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Avirulence/virulence reaction of blast fungus on finger millet: A study on F_1 culture of a cross derived from host specific isolates of finger millet and foxtail millet

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Abstract

Host specificity of blast fungus (*Magnaporthe grisea*) was examined on four finger millet (*viz.*, K7, GE5230, GPU26, PR202) and one local landrace of foxtail millet using F_1 progenies of a cross between two highly fertile and host specific pathogenic cultures isolated from a collection of field samples at central Himalayan region of Uttarakhand state in India. Parental isolate VII739 was virulent on finger millet and avirulent on foxtail millet cultivars, contrarily VII769 showed virulent reaction on foxtail millet and avirulent on finger millet cultivars. Data revealed that pathogenicity developed from the cross between finger millet isolates and foxtail millet isolate was conditioned by one (on K7 and PR202) and two (on GE5230) genes. The segregating ratio for 1:1:1:1 and 3:1:3:1 in combined analysis between K7 and PR202 and between PR202 and GE5230, respectively suggested that genes present in the cultivars were different and independent, contrarily 2:0:1:1 ratio between cultivars K7 and GE5230 demonstrated that one gene in GE5230 was identical to the one in K7. Avirulent genes for pathogen on K7, PR202 and GE5230 were designated respectively as AVR_1, AVR_2 and (AVR_1, AVR_3) and their corresponding resistance genes in cultivars as R_1, R_2 and (R_1, R_3). Avirulent reaction on GPU26 and foxtail millet cultivar explained that complex host species specificity of *M. grisea* existed in nature.

Key words: *Magnaporthe grisea*, finger millet, foxtail millet, resistance

Introduction

Magnaporthe grisea (Hebert) Berr, ( ) Sace.(= Cavara), is the casual agent of blast disease, which is probably the most serious fungal disease of many gramenicius species including rice, finger millet, foxtail millet, etc. Resistance breeding, an economical and viable disease management strategy, is often challenged by the occurrence of resistance breaking fungal variants and lead to premature withdrawal of high yielding varieties. It is presumed that considerable amount of genetic variability of blast pathogen has resulted in the rapid adaptation of the pathogen to many host varieties. Occurrence of recombinants among haploids segregating from diploids suggested that para-sexuality also contributes to variation in the pathogen [1], however, discovery of development of perithecia through sexual reproduction [2] has tremendously aided in genetic analysis of the blast pathogen. Yaegashi [3] suggested that the pathogenicity of F_1 progenies developed from a cross between finger millet isolates and weeping lovegrass isolate on finger millet and weeping lovegrass was conditioned by a single gene. Valent [4] identified two major genes involved in the species specificity of an phenotype and type, towards weeping lovegrass. Subsequently, it was shown that they had characteristics similar to avirulent genes [5,6] that are known to condition cultivar specificity, while studies on the host range, sexual fertility [7], and fingerprinting with repetitive elements [8] indicated that the finger millet isolates were distinguishable from the other host specific pathotypes. In the present study F_1 progenies were developed by crossing finger millet derived and

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foxtail millet derived isolates to elucidate number of genes involved in controlling the pathogenecity on finger millet cultivars.

Materials and Methods

Field samples of were collected from central Himalayan hills in Uttarakhand state in India. Two pathogenic cultures, one each from finger millet (VII739) and foxtail millet (VII769) were isolated on the basis of high host specificity and cross fertility and used as parental isolates in the crossing programme. Isolate VII769 was virulent on finger millet (L) and avirulent on foxtail millet (L.), while isolate VII769 was avirulent on finger millet and virulent on foxtail millet. Isolates VII769 and VII739 were of the mating type MATI-1 and MATI-2, respectively. The crosses between two isolates were made by pairing agar blocks, cut from the margins of growing colonies, about 3 cm apart on RYLA medium (Ragi Yeast Lactose Agar medium containing per liter 20 grams ground ragi powder, 20 grams agar, 5 grams lactose and 1 gram yeast extract). Sealed agar plates were incubated at 28°C. After 15 days of incubation, perithecia were examined at the junction of two opposing isolates under a stereo binocular microscope. The bulbous base shaped, large size mature perithecia were placed on 4% water agar medium and pressed gently with fine needle to release F_1 ascospores. A glass needle with fine curved tip as used to separate asci from the ascal fan. Free ascospores were germinated on 2% water agar medium at 28°C. After 2-6 hours, germinated ascospores were transferred to RYLA slants and incubated at 28°C.

The pathogenicity test of F_1 progenies was performed on four finger millet cultivars ., GE5230, GPU26, K7, PR202 and one local land race of foxtail millet, which were thoroughly homogenized by self pollination and used for screening against pathogen for three successive generations. Seeds of the test cultivars were sown in five separate blocks within a seedling tray with one cultivar in each tray. The trays were filled with soil mixed with vermicompost (3:1) and grown at 25°C in a screen house with 12 hours photoperiods for 21 days.

Parental isolates of and their F_1 progenies were grown on RYLA medium in petridishes for four days. Subsequently, the culture were washed off by rubbing mycelium surface with a brush. The colonies were exposed to fluorescent light (40W) at 25°C for 4 days to induce sporulation. The conidia thus produced were suspended in water and adjusted to a concentration of 5×10^5 spores per ml. Fifty milliliter of spore suspension with 0.01% Tween 20 was sprayed on the adaxial surface of primary leaves of four finger millet cultivars ., GE5230, GPU26, K7, PR202 and one local landrace of foxtail millet in a plastic cage using an automizer. The cages were sealed to maintain high humidity and placed in dark for 36 hours. All the plants were inoculated with 270 F_1 progenies of the two parental isolates. The inoculated plants were then returned to screen house and incubated further at 25°C. After seven days of incubation the symptoms developed on leaf surface of inoculated plants and were evaluated on the basis of lesion type, lesion size, number of lesions and affected area on leaf surface following the method of Murakami [9] with modifications. Average score of disease on two leaves over five replications was used to distinguish reaction between avirulence and virulence type of pathogen culture among F_1 progeny. Isolates exhibiting no detectable disease symptoms were considered as avirulent, where as those exhibiting visible disease symptoms were treated as virulent. Chi square (χ^2) analysis was employed to observe the segregation pattern of F_1 progeny of on individual cultivars. A combined analysis involving the two cultivars was performed to observe the presence of identical genes between them. It was assumed that one gene of each cultivar would segregate for 1:1:1:1 if they were independent to each other while 3:1:3:1 ratio would be observed when pathogenic reaction would be independent and one of the two cultivars was controlled by single gene and the other by two gene. Contrarily, 2:0:1:1 segregating ratio would explain the possibility of one gene being identical in later case when pathogenic reaction of one of the two cultivars was controlled by single gene and other by two genes [10].

Results and discussion

Virulent or avirulent reaction of the parental isolates of revealed contrasting results on the two hosts (finger millet and foxtail millet) 7 days after inoculation. Pathogenic culture VII739 that was isolated from finger millet produced visible virulent symptoms on finger millet and avirulent reaction on foxtail millet cultivar. On the other hand, foxtail millet derived isolate VII769 produced no detectable virulence symptoms on finger millet but showed virulence reactions on foxtail millet. The F_1 progenies developed through

cross between the two parental isolates (VII739 and VII769) exhibited avirulent reaction on foxtail millet cultivar. Virulent or avirulent reaction of F_1 progenies, however, segregated on finger millet cultivars suggesting that host-pathogen interaction between avirulence genes in F_1 progeny and their corresponding resistance genes in the host [11] perhaps determined diversity with respect to resistance and susceptibility in finger millet cultivars. It was presumed that avirulence factors present in foxtail millet possibly introgressed in F_1 progenies through recombination and exchange of genetic materials during sexual union between the two parental isolates [10, 12].

Haploid nature of is advantageous in studying segregation of genes involved in expression of disease on cultivars immediately in F_1 generation after recombination and genetic exchange between two parental isolates because recombinant type of F_1 progeny in haploid isolates of fungal culture does not exhibit heterozygous state in gene expression. The segregation pattern on individual finger millet cultivars (Table 1) suggested that pathogenic reactions of F_1 progeny was host specific and the expression of disease symptom varied with the specificity of pathogenic reaction on host. In this study no virulent symptom was detected on GPU26. Avirulent and virulent cultures segregated in 1:1 ratio on K7 and PR202. This suggested monogenic control of pathogenicity in F_1 isolates on finger millet cultivars. Pathogenic reaction on GE5230 was segregated for 3:1 ratio. This led to suggest that two unlinked independent loci were involved in controlling the pathogenicity reaction on the cultivar.

The cultivar specific pathogenicity of F_1 haploid isolates in combined analysis showed 1:1:1:1 ratio between K7 and PR202 (Table 2). This explained that the out come of an apparent infection on K7 and PR202 by a potential pathogen (F_1 isolate) possibly depends on the genotype of plants as well as that of F_1 isolates. F_1 cultures avirulent on K7 segregated for avirulence and virulence on PR202, conversely all virulent cultures on PR202 also segregated for avirulence and virulence on K7. It was inferred that for each gene conditioning avirulence in the pathogen (F_1 isolates) there was a specific gene conditioning resistance in finger millet cultivars [13]. The avirulent reaction of the progeny of cross between finger millet and foxtail millet isolates on K7 and PR202 in the present investigation, seemed to be controlled by AVR_1 and AVR_2 genes and their corresponding virulence loci as avr_1 and avr_2 respectively. Hence, the cultivar specific operation of the avirulance gene AVR_1 could be associated with the presence of resistance gene R_1 in K7 cultivar and that for AVR_2 locus could be attributed to R_2 gene carried by PR202 finger millet cultivar.

A 2:0:1:1 segregation ratio was obtained in combined analysis between K7 and GE5230 suggesting that avirulent gene present in GE5230 segregated on avirulence and virulence loci of K7, contrarily no detectable compatible symptoms appeared against avirulent locus in K7 even by the presence of virulence genes in GE5230. This demonstrated that one of the two genes present in GE5230 was perhaps identical to the one in K7 and the avirulent reaction on GE5230 was probably expressed by AVR_1 and AVR_3 loci. The gene interaction on GE5230 possibly emanated by the presence of both R_1 and R_3 genes corresponding to the AVR_1 and AVR_3 loci in F_1 isolates. Virulent reaction on PR202 and GE5230 segregated in 3:1:3:1 ratio indicating that digenic control of pathogenicity on GE5230 segregated in 3:1 ratio to both avirulent and virulent loci present in PR202 and the genes involved

Table 1. Pathogenicity reaction of F_1 (haploid) blast fungus isolates on individual finger millet cultivars

Cultivar	Number of F_1 progenies			
	Avirulent	Virulent	Total	χ^2
GE5230	195	75	270	3:1
GPU26	270	0	270	-
K7	125	145	270	1:1
PR202	136	134	270	1:1

F_1 isolate with less than virulence index score was considered as avirulant, isolate with higher values were considered virulant

Table 2. Combined analysis of pathogenicity reaction of F_1 blast fungus isolates involving two finger millet cultivars

Cultivar 1	Cultivar 2	A_1A_2	A_1v_2	v_1A_2	v_1v_2	χ^2
K7	PR202	65	60	71	74	1:1:1:1
	GE5230	125	0	70	75	2:0:1:1
PR202	GE5230	106	35	89	40	3:1:3:1

A_1 = avirulent reaction on cultivar 1, v1 = virulent reaction on cultivar 1, A_2 = avirulent reaction on cultivar 2, v_2 = virulent reaction on cultivar 2

in pathogenic reaction seemed to be independent and different between the two cultivars.

Avirulent reaction of F_1 isolates on GPU26 indicated that a complex host specificity of existed towards finger millet cultivars analogous to that observed in rye mildew fungus, which carried avirulence genes corresponding to wheat gene for resistance to wheat mildew fungus [5]. It is inferred that foxtail millet also carried similar avirulence genes corresponding to the resistance gene present in GPU26. Nevertheless, further study is required to observe the range of complex host species specificity of widely adapted pathogen on finger millet and foxtail millet cultivars in central Himalayan region.

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