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Variability studies of rice blast fungus in Konkan region of Maharashtra

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Abstract
Of the 5 RAPD primers used OPD-08 showed 100% polymorphism. All the isolates exhibited over all polymorphism of about 89.29%. The similarity degree value ranged from 0.02 to 0.57. Isolates BLS-5 and BLS-10 were found closely related with high SD value (0.57) followed by BLS-7, BLS-10 (0.53) and BLS-5, BLS-9 (0.52) whereas least similarity was observed between BLS-4 and BLS-12 (0.02) followed by BLS-4, BLS-5 (0.08) and BLS-3, BLS-11 (0.09).

Keywords: RAPD, rice blast, polymorphism, genetic variability

Introduction
Rice (*Oryza sativa* L.) is the most important staple food grain crop of the world which constitutes the principle food for about 60 per cent of the world's population. It contributes 43 per cent of total food grain production and 46 per cent of total cereal production in India. In Maharashtra state, rice is cultivated on an area of 15.13 lakh hectares in four regions *viz.*, Vidharbha (7.95 L ha.), Konkan (3.83 L ha.), Western Maharashtra (3.23 L ha.) and Marathwada (0.12 L ha.) with annual production of 41.71 L tonnes unmilled (brown rice) and 28.78 L tonnes milled rice. Highest productivity was recorded in Konkan region *i.e.* 2.75 tonnes ha milled rice and 3.83 tonnes ha unmilled (brown rice) with total production of 15.26 L tonnes unmilled (brown rice) and 10.53 L tonnes milled rice from 3.83 L ha area (Anonymous, 2015). Rice is a crop of Asian origin. It belongs to family Poaceae with two species, *Oryza sativa* and *O. glaberrima*. *O. sativa* is a native of tropical and subtropical southern Asia while *O. glaberrima* is a native of West Africa. Studies have suggested that there are three races of *Oryza sativa* cultivars *viz.* Indica, Javonica and Japonica. The blast pathogen *Pyricularia grisea* (Cooke) Sacc. is the anamorph of *Magnaporthe grisea* Barr which is an Ascomycetes. It has potential to attack the plant in all stages of growth. The infected host initially manifests the infection in form of typical elliptical to spindle shaped spots with dark reddish brown margin and ashy grey centre. Lesions may enlarge and coalesce, growing together, to kill the entire leaves. The disease primarily occurs on nursery seedlings. In advanced stages, infection to the neck region culminates into neck blast. The fungus forms pyriform (pear shaped), light brown, three celled, discrete macro-conidia on light brown conidiophores. The conidiophores and conidia are pigmented due to melanin (Kato, 2001).

Materials and Methods
All the isolates of *P. grisea* were grown separately in 100 ml flasks containing 30 ml oat meal broth for 10 days at 27 ± 1^0C. The mycelial mat of each isolate was harvested and placed separately on the sterilized Whatman No. 1 filter paper for 30 min to drain out excess medium. The collected mats were stored overnight in a deep freeze (-20 ^{0}c). Later, such dried, frozen mycelial mats were ground separately in desired extraction buffer with pre-cooled mortar and pestle and then total DNA was collected.
The total genomic DNA was extracted by adopting CTAB method (Reader and Baroda, 1985) [6]. The harvested mycelia (25 mg) were freeze-dried, lyophilized and macerated in liquid nitrogen using extraction buffer with the help of pre-cooled mortar and pestle. Such macerated tissue was suspended in extraction buffer in 1.5 ml eppendorf tube and vortexed until evenly suspended. Fifty µl of 10% SDS was added and gently shaken at 37 ^{0}c for 1 hr.

To this 75 µl of 5 M NaCl was added and mixed thoroughly but gently. To the same mixture, 65 µl of CTAB (Cetyl trimethyl ammonium bromide) solution was added and mixed thoroughly. The mixture was incubated at 65^0c for 10-20 min. The mixture was emulsified with equal volume of chloroform iso-amyl alcohol (24:1) for 5 min.

The mixture was centrifuged at 10,000 rpm for 12 min. with cooling centrifuge. DNA was precipitated in 200 µl of cold isopropanol, washed with 70% ethyl alcohol and dried under vacuum. The supernatant in freezer left inside was kept at -20^0C for overnight. The precipitated DNA was resuspended in 10 mg/ml TE buffer and incubated at 37 ^{0}C for 30 min. The DNA concentration was estimated by fluorimeter adjusted to 10 ng ml.

The amplified profiles for all the primers were compared with each other and the bands of DNA fragment were scored as '1' for the presence and '0' for the absence of a band generating the '0', '1' matrices and per cent polymorphism was calculated by using the following formula.

$$\text{Per cent polymorphism} = \frac{\text{No. of polymorphic bands}}{\text{Total number of bands}} \text{ x } 100$$

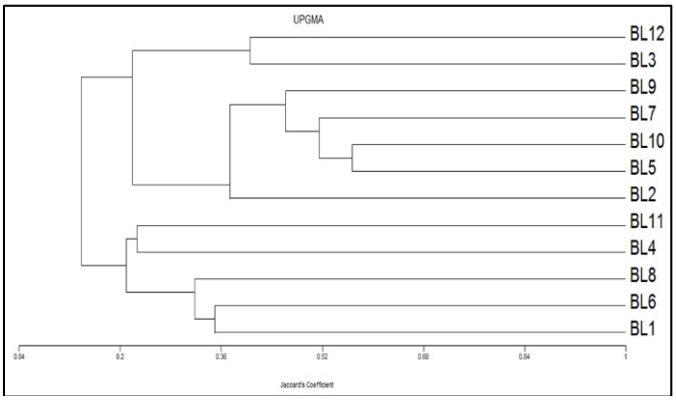
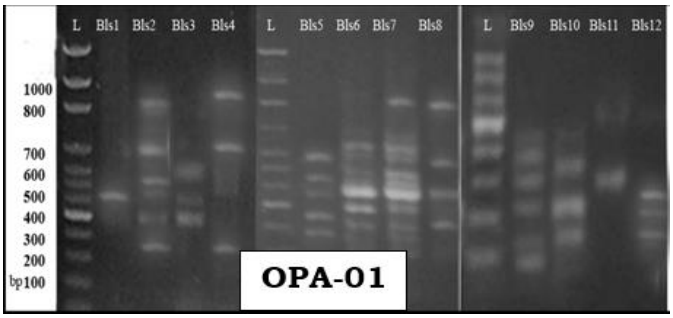
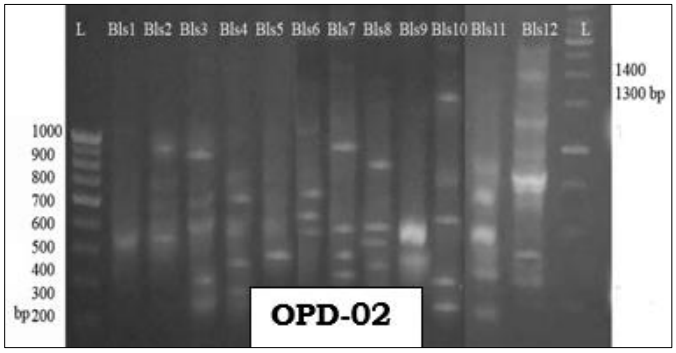


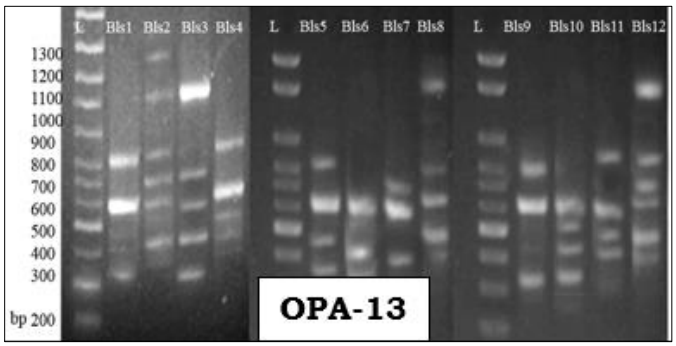
Fig 1: Dendrogram based on RAPD analysis of 12 isolates of *Pyricularia grisea*.

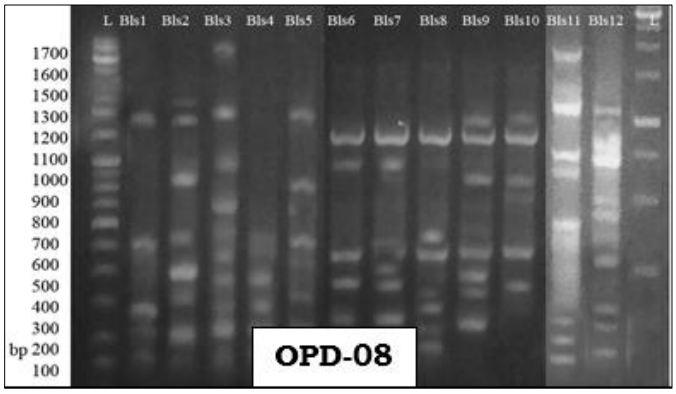
Table 1: Variation in banding profile of *P. grisea* using different RAPD primers.

Sr. No.	Primers	Total Bands	Polymorphic Bands	% Polymorphism
1.	OPA-01	10	8	80.00
2.	OPA-13	13	12	92.31
3.	OPD-02	15	13	86.67
4.	OPD-08	16	16	100.00
5.	OPD-20	16	14	87.50









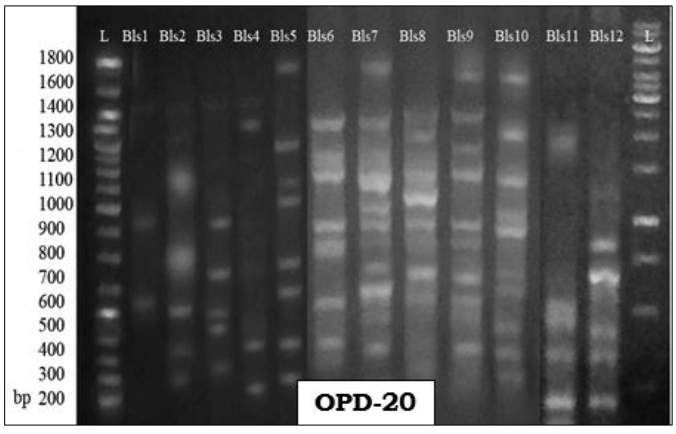


Plate I: Banding profile of twelve *P. grisea* isolates based on RAPD markers.

Result and Discussion

Of the five primers used for amplification OPD-08 showed hundred per cent polymorphism followed by OPA-13 (92.31%), OPD-20 (87.50%), OPD-02 (86.67%) and OPA-01

(80.00%). Since out of a total of 70 bands, 63 polymorphic bands were obtained, the isolates exhibited over all polymorphism of about 89.29%. Above results are in confirmity with those of Srivastava *et al.* (2014) [1] who reported that, in RAPD profile, minimum and maximum per cent similarities among the isolates were in the range of 0.35 to 0.80 degree. Vanaraj *et al.* (2013) [2] observed 52.9% similarity among the leaf and neck blast isolates of Tirunelveli region, while isolates from Coimbatore and Gudalore region exhibited 32.7% similarity.

A dendrogram was generated based on SM (Similarity Co-efficient). The SD (Similarity Degree) value for the isolates ranged from 0.02 to 0.57 (Table 2). The isolates BLS-5 and BLS-10 were closely related with high SD value (0.57) followed by BLS-7 and BLS-10 (0.53) and BLS-5 and BLS-9 (0.52), whereas least similarity was observed between BLS-4 and BLS-12 (0.02) followed by BLS-4 and BLS-5 (0.08) and BLS-3 and BLS-11 (0.09).

Based on the SD value higher than 0.01, the twelve isolates were divided into two major clusters A and B. The cluster A was divided into A1 and A2. The sub group A1 was divided into A3 and A4. The sub group A3 was comprised of BLS-1 and BLS-6 isolates while the A4 comprised of isolate BLS-8. The sub group A2 was comprised of BLS-4 and BLS-11 isolates. The cluster B was further divided into two sub clusters B1 and B2. The cluster B1 was divided into sub group B3 and B4. The sub group B3 was comprised of BLS-2 isolate. Sub group B4 was further divided into B5 and B6. The sub group B6 was comprised of BLS-9 isolate. The B5 was divided into B7 and B8 sub groups, while B8 sub group comprised of BLS-7 isolate. Isolates BLS-5 and BLS-10 were included in B7 sub group. The sub cluster B2 was comprised of BLS-3 and BLS-12 isolates. The genetic relatedness and probable mechanisms of genetic variation among the Indian isolates of rice blast pathogen by using 171 polymorphic markers were scored using 33 selected random decamer primers (Chadha and Krishna, 2005) [3]. It was concluded that isolates exhibited about 64 per cent polymorphism and similarity degree value ranged from 0.76 to 0.92.

Table 2: Similarity co-efficient of *Pyricularia grisea* isolates based on RAPD analysis.

Isolates	BLS-1	BLS-2	BLS-3	BLS-4	BLS-5	BLS-6	BLS-7	BLS-8	BLS-9	BLS-10	BLS-11	BLS-12
BLS-1	1.00											
BLS-2	0.13	1.00										
BLS-3	0.12	0.28	1.00									
BLS-4	0.17	0.14	0.11	1.00								
BLS-5	0.10	0.38	0.23	0.08	1.00							
BLS-6	0.35	0.08	0.11	0.19	0.18	1.00						
BLS-7	0.15	0.44	0.20	0.16	0.50	0.13	1.00					
BLS-8	0.35	0.12	0.11	0.29	0.12	0.29	0.27	1.00				
BLS-9	0.13	0.33	0.28	0.18	0.52	0.14	0.37	0.14	1.00			
BLS-10	0.13	0.33	0.28	0.18	0.57	0.14	0.53	0.14	0.50	1.00		
BLS-11	0.16	0.17	0.09	0.23	0.21	0.29	0.15	0.17	0.17	0.13	1.00	
BLS-12	0.16	0.25	0.41	0.02	0.23	0.14	0.17	0.22	0.11	0.16	0.12	1.00

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