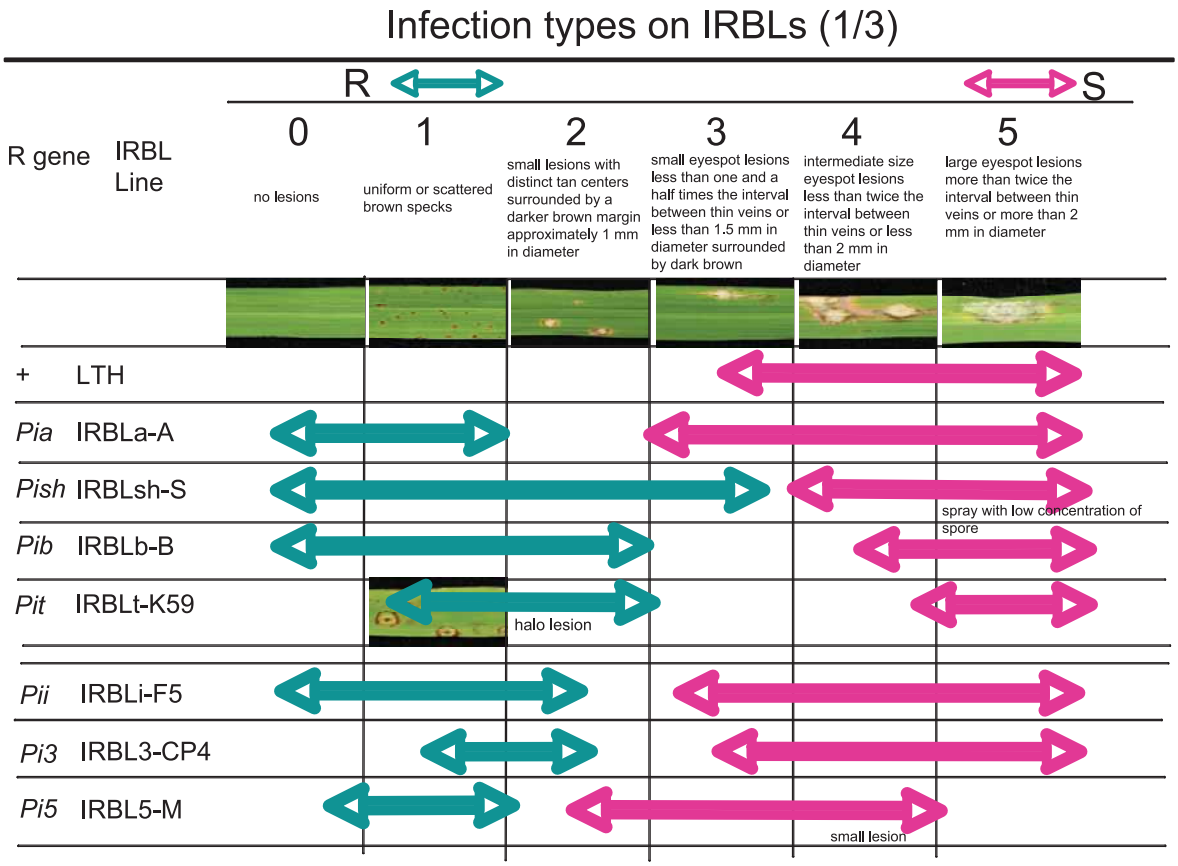
4 = Typical spindle shaped blast lesion capable of sporulation, 3 mm or longer with necrotic gray centers and water soaked brown margins little or no coalescence of lesion.
Intermediate size eyespot lesions less than twice the interval between thin veins or less than 2 mm in diameter.

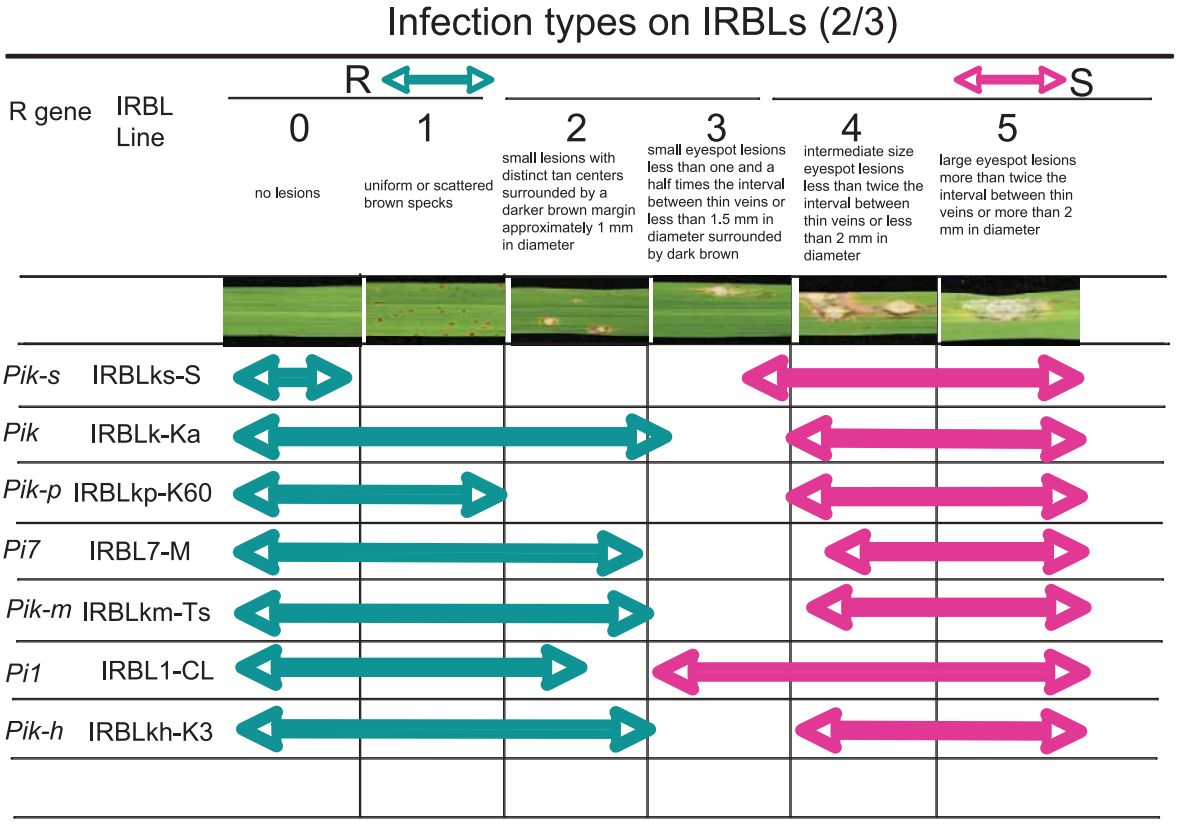
5 = Lesions as in 4 but about half of one or two leaf blade killed by coalescence of lesion.
Large eyespot lesions more than twice the interval between thin veins or more than 2 mm in diameter.

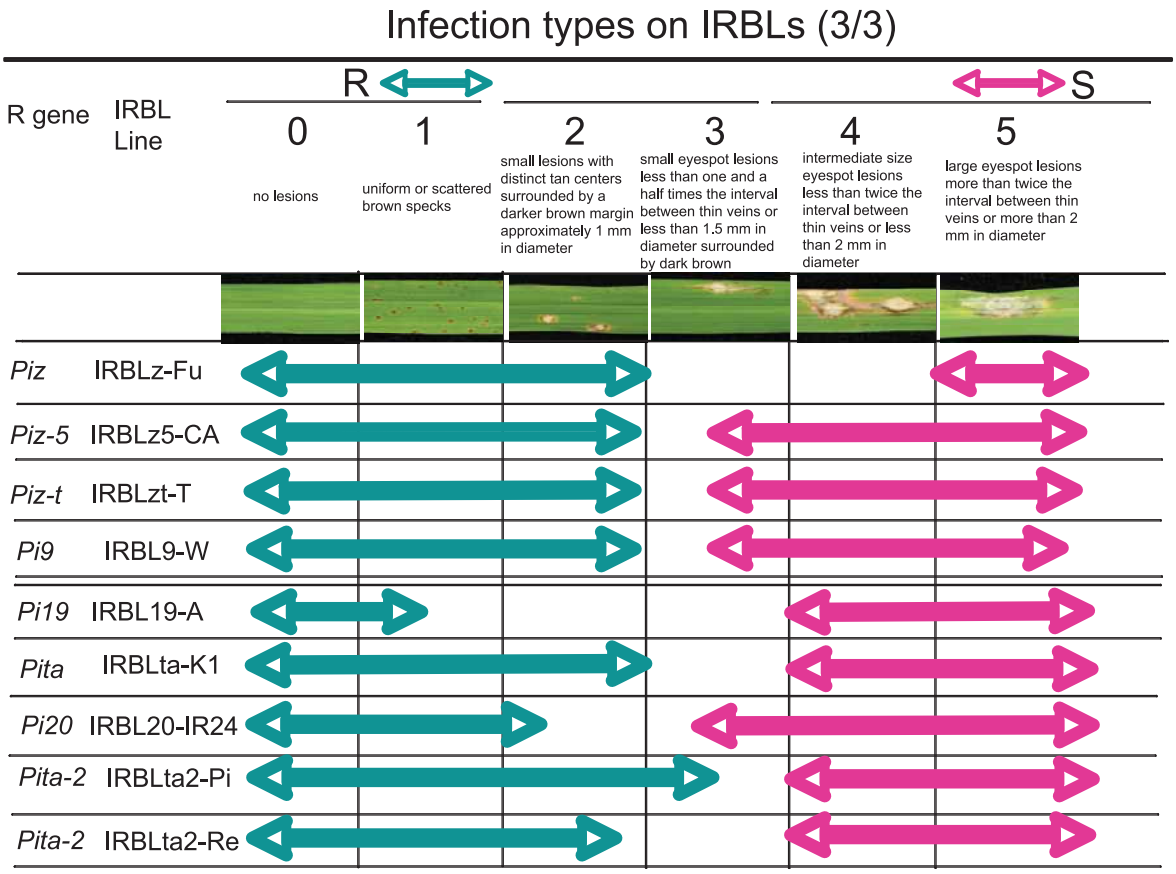
1) Infection type 0 to 2 is resistance reaction, and infection type 3 to 5 is susceptible reaction basically.
2) When we inoculated it into the rice plant which the most upper leaf extremely slightly emergence, the lesion developed on a tip of the leaf becomes sensitive, and there is a case more than infection type 3, but does not determine as the susceptible lesion.

Also record the presence or absence of sporulation on lesions after incubation for 24 h at 27 ℃.

Table 1. A standard decisions for susceptible or resistance reaction each LTH monogenic line







X. Designation of new international differential system

1. Each race code number has following five parts divided by hyphen (i.e. 1st-2nd-3rd-4th -5th).
2. The 1st part of the race code number has a two-digit number and is composed of LTH, and IRBLa-A in ones place, IRBLsh-B (or IRBLsh-S), IRBLb-B and IRBLt-K59 in tens place.
3. The 2nd part has one digit number and is composed IRBLi-F5, IRBL3-CP4, and IRBL5-M, which are multiallelic or closely linked.
4. The 3rd part has a three-digit number and is composed of IRBLk-Ka, IRBLkp-K60 and IRBL7-M in ones place, IRBLkm-Ts, IRBL1-CL and IRBLkh-K3 in tens place, IRBLks-S, in hundreds place, which are multiallelic or closely linked.
5. The 4th part has a two-digit number and is composed IRBLz-Fu, IRBLz5-CA, and IRBLzt-T in ones place, IRBL9-W in tens place, which are multiallelic or closely linked.
6. The 5th part has a three-digit number and is composed IRBL19-A, IRBL20-IR24 in ones place, IRBLta-K1, IRBLta-CP1 in tens place, IRBLta2-Pi, IRBLta2-Re, IRBL12-M in hundreds place, which are multiallelic or closely linked.
7. We put in front of 2nd to 5th part i, k, z, ta which showed locus of the multiple allele.
8. A race number is sum of a code number showing a susceptible reaction.
9. "U" mentioned before a number shows an international blast pathogenicity race.

Table 2. New designation system for blast races based on the reaction of monogenic line with LTH genetic background

Group	I		II	III			IV		V		
Locus	-		*Pii*	*Pik*			*Piz*		*Pita*		
Target resistance gene	*Pish*	+	*Pii*	*Pik-s*	*Pik*-m	*Pik*	*Pi9*(t)	*Piz*	*Pita-2*	*Pita*	*Pi19*
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20*(t)
	Pit	-	*Pi5*(t)	-	*Pik-h*	*Pi7*(t)	-	*Piz-t*	*Pi12*(t)	-	-
Monogenic line (IRBL)	sh-S	LTH	i-F5	ks-S	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A
	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24
	t-K59	-	5-M	-	Kh-K3	7-M	-	zt-T	12-M	-	-
Code	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	-	2	2	-	2	2	2	2
	4	-	4	-	4	4	-	4	4	-	-
Ex. Blast isolates virulence to all genes	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	-	S	S	-	S	S	S	S
	S	-	S	-	S	S	-	S	S	-	-
	7	3	7	1	7	7	1	7	7	3	3

Example blast race No. of isolate that is virulence to all differential varieties (genes): U73-i7-k177-z17-ta733.

Table 3. Comparison of a race number of standard rice blast strains of Japan by the Japanese differential system and the international differential system

Blast strain	Differentiation system	
	Japanese race	International race
Mu-95	J001.2	U31-i0-k100-z00-ta401
Kyu89-246	J003.0	U13-i0-k100-z00-ta001
95Mu-29	J003.2	U33-i0-k100-z00-ta401
Shin83-34	J005.0	U11-i7-k100-z00-ta001
Ina86-137	J007.0	U13-i7-k100-z02-ta001
31-4-151-11-1	J007.2	U33-i7-k100-z00-ta001
Kyu92-22	J017.1	U13-i7-k107-z00-ta001
1804-4	J031.1	U11-i0-k177-z00-ta001
TH68-126	J033.1	U13-i0-k177-z00-ta001
TH68-140	J035.1	U11-i7-k177-z00-ta001
24-22-1-1	J037.1	U13-i7-k177-z00-ta001
Ai79-142	J037.3	U33-i7-k177-z00-ta001
Kyu9439013	J047.0	U13-i7-k100-z01-ta001
TH69-8	J071.1	U11-i0-k177-z01-ta001
Sasamori121	J077.1	U13-i7-k177-z01-ta001
Ina93-3	J301.0	U11-i0-k100-z00-ta331
GFOS8-1-1	J303.0	U13-i0-k100-z00-ta333
0528-2	J333.1	U13-i0-k107-z00-ta331
Ao92-6-2	J337.1	U13-i7-k177-z00-ta331
Mu-183	J337.3	U33-i7-k177-z00-ta331
IW81-04	J437.1	U13-i7-k177-z06-ta001
Ai74-134	J477.1	U13-i7-k177-z05-ta001

Table 4. List of monogenic lines as the differential varieties

Variety Name	Target resistance gene	Glume color
IRBLt-K59	*Pit*	Purple
IRBLsh-S	*Pish*	Brown
IRBLsh-B	*Pish*	Brown
IRBLb-B	*Pib*	Purple
IRBLz-Fu	*Piz*	Brown
IRBLz5-CA-1	*Piz-5* = *Pi2* (t)	Purple
IRBLz5-CA-2	*Piz-5* = *Pi2* (t)	Brown
IRBLzt-T	*Piz-t*	Brown
IRBL9-W	*Pi9*	Brown
IRBLi-F5	*Pii*	Brown
IRBL3-CP4	*Pi3*	Purple
IRBL5-M	*Pi3*	Brown
IRBLa-A	*Pia*	Purple
IRBLks-F5	*Pik-s*	Purple
IRBLk-K	*Pik*	Purple
IRBLkp-K60	*Pik-p*	Brown
IRBLkh-K3	*Pik-h*	Purple
IRBLkm-Ts	*Pik-m*	Brown
IRBL1-CL	*Pi1*	Brown
IRBL7-M	*Pi7* (t)	Purple
IRBL12-M	*Pi12* (t)	Purple
IRBL19-A	*Pi19*	Brown
IRBLta-K1	*Pita* = *Pi4* (t)	Purple
IRBLta-CT2	*Pita* = *Pi4* (t)	Purple
IRBLta-CP1	*Pita* = *Pi4* (t)	Purple
IRBLta2-Pi	*Pita-2*	Purple
IRBLta2-Re	*Pita-2*	Brown
IRBL20-IR24	*Pi20* (t)	Brown
LTH	Susceptible	Purple

This list was modified form the Tsunematsu et al., 2000, Fukuta et al. 2004, and Kobayashi et al., 2007, partially.

Acknowledgements

This protocol was written and would be distributed to these scientist who were facing the problems of blast disease and wanted to start the study, under the JIRCAS research project, Blast Network for Stable Rice Production. IRRI-Japan Collaborative Research Project Phase V has also supported the preparation of the manuscript. Several parts in IRRI methods were referred from protocols in Host Plant Resistance-Disease and Molecular Plant Pathology Units at Plant Breeding, Genetics, and Biotechnology (PBGB), IRRI. The IRRI protocols had been accumulated over the years of scientists and research staffs who worked on blast studies from 1986-1996. They are including J. Bonman, H. Leung, R. Nelson, R.

Zeigler, D. Mackill, J. Bandong, M. Bernardo, E. Borromeo, A. Bordeos, R. Scott, C. Dahu, T. Inukai, T. Vergel de Dios-Mew, A.O. Mackill, P. Reimers, B. Consignado, G.L. Wang, E. Silab, and B. Consignado and P. Reimers compiled it in 1992.

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