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ROLE OF *BIPOLARIS ORYZAE* IN PRODUCING ABNORMAL SEEDLING OF RICE (*Oryza sativa*)

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ABSTRACT

Two rice hybrids and their parental lines were collected from Haryana and Andhra Pradesh of India in 2003. These seed lots were used to study on role of Bipolaris oryzae in producing abnormal seedlings. At the end of germination test, abnormal seedlings were separated and classified into different categories of abnormal seedlings according to their external morphology. These abnormal seedlings were transferred to the top of wet blotters in Petri dishes and incubated at 20ºC under 12 h alternating cycles of NUV light and darkness. Generally, 3 to 4 days were optimal for the fungi to sporulate for recording the fungus associated with the different categories of abnormal seedlings.

Nine categories of seedling abnormality were recorded after germination test as follows: No root(s); no root, decay in shoot; short root; decay in shoot; decay in shoot and root; browning primary root and weak shoot; coiling or twisted shoot; spindly, pale or watery shoot; and weak, spindly primary roots. Seedlings with decay in shoot and seedlings with decay in both root and shoot were encountered most frequently in 33.33% and 21.17% of the total abnormal seedlings respectively. Incidence of Bipolaris oryzae, Alternaria padwickii and Curvularia lunata on the abnormal seedlings was 43.2%, 48.7% and 50.9 % respectively. Bipolaris oryzae, Alternaria padwickii and Curvularia lunata were present in 61.7 %, 68.1% and 53.6% respectively of the seedlings with decay in shoot and root, and in 56.8%, 55.6% and 55.4% respectively of the seedlings with decay in shoot.

INTRODUCTION

Brown spot of rice, a fungal disease is caused by Bipolaris oryzae Breda de Haan that is prevalent in all the rice growing countries of the world and most of the cultivars grown in the world are susceptible to this pathogen. Though it is considered as a minor disease, it is known to cause considerable economic losses during normal years and at times like the great Bengal famine of 1942 (Padmanabhan, et al., 1948).

The pathogen is known to cause damage at different stages like: storage, seed germination and seedling establishment, vegetable growth and reproductive phase. The nature of damage caused by the pathogen differs with different stages. At the time of storage it affects seed quality parameters like germination, viability, and vigor. At the time of seed germination, it affects both root and shoot system and affects the survivability of seedlings by causing seedling blight. Due to formation of brown spots and blight symptoms on leaves, total photosynthetic area gets reduced during vegetative phage. At reproductive phase, the nature of damage is in the form of grain discoloration, poor grain filling and reduced yield. Hence, the present study of role of Bipolaris oryzae in producing abnormal seedling of rice needs to be undertaken to confirm its potential damages.

MATERIALS AND METHOD

There were two rice hybrids along with their parental lines viz. PRH 10, P6-A, P6-B, PRR-78 and DRRH1, IR 28025-A, IR 28025-B, IR40750-R were collected from Karnal-Haryana and Hyderabad-Andhra Pradesh respectively of India in Kharif season 2002 and used in the study.

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The role of *Bipolaris oryzae* in producing abnormal seedlings was determined by using the between paper method and standard blotter method (ISTA, 2003). They are described as follows:

Four hundred seeds in four replicates of hundred seeds each were placed between two layers of moist germination papers with the help of counting board. Then the germination papers were folded along one edge and then rolled up carefully ensuring that no excess pressure is placed on the seeds. These were wrapped with the sheet of wax paper to reduce surface evaporation and placed in a germinator at 25^0C temperatures in an upright position. After 7 days' incubation, the seedlings were evaluated for normal, abnormal seedlings, fresh ungerminated and dead seeds according to the International Rules for Seed Testing (2003). Abnormal seedlings separated from germination test were classified into different categories of abnormal seedlings according to their external morphology (Guerrero et al., 1972). Abnormal seedlings had no mycelial growth and also those having only mycelium of a fungus, were transferred to the top of wet blotters in Petri dishes and incubated at 20ºC under 12 h alternating cycles of NUV light and darkness. Generally, 3 to 4 days were optimal for the fungi to sporulate for recording the fungus associated with the different categories of abnormal seedlings.

RESULTS AND DISCUSSION

Abnormal seedlings

As usual, germination test evaluates percentage of normal seedlings, abnormal seedlings, fresh ungerminated and dead seeds according to the International Rules for Seed Testing (2003) at the end of the test, but in the present study, abnormal seedlings were further separated and classified into different categories as follows (plate 1):

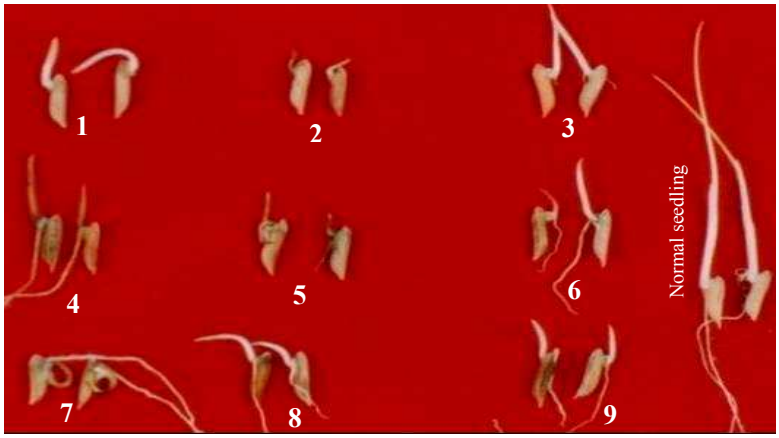

Plate 1: Different categorie of abnormal seedlings

Category 1: No root(s)
Category 2: No root, decay in shoot
Category 3: Short root
Category 4: Decay in shoot (shoot usually weak and showing decay near the point of attachment to the seed)
Category 5: Decay in shoot and root
Category 6: Primary root showing browning, no branching or secondary development, usually shoot appearing weak
Category 7: Coiling or twisted shoot (shoot appearing weak and showing browning)
Category 8: Spindly, pale or watery shoot
Category 9: Weak, spindly primary roots

The frequency of the different categories of abnormalities was observed in the following descending order: 4, 5, 2, 8, 6, 9, 7, 1, and 3. Seedlings with decay in shoot and seedlings with decay in root and shoot both were encountered most frequently in 33.33% and 21.17% of the total abnormal seedlings respectively. The seedlings with no root but having decayed shoot as well as seedlings with watery shoot, seedlings with browning in the primary root, and seedlings with weak, spindly primary root occurred by 13.51%,

11.71%, 9.91%, and 5.41%, respectively. Other abnormalities were encountered in range of 0.9 – 2.25% (table 1 and Fig. 1). Therefore, Abnormal seedlings with decay in shoot and seedlings with decay in both shoot and root were found commonly and highly in the study, the result of the study was also confirmed by Guerrero et al., (1972).

Table 1: Distribution of abnormal seedlings (AS) in eight seed lots (400 seeds)

Categories of AS	Rice hybrids/ parental lines									
	IR 58025-A	IR 58025-B	IR 40750-R	DRRH-1	P 6-A	P 6- B	PRR-78	PRH-10	Total	(%)
No root(s)	1	0	0	0	1	1	1	0	4	1.80
No root, decay in shoot	3	8	0	4	0	7	8	0	30	13.51
Short root	2	0	0	0	0	0	0	0	2	0.90
Decay in shoot	16	14	1	11	5	10	7	10	74	33.33
Decay in shoot and root	8	6	0	3	2	10	13	5	47	21.17
Browning primary root	3	6	0	2	5	3	2	1	22	9.91
Coiling or twisted shoot	0	0	0	1	0	0	2	2	5	2.25
Spindly, pale or watery shoot	1	1	0	1	6	7	5	5	26	11.71
Weak, spindly primary root	2	1	1	1	1	1	4	1	12	5.41
Total abnormal seedling	36	36	2	23	20	39	42	24	222	
%	9	9	0.5	5.75	5	9.75	10.5	6		

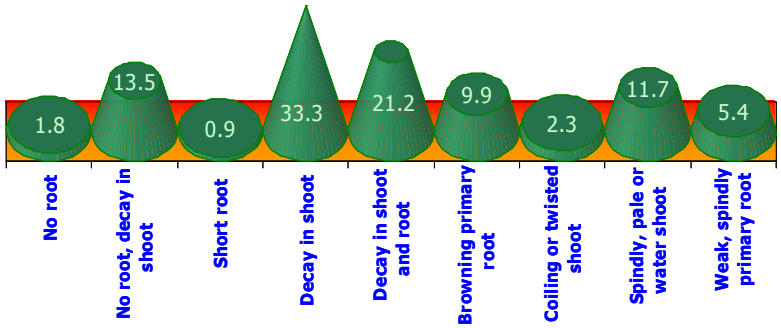

Fig 1: Distribution of abnormal seedlings in seed lots

Associated fungi and their role:

Ten seedborne fungi were detected by using the blotter method in the study. They were *Bipolaris oryzae*, *Alternaria padwickii*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Curvularia lunata*, *Alternaria alternata*, *Sarocladium oryzae*, *Rhizopus sp.*, *Aspergillus sp.*, and *Penicilium sp.* The most predominant seedborne fungi were *Bipolaris oryzae*, *Alternaria padwickii* and *Curvularia lunata*, *Bipolaris oryzae* appeared on 43.24% of abnormal seedling, *Alternaria padwickii* and *Curvularia lunata* appeared on 48.65% and 50.90% respectively of abnormal seedlings, while other seedborne fungi

occurred in range of 0.9 - 11.26% (table 2 and Fig. 2). Zad and Khosravi (2000) also reported similarly the association of seedborne fungi with abnormal seedlings of rice, which supports our study. *Bipolaris oryzae*, *Alternaria padwickii* and *Curvularia lunata* were present in 56.76%, 44.59% and 55.41% respectively of the seedlings with decay in shoot and 61.7%, 68.09% and 59.57% respectively of the seedlings with decay in shoot and root (table 3), the result of the study was also confirmed by Guerrero et al. (1972). Hence, three seedborne fungi *Bipolaris oryzae*, *Alternaria padwickii* and *Curvularia lunata* were considered as the important agents in producing the abnormal seedlings. This is because they associated consistently with the abnormal seedlings in general, and with abnormal seedlings with decay in shoot and abnormal seedlings with decay in shoot and root in particular. Role of important seedborne fungi is as follows.

Table 2: Number of fungal infected seeds on different categories of abnormal seedlings

Categories of abnormal seedlings	No. infected seeds per sample (400 seeds)									
	Bo	Ap	Fm	Fp	Cl	Rhi	Asp	Aa	Pen	So
No root(s)	0.00	0.38	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
No root, decay in shoot	1.50	2.50	0.00	0.00	1.88	0.00	0.13	0.63	0.13	0.25
Short root	0.13	0.13	0.00	0.00	0.13	0.00	0.13	0.00	0.00	0.00
Decay in shoot	5.25	4.13	0.00	0.00	5.13	0.63	0.38	1.13	0.25	0.38
Decay in shoot and root	3.63	4.00	0.13	0.13	3.50	0.13	0.13	0.50	0.13	0.50
Browning primary root	0.38	1.00	0.00	0.13	1.00	0.00	0.00	0.25	0.25	0.00
Coiling or twisted shoot	0.13	0.25	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
Spindly, pale or watery shoot	1.00	1.00	0.00	0.50	1.63	0.00	0.00	0.38	0.00	0.00
Weak, spindly primary root	0.00	0.13	0.13	0.00	0.38	0.00	0.00	0.25	0.13	0.00
Total	12.00	13.50	0.25	0.75	14.13	0.75	0.75	3.13	0.88	1.13

Table 3: Incidence of seedborne fungi associated with the different categories of abnormal seedlings

Categories of abnormal seedlings	Incidence of seedborne fungi (%)									
	Bo	Ap	Fm	Fp	Cl	Rhi	Asp	Aa	Pen	So
No root(s)	0.00	75.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00
No root, decay in shoot	40.00	66.67	0.00	0.00	50.00	0.00	3.33	16.67	3.33	6.67
Short root	50.00	50.00	0.00	0.00	50.00	0.00	50.00	0.00	0.00	0.00
Decay in shoot	56.76	44.59	0.00	0.00	55.41	6.76	4.05	12.16	2.70	4.05
Decay in shoot and root	61.70	68.09	2.13	2.13	59.57	2.13	2.13	8.51	2.13	8.51
Browning primary root	13.64	36.36	0.00	4.55	36.36	0.00	0.00	9.09	9.09	0.00
Coiling or twisted shoot	20.00	40.00	0.00	0.00	40.00	0.00	0.00	0.00	0.00	0.00
Spindly, pale or watery shoot	30.77	30.77	0.00	15.38	50.00	0.00	0.00	11.54	0.00	0.00
Weak, spindly primary root	0.00	8.33	8.33	0.00	25.00	0.00	0.00	16.67	8.33	0.00
Total abnormal seedling	43.24	48.65	0.90	2.70	50.90	2.70	2.70	11.26	3.15	4.05

Ap: *Alternaria pawickii*; Bo: *Bipolaris oryzae*; Cl: *Curvularia lunata*; Rhi: *Rhizopus spp.*; Aa: *Alternaria alternata*, Fm: *Fusarium moniliforme*; Fp: *Fusarium pallidoroseum* [Fusarium semitectum]; So: *Sarocladium oryzae*; Asp: *Aspergillus ssp*; Pen: *Penicilium ssp.*

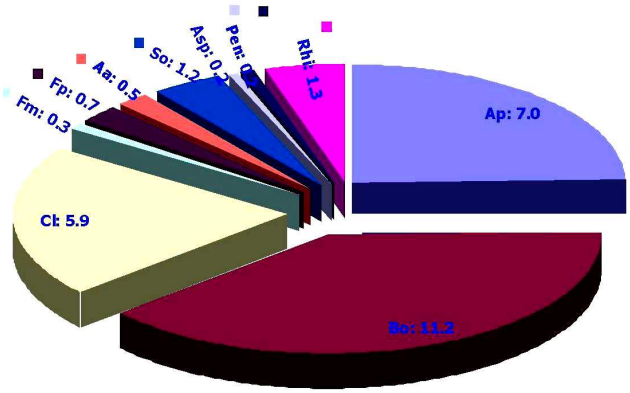

Fig 2: Incidence of seedborne fungi associated with the abnormal seedlings

Bipolaris oryzae L. (Breda de Haan):

Generally, on the 8th day of incubation in the rolled towel method, mycelium and spores of the fungus were observed on decaying tissues of shoot and root of severely infected abnormal seedlings and even on the substrate where these abnormal seedlings were located. This association indicated a direct link between the pathogen and abnormalities, in case of mild decay in shoot and root. The fungus in some cases sporulated only on the diseased portions. The fungus was also found growing on the seed coat. Most of the seedlings of this category were transferred to the top of blotter paper and incubated under NUV light for inducing sporulation. *Bipolaris oryzae* was seen abundantly after incubation (plate 2), sometimes in few cases, *Alternaria padwickii* was also seen simultaneously along with *Bipolaris oryzae*.

Alternaria padwickii

The fungus was seen on different categories of abnormal seedlings. Mycelium and spores of the fungus were frequently found at the embryonal portion, and sometimes they appeared on seed coat. In case of heavy infection, mycelium and spores covered entire surface of seed coat and even on roots and shoot. The towel substrate under seed in a few cases was stained purple or pink in color, these abnormal seedlings were ultimately killed. Some abnormal seedlings, which had no fungal growth when transferred to the top of blotter, developed abundantly characteristic mycelium and conidia of *Alternaria padwickii* within few days of incubation (plate 3).

Curvularia lunata

The fungus was found on different categories of abnormal seedlings. Conidia of the fungus appeared mainly on seed coat, especially on discolored and cracked seed coat. Normally, the fungus has not caused severed damages to seedlings, but in a few cases, heavy growth of the fungus caused abnormal seedling and even death of seedling.

Roles of other fungi associated with abnormal seedlings have not been indicated unknowingly in producing abnormal seedlings, because of their low and inconsistent frequency of occurrence (plate 4).

The standard blotter method with near Ultraviolet light permits better growth and sporulation of many fungi. This method gave a good picture pertaining to the health status of the seed samples. The level of moisture in the blotter during the course of the test was not optimal for germination and seedling evaluation. That is the main reason why the rolled towel method (BP) was conducted for recording germination. Therefore, germination test and standard blotter method cannot be combined into a single test and both have to be used in order to access adequately the planting value of seed lots.

Plate2 Conidia of *Bipolaris oryzae* x 400

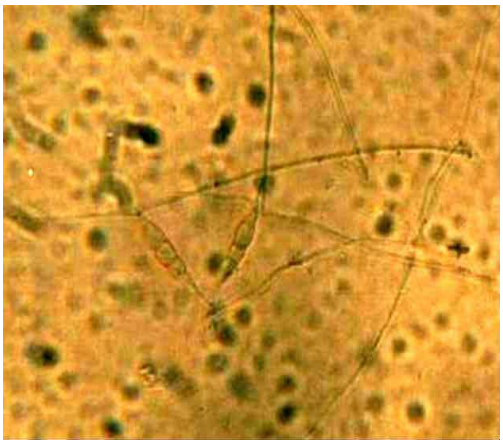

Plate3 Conidia of *Alternaria padwickii* x 400

Plate 4 Conidia of *Curvularia lunatax* 400

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SUMMARY IN VIETNAMESE

Hai giống lúa lai cùng với các dòng bố mẹ của chúng được thu thập từ hai tiểu bang Haryana và Andhra Pradesh của Ấn Độ trong năm 2003. Các lô hạt giống này được sử dụng để nghiên cứu vai trò của nấm gây bệnh đốm nâu trên lúa (Bipolaris oryzae) trong việc tạo ra cây mạ bất thường. Sau khi đánh giá khả năng nẩy mầm của hạt giống, các cây mạ bất thường sẽ được tách riêng và phân ra thành các loại mạ bất thường dựa trên các đặc điểm hình thái bên ngoài của chúng. Các loại mạ bất thường này sẽ được đặt trên giấy thấm trong đĩa Petri và được ủ ở nhiệt độ 20^0C dưới chế độ 12 giờ sáng và 12 giờ tối. Thời gian ủ thường 3 đến 4 ngày đủ để nấm phát sinh bào tử và lấy chỉ tiêu.

Chín loại mạ bất thường được ghi nhận như sau: Mạ bất thường không có rễ; không có rễ, thối chồi mầm; rễ ngắn; thối chồi mầm; thối chồi mầm và rễ mầm; rễ thứ cấp ngã vàng và chồi mầm yếu; chồi mầm xoắn; chồi mầm yếu, nhạt màu; và rễ thứ cấp yếu. Cây mạ thối chồi mầm và cây mạ thối chồi và rễ mầm chiếm tỷ lệ khoảng 33.33% và 21.17% trong tổng số cây mạ bất bình thường. Tỷ lệ nhiễm của *Bipolaris oryzae*, *Alternaria padwickii* và *Curvularia lunata* trên tổng cây mạ bất bình thường là 43.2%, 48.7% và 50.9%, tỷ lệ nhiễm trên cây mạ bị thối chồi và rễ mầm là 61.7%, 68.1% và 53.6%, còn trên cây mạ bị thối chồi mầm là 56.8%, 55.6% và 55.4%.