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Evaluation of Different Rice Cultivars/Lines by Direct Sheath Inoculation against *Rhizoctonia oryzae* (Ryker et Gooch)

Mohammad Khurshed Alam BHUIYAN and Kei ARAI
(*Laboratory of Plant Pathology and Entomology*)
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Introduction

Rice sheath blight occurs throughout temperate and tropical production areas and is most prominent where rice is grown under intense production systems[17]. Several kinds of sclerotial fungi namely-*Rhizoctonia solani* (Kühn), *Rhizoctonia oryzae* (Ryker et Gooch), *Rhizoctonia oryzae-sativae* (Sawada) Mordue, *Sclerotium hydrophilum* (Saccard) and *Sclerotium fumigatum* (Nakata) are producing sheath blight like lesions on rice leaf sheath. Of these, *R. oryzae* causing bordered sheath spot disease of rice is second only to *R. solani* in aggressiveness[19,26]. *R. oryzae* has been frequently isolated from sheath blight like lesions of rice plants throughout the rice growing parts of the world[1,2,7,9,14,19,22,24]. The impact of bordered sheath spot as rice disease appears to be increasing worldwide, causing yield losses more than 20% in susceptible varieties[23].

Resistance, the most desirable control measures, has been investigated against sheath blight disease caused by *R. solani* by the reseachers in several countries[10,11,12]. Thousands of entries have been evaluated under disease pressure obtained by various inoculation techniques in green house or in field tests. International sheath blight nurseries providing simultaneous entry evaluation in several countries are conducted by researchers at the International Rice Research Institute, Los Baños, Philippines. Results from these programs are similar in that a source of immunity or high level resistance has not been found[17]. However, several other investigations showed that varietal differences in resistance were significant and several cultivars/lines were found as resistant[5,10,15,18].

Recently, Lakshmanan evaluated 87 breeding lines derived from *Oryza officinalis* for resistant to sheath blight caused by *R. solani* and found five highly resistant lines[16]. On the contrary, there has been no report of evaluation of different rice cultivars/lines against bordered sheath spot disease caused by *R. oryzae,* except a limited trial of cv. Nizersail and 48 γ ray induced mutants against *R. oryzae*[13]. Up to date, there is no detailed information about the level of resistance of different established cultivars/lines against bordered sheath spot disease. We evaluated 89 cultivars/lines comprising Japonica, Indica and Javanica types, of which some have noticeable resistance level against *R. solani,* collected from Japan and other countries. The purpose of the present study is to document the status of the tested cultivars/lines against *R. oryzae.* A preliminary report of this experiment has been published[3].

Materials and Methods

Fungus isolate and rice cultivars/lines

The virulent isolate ROl of *R. oryzae* collected from the Laboratory of Plant Pathology, Saga

University was used as inoculum. The fungus was maintained on potato sucrose agar (PSA) medium at 10℃. A total of 89 cultivars/lines comprising Japonica, Indica and Javanica types was evaluated for their reaction to bordered sheath spot disease. The cultivars/lines were collected from different Universities and Reseach Institutes, namely-Kagoshima University, Saga University, National Institute of Agricultural Research Centre, Kagoshima Agricultural Experimental Station, Kumamoto Agricultural Reaearch Centre, Okinawa Agricultural Research Centre, Miyazaki Agricultural Research Centre, Oita Agricultural Research Centre, Saga Agricultural Experimental Research Centre and Nagasaki Agricultural Experimental Station.

Cultivars/lines growth conditions

The experiment was conducted during May-August, 1992. Rice seeds were surface sterilized in 70% ethanol for five minutes, rinsed in sterile distilled water (SDW), and placed in Petri-dishes containing SDW for 24 h at room temperature to allow inhibition of water. The seeds were planted in the sterilized loam soil in plastic tray and placed in the glass house. A 12 h day temperature of 30 ± 5℃ and a night temperature of 25 ± 3℃ were maintained in the glass house. Three weeks old seedlings were transplanted in 20 x 20 x 16 cm^3 plastic pots. The pots were placed in the natural condition. Watering was done twice in a day.

Inoculation and observation

Inoculation was done to rice leaf sheaths of 7-wk old rice plants. Inoculation was made by placing a 4mm in diameter mycelial agar disk from the growing edge of the colony on PSA medium inside the leaf sheaths. The pots with inoculated rice plants were then kept in a growth chamber at 30℃ under saturated humidity for 48 h and again transferred in natural condition. Each cultivar /line had three replicated pots and 20 tillers have been inoculated per replicated pot. Observation was made from one day after inoculation and the symptom development was recorded until growth stage code 58[6].

Selected diseased sheaths were checked for the presence of the test pathogen by culturing on PSA. Based on the results of the previous year the same experiment was repeated during May-August, 1993 with selected cultivars from each class of the resistance level, and similar growth conditions of the cultivars were maintained, following the similar method of inoculation.

Disease rating and disease index

Approximately three weeks after inoculation each inoculated pot was rated for the amount of bordered sheath spot infection, based on the symptoms development and disease progressions on a 0 to 5 scale as follows:

0 : Inoculated plants have no symptoms.

1 : Restricted water soaked lesions at inoculation points, lesion centres grey green to nearly white, margin or lesion abroad red brown or purple border usually broader than necrotic centre, symptoms enlarged less 3.0cm^2.

2 : Lesions with large necrotic centre and narrow sunken or red brown border, symptoms enlarged 3.1-5.0cm^2.

3 : Lesions with a large necrotic centre and dark brown borders, lesions extending to blades of lower leaves, symptoms enlarged 5.1-8.0cm^2.

4 : Coalesced lesions with a large necrotic centre and dark brown borders, leaves became drying, culms became water soaked, symptoms enlarged 8.1-12.0cm^2.

5 : Sheaths and leaves became drying or dead, culms got water soaked, brown or collapsing, most of the tillers lodged, symptoms enlarged more then 12.0cm^2.

The formula for calculation of Disease Index (DI) was used as described by Osullivan and Kavanagh with slight modifications[21]

$$DI\% = \frac{100\ (a+2b+3c+4d+5e)}{5\ (w+a+b+c+d+e)}$$

Where, w represents inoculated tillers class 0 and a, b, c, d, and e are class 1, 2, 3, 4, and 5, respectively. Disease Index (DI) 0-100, expressed as a percentage of the maximum possible level of infection.

Results

There was no difference in the results of the first year and the second year experiments. The results of the experiment presented in the Tables 1-6 and Fig. 1 are based on the data of the first year experiment. All the tested cultivars/lines were classified into six different levels of resistance. Only five cultivars/lines namely-Tetep, Zenith, Mizuho, Tamakei 74 and 893 (Korea) showed highly resistant reaction (Table 1). A total of 14 cultivars/line showed resistant reaction (Table 2), 18 cultivars/lines appeared as of moderately resistant reaction (Table 3) and 30 cultivars/lines were considered to be moderately susceptible (Table 4). Remaining 15 cultivars/lines showed susceptible reaction (Table 5) and seven cultivars/lines, namely 585, 564, 610, Akamai, Taichu 65, Yumihikari and Minamihikari showed highly susceptible reaction (Table 6) against *R. oryzae*. No cultivars/lines have been proved immune against *R. oryzae*.

Representative symptoms of disease rating scale: class 0-5 derived from different level of resistance are documented in Fig. 1 (A-F). Different disease rating classes 0-5 have also existed in the cultivars/lines catagorized as different level of resistance; and the frequency of individual disease rating class also differed among the different level of resistance. In the highly susceptible cultivars/lines, the symptom development was quite severe, and the entire sheaths and leaves became drying within two weeks of inoculation, and all the inoculated tillers represented the disease rating class, counting either 4 or 5 (Fig. 1 F).

Table 1. Highiy resistant reaction to *Rhizoctonia oryzae* causing bordered sheath spot disease of different rice cultivars/line

Cultivars/line	Grain type	Disease index* (%)
893	Japonica (Korea)	4.7
Zenith	Indica	5.7
Tetep	〃	6.7
Tamakei 74	Japonica	8.3
Mizuho	〃	8.7

* DI: 1-10% considered as highly resistant

Table 2. Resistant reaction to *Rhizoctonia oryzae* causing bordered sheath spot disease of different rice cultivas/line

Cultivars/line	Grain type	Disease index* (%)
Kurenaimochi	Japonica	12.3
Homarenishiki	〃	13.3
Fujisaka 5	〃	13.3
Reiho	〃	14.3
Akitakomachi	〃	14.3
Kinoshitamochi	〃	14.7
Hiyokumochi	〃	14.7
C 206	Javanica	15.3
Toyonishiki	Japonica	15.3
Koganemasari	〃	16.0
Yashiromochi	〃	16.0
Shimokita	〃	17.0
Kagahikari	〃	17.0
Taducan	Indica	17.3

* DI: 11-20% considered as resistant

Table 3. Moderately resistant reaction to *Rhizoctonia oryzae* causing bordered sheath spot disease of different rice cultivars/lines

Cultivars/lines	Grain type	Disease index* (%)
Natsuhikari	Japonica	20.7
Kuromai	〃	22.0
599	Javanica (Cambodia)	22.3
Toyokogane	Japonica	23.3
K-1	〃	24.3
Nishihikari	〃	24.6
Saiwaimochi	〃	25.7
Kinuhikari	〃	25.7
Chosen	〃	25.7
Kuju	〃	26.0
Toyosachi	〃	26.0
Sakaemochi	〃	26.3
Ginga	〃	26.3
Todorokiwase	〃	26.3
Toride 1	〃	26.7
Tenryo	〃	27.0
Ishikarishirage	〃	27.7
827	Indica (Myanmar)	29.3

* DI: 21-30% considered as moderately resistant

Table 4. Moderately susceptible reaction to *Rhizoctonia oryzae* causing bordered sheath spot disease of different rice cultivars/lines

Cultivars/lines	Graintype	Disease index* (%)
Umehikari	Japonica	32.3
Seinanmochi	〃	32.3
Minamihikari	〃	33.3
Tsukushihomare	〃	33.3
Kyotoasahi	〃	34.0
Norin22	〃	34.3
Koshihikari	〃	34.3
Fukunishiki	〃	34.3
Hoshiyutaka	〃	35.0
Ashahi 1	〃	35.3
Saikai-134	〃	35.7
Hakutomochi	〃	35.7
Chokoine	〃	36.7
Chiyonishiki	〃	38.3
WSS-2	〃	38.3
Shinrei	〃	39.3
BL-1	〃	40.7
Wasetoramochi	〃	40.7
586	Japonica (India)	42.3
609	Indica (China)	43.7
Nipponbare	Japonica	44.3
Ogonbare	〃	44.7
Mineasahi	〃	45.0
Zuiho	〃	45.3
WSS-3	〃	45.3
Misatohatamochi	〃	45.3
Akiyutaka	〃	46.3
K-59	〃	46.7
Yamadanishiki	〃	47.0
Tsuyuake	〃	47.0

* DI: 31-50% considered as moderately susceptable.

Table 5. Susceptable reaction to *Rhizoctonla oryzae* causing bordered sheath spot disease of diffferent rice cultivars/lines

Cultivars/lines	Grain type	Disease index* (%)
Nishihomare	Japonica	52.3
733	Indica (India)	53.0
Kusabue	Japonica	54.3
Higonohana	〃	57.7
556	Javanica (Indonesia)	59.7
817	Indica (India)	60.0
Somewake	Japonica	60.3
Akanemochi	〃	61.0
Saga-1	〃	62.3
Aichiasahi	〃	63.0
Hinohikari	〃	64.3
Pi-14	〃	64.3
C205	Indica	64.3
Kanto 51	Japonica	65.0
Seinan-89	〃	68.7

* DI: 51-70% considered as susceptible

Table 6. Highly susceptible reaction to *Rhizoctonia oryzae* causing bordered sheath spot disease of different rice cultivars/lines

Cultivars/lines	Grain type	Disease index* (%)
Taichu 65	Japonica (Taiwan)	73.0
Akamai	Japonica	73.7
Yumehikari	〃	74.7
Minamihikari	〃	76.3
610	Indica (China)	80.3
564	Japonica (Veitnum)	81.7
585	Indica (India)	82.3

* DI: 71-100% considered as highly susceptible

Discussion

Bordered sheath spot disease caused by *R. oryzae* is second only to *R. solani* in aggressiveness among the sheath blight like lesions producing fungi of rice[19,26], but there was no detailed study of varietal reaction against *R. oryzae,* except a limited trial by Hossain *et al*[13]. Whereas several varietal screening experiments have been done against sheath blight disease caused by *R. solani* throughout the world[4,5,10,12,15,18]. No varieties were found immune to *R. solani,* but a number of varieties showed resistant reactions to the sheath blight disease. We evaluated 89 cultivars/lines comprising Japonica, Indica and Javanica types against *R. oryzae.* The results of our experiments suggested that there was a great varietal difference in the level of resistance which supports the results of Kim *et al.*[15] who found the fact that varietal difference in resistance was significant in the case of *R. solani.* We found that the cultivars Tetep and Zenith showed highly resistant reaction and Taducan showed resistant reaction against *R. oryzae.* Tetep and Taducan were resistant, and Zenith was moderately susceptible, against *R. solani*[10]. In the true sense, it is not rational to make comparison between the level of resistance of the tested cultivars of the current study and the known level of resistance by other investigators, owing to the fact that the fungus, as well as stage and method of inoculation are different. Our preliminary study suggested that inoculation at the tillering stage incurred maximum level of infection, similar observation was made by Yoshimura and Nishizawa[27].

Despite differences in green house and field cultures, green house screening techniques appear to be adequate for use in a breeding program[20]. The present study was performed at the growth chamber under controlled temperature and humidity and we believe that our method of direct sheath inoculation allows the maximum level of infection. Lee and Rush mentioned in their research article that Indica types of rice were more tolerant against *R. solani* than Japonica types[17], but we could not find such differences between Indica and Japonica types against *R. oryzae* and both the types were found as highly susceptible to highly resistant. Rice sheath blight fungus and bordered sheath spot fungus were substantially similar in respect to their mode of invasion and infection to rice leaf sheaths[8]. Thus the results of the present investigation might provide valuable guideline concerning the resistance, the most desirable control measures against other sclerotial fungi prodeucing sheath blight like lesions in the rice, including *R. solani.*

However, only a limited cultivars/lines were tested in this study. Successful breeding for disease resistance requires production of repeatable severity levels that allows expression of a full spectrum of resistance levels under realistic production conditions. Further evaluations of large

number of entries against all the sclerotial fungi producing sheath blight like lesions in the rice both in the green house and under the field conditions are needed, and are anxiously anticipated.

Summary

An evaluation of 89 cultivars/lines comprising Japonica, Indica and Javanica types from Japan and other parts of Asia has been done against bordered sheath spot disease of rice caused by *Rhizoctonia oryzae* during May-August, 1992 and 1993. Rice seedlings were transplanted in plastic pots and were placed under natural epiphytotic condition. The severe isolate RO1 of *R. oryzae* was used as inoculum and inoculations were made by placing mycelial agar disk inside the leaf sheaths. The inoculated pots were kept in a growth chamber at 30℃ under saturated humidity for 48 h. Disease index was expressed as percentages calculated from the 0-5 scales determined on the basis of disease severity. Among the cultivars/lines tested only five cultivars/lines namely-Tetep, Zenith, 893 (Korea), Mizuho and Tamakei 74 showed highly resistant reaction and seven cultivars /lines, nemely-Yumihikari, Minamihikari, Taichu 65, 585 (Assam, India), 610 (China),564 (Mekong,Vietnum) and Akamai, showed highly susceptible reaction. A total of 14 cultivars/lines showed resistant reaction, 18 cultivars/lines showed moderately resistant reaction and 30 cultivars /lines showed moderately susceptible reaction. Remaining 15 cultivars/lines have showed susceptible reaction to the bordered sheath spot disease.

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Explanation of Fig. 1

A. Representative symptoms of highly resistant cultivar (cv. Zenith), only water soaked (arrow) area rarely observed, but most of the inoculated tillers fall under disease rating class 0. B. Representative symptoms of resistant cultivar (cv. Taducan) disease rating class varied from 0 to 3, arrows indicates disease rating scale 3, C. Representive symptoms of moderately resistant cultivars (cv. Natsuhikari), lesions with necrotic centre and red brown border considered as disease rating class 2 (big arrow), disease rating class also varied from 0-3 (other arrows). D. Representative symptoms of moderately susceptible cultivars (cv. Shinrei), disease rating class varied from class 1-4, big arrow indicate disease rating class 1 and typical early symptom of bordered sheath spot disease, double arrows indicates disease rating class 4 and single small arrow indicates disease rating class 3. E. Symptoms representing susceptible cultivar (cv. Hinohikari), disease rating scale of inoculated tillers varied from class 1-5, arrows indicate symptoms of different disease rating class. F. Symptoms representing highly susceptible cultivar (cv. Taichu-65), all the inoculated tillers showed disease rating scale class 4-5, big arrow indicates disease rating class 5 and other small arrows indicate disease rating class 4.

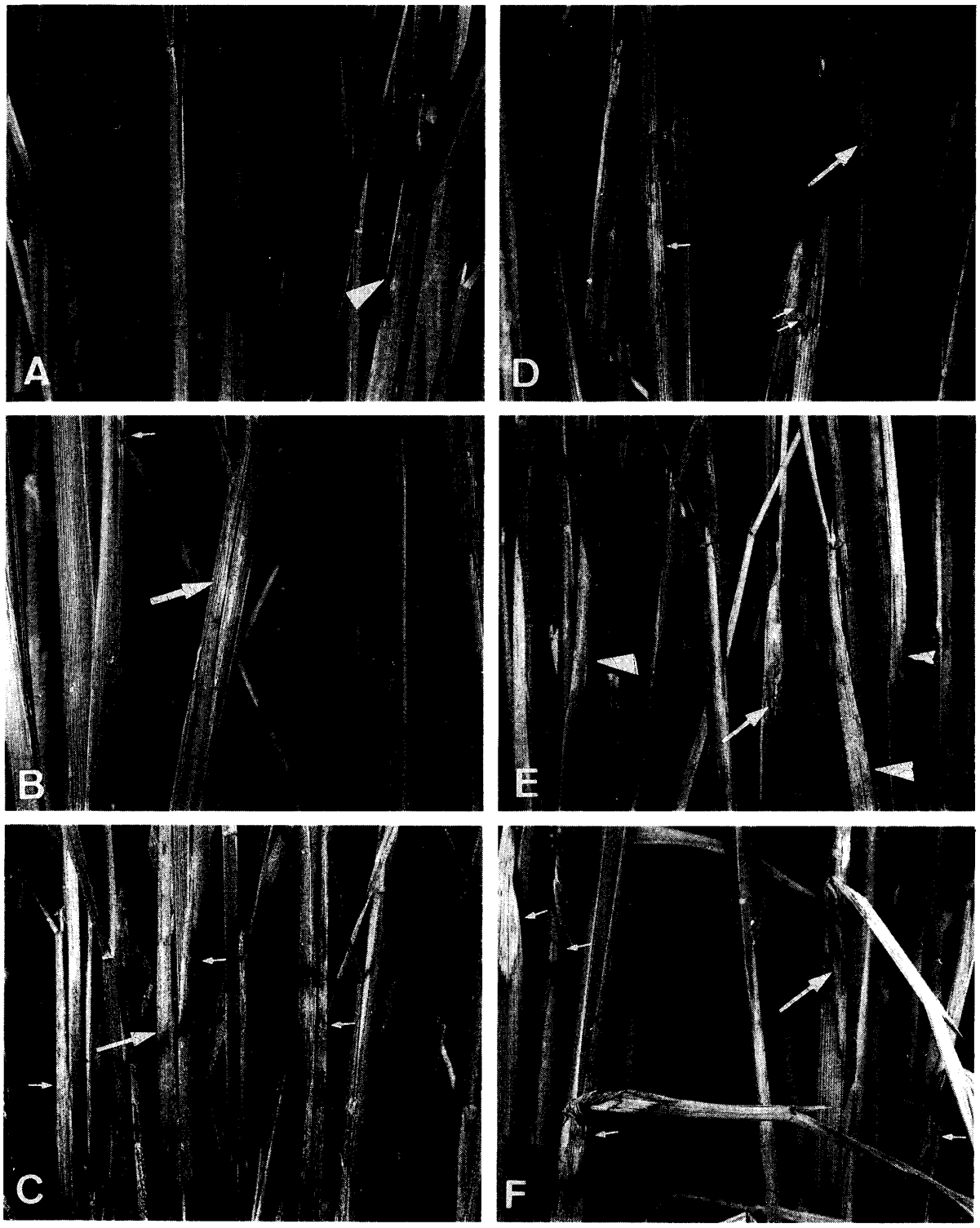

Fig. 1 (A-F). Representative symptoms of bordered sheath spot disease showing disease rating scale 0-5, from different level of resistance.

References

1) Atkins, J. G.: Rice diseases of the Americas. A review of literature. U. S. Dep. Agric., Agric. Hdbk. **448**, p.106. (1974)
2) Bernaux, P.: Action *in vitro* of a herbicide (molinate) on three fungal parasites of rice. *Sclerotium oryzae* Catt., *S. hydrophilum* Sacc. and *Rhizoctonia oryzae* Ryker et Gooch. *Agronomie*, **2**, 301-304 (1982)
3) Bhuiyan, K. A. and Arai, K.: Screening of rice cultivars/lines against bordered sheath spot disease. *Ann. Phytopath. Soc. Japan*, **59**, 295 (abstract, 1993)
4) Binesh, H, and Torabi, M.: Mode of transmission of rice sheath blight through seeds and reaction of rice cultibars to the disease. *Iranian J. Plant Path.*, **21**, 15-25 (1985)
5) Borthakur, B. K. and Addy, S. K.: Screening of rice germplasm to sheath blight (*Rhizoctonia solani*). *Indian J. Agric. Science*, **58**, 537-538 (1988)
6) Chin, K. M., Leohken, A., Sozzi, D. and Williams, R. J.: A modified Zadoks decimal code for the growth stage of rice. *Tropical Pest Managenment*, **37**, 277-280 (1991)
7) Hashioka. Y. and Makino, M.: *Rhizoctonia* group causing the rice sheath spot in the temperate and tropical regions with special reference to *Pellicularia sasakii* and *Rhizoctonia oryzae*. *Res. Bull. Fac. Agric. Gifu Univ.*, **28**, 51-63 (1969)
8) Hashioka, Y. and Okuda, K.: Scanning and transmission electron microscopy on the initial infection of rice sheaths by *Pellicularia sasakii* and *Rhizoctonia oryzae*. *Res. Bull. Fac. Agric. Gifu Univ.*, **31**, 99-111 (1971)
9) Hirayama, S., Shoji, H., Tanaka, T. and Kimura, K.: On the outbreak feature of brown *Sclerotium* disease and bordered sheath spot of rice plant (*Sclerotium oryzae-sativae* and *Rhizoctonia oryzae*). *Ann. Rep. Soc. Pl. Prot. North Japan*, **31**, 40-41 (1980) (in Japanese)
10) Hoff, B. J., Rush, M. C., Mcllrath, W. O. and Morgan, A.: Disease nurseries (a preliminary report). 68th Annu. Prog. Rep., Rice Expt. Stn., Crowley, L. A., p.142-192 (1976)
11) Hori, M. and Anraku, M.: Susceptibility of genus *Oryzae* to rice sheath blight (*Rhizoctonia solani* Kühn). *Chugoku Agric. Res.*, **61**, 6-8 (1981) (in Japanese)
12) Hori, M., Anraku, M. and Matsumoto, K.: Virulence and morphological and physiological characteristics of isolates of rice sheth blight fungus in Japan. *Chugoku Agric. Res.*, **62**, 10-14 (1981) (in Japanese)
13) Hossain, I., Jalaluddin, M., and Ashrafuzzaman, M.: Resistance of mutant strains of rice to sheath blight disease. *Bangladesh J. Botany*, **9**, 125-129 (1980)
14) Inagaki, K.: Sheath blight and other rice sclerotial diseases caused by *Rhizoctonia oryzae* and 3 *Sclerotium* species with respect to their concurrent occurences in mature rice plants. *Ann. Phytopath. Soc. Japan*, **49**, 736-738 (1983) (in Japanese)
15) Kim, K. H., Yang, K. J. and Lee, S. B.: Studies on varietal resistance to sheath blight disease in rice. II. Varietal differences of resistance. *Korean J. Plant Path.*, **32**, 302-309 (1987)
16) Lakshmanan, P.: Resistance to sheath blight (ShB) and brown spot (BS) in lines derived from *Oryza officinalis*. *International Rice Res. Newsletter*, **16** (6), 8 (1991)
17) Lee, F. N. and Rush, M. C.: Rice sheath blight: A major rice disease. *Plant Dis.*, **67**, 829-832 (1983)
18) Miah, A. A., Haque, M. A. and Nahar, M. A.: Comparative reaction to high yielding short and traditional tall Indica rice strains to sheath blight disease. *Bangladesh J. Botany*, **8**, 106-112 (1979)
19) Nonaka, F.: Causal fungi for lesion formation of various types of sheath of rice plant. *Proc. Assoc. Pl. Prot., Kyushu*, **23**, 15-17 (1977) (in Japanese)
20) Oster, J. J.: Screening techniques for stem rot resistance in rice in California. *Plant Dis.*, **74**, 545-548 (1990)
21) O'sullivan, E, and Kavanagh, J. A.:Characteristics and pathogenicity of *Rhizoctonia* spp. associated with damping-off of sugar beet. *Plant Pathology*, **40**, 128-135 (1991)
22) Ou, S. H.: Rice diseases (2nd ed.). Commonw. Mycol. Inst. Kew, Surry, England. p.380. (1985)
23) Ra, D. S. and Oh, J. H.: Development of rice bordered sheath spot caused by *Rhizoctonia oryzae* and its effects on yield as influenced by infection time. *Korean J. Plant Path.*, **4**, 278-283 (1988)
24) Ryker, T. C. and Gooch, F. S.: *Rhizoctonia* sheath spot of rice. *Phytopathology*, **28**, 233-246 (1938)

25) Shahjahan, A. K. M. and Mew, T. W.: Sheath spot of rice in the Philippines. *International Rice Res. Newsletter,* **11**(3), 17 (1986)
26) Shangzhi, Y. and Mew, T. W.: Sheath blight diseases in tropical rice fields. *International Rice Res. Newsletter,* **12**(5), 19-20 (1987)
27) Yoshimura, S. and Nishizawa, T.: Studies on the method of testing varietal resistance of upland rice plants caused by sheath blight, *Hypochnus sasakii* Shirai (Preliminary report). *Bull. Kyushu Agric. Exp. Sta.,* **2**, 361-378 (1954) (in Japanese)