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Random amplified polymorphic DNA (RAPD) polymorphism in *Puccinia sorghi* Schw. causing common rust of maize

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An experiment was conducted at University of Agricultural Sciences, Dharwad, Karnataka to detect molecular variation in *Puccinia sorghi* Schw. through molecular tool *viz*., random amplified Polymorphic DNA (RAPD). In this study, molecular variation in the pathogen served as a guideline for breeding suitable maize varieties against common rust disease which seriously affects maize productivity. It was reported that **losses in total yield ranged from 0 in the more resistant entries to nearly 50% in more susceptible entries. Losses in total yield in late-planted sweet corn were 18, 26, and 49% for cv. Sugarloaf (most resistant), cv. Jubilee (intermediate) and cv. Style Pak (most susceptible), respectively. Results revealed that a variety of polymorphic bands were found from the specimens by polymerase chain reaction (PCR) amplification using 10 primers: OPA-19, OPB-08, OPB-15, OPB-17, OPC-08, OPD-07, OPD-13, OPD-18, OPF-02 and OPG-05. These bands were used to construct unweighted pair group method with arithmetic mean (UPGMA) dendogram, which cluster analysis divided the 15 specimens into major 2 groups: Groups A and B. Out of 10 primers used, 3 primers *viz*., OPA-19 (11.11%), OPB-17 (20%) and OPF-2 (14%) showed polymorphism. The similarity coefficient values revealed that, the least similarity (81%) was between Tamil Nadu-Andhra Pradesh, Haryana-Tamil Nadu and Madhya Pradesh-Haveri isolates. The maximum similarity (100%) was found between Andhra Pradesh-Haryana and Belgaum-Jammu and Kashmir isolates. Bihar isolate formed entirely separate cluster within Group A, whereas Tamil Nadu-Maharashtra and Bijapur-Haveri isolates formed separate clusters within Group A. In Group B, Sikkim, Uttar Pradesh, West Bengal and Bengaluru isolates formed entirely separate clusters, respectively.**

Key words: Genetic variability, *Zea mays,* common rust, *Puccinia sorghi*, random amplified polymorphic DNA (RAPD).

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops that expand its adaptation from very high north and south latitudes in temperate areas, through subtropical and tropical environments in north and south of the equator. It is the potential crop of Karnataka, which has come up on large areas in different districts both under assured rainfed areas and in the irrigated command areas of Ghataprabha, Malaprabha, Tungabhadra, Bhadra and Upper Krishna. The maximum acreage and production of maize is in Uttar Pradesh and highest

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acreage yield per hectare is in Andhra Pradesh (3182 kg) followed by Punjab (2574 kg). The average maize yield in India is 1785 kg/ha. The Karnataka State has maximum area of 12.37 lakh ha with production of 30.07 lakh tones and productivity 2540 kg/ ha (Anonymous, 2010). In India, it is grown over an area of 6.5 million ha with total production of about 11.5 metric tons. In Karnataka, about 6% land is under maize production with 12% share in India's production. The main season for growing maize is *kharif*, covering an area to the extent of 86%, out of which 60% comes under irrigation and about 90% area is covered by hybrids. An anticipated increase in harvested area and yields is expected to lift world maize production in 2011/12 by 33 m tons to 858 m. The forecast is 15 m tons above the 6 June GMR Update, mainly reflecting the adoption of official 2010 production data for China, but also larger US plantings and improved Black Sea crop prospects. Global consumption, also revised up to take into account the new figures for China, is expected to remain firm, with feed, industrial and food demand forecast to be higher than last year, especially in developing countries (Anonymous, 2011).

The crop is grown in an area of 140 million ha. Among the cereals, maize is wide spread crop next to wheat and rice in the world and ranks fourth after rice, wheat and sorghum. As many as 18 foliar diseases are reported to occur in India, but common rust of maize caused by *Puccinia sorghi* Schw. is considered to be a major disease. Common rust of maize appears in severe form in several areas of the country, resulting in grain yield reductions by 32.18% (Sharma et al., 1982) and up to 60.53% yield loss in susceptible cultivar CM- 202 (Dey et al., 2012). The disease appears from the early stages of crop growth and causes long lasting effect on the crop growth and yield potential of the crop if proper management is not adopted in time. In India, the disease has become a potential threat to maize cultivation.

Since there is no effective chemical control of common rust of maize disease, the development of resistant cultivars has become the chief aim of disease control programs. In spite of great efforts, resistant cultivars, especially in maize culture, have on the average, a useful life restricted to 2 or 3 years owing to the introduction of new pathogen strains which disrupt the acquired specific resistance (Correa-Victoria and Zeigler, 1993). Due to the high virulence of *P. sorghi* Schw. in maize field populations, genetic variability of this fungus has been accounted as one of the chief causes of disruption in cultivar resistance (Bruno and Urashima, 2001; Mehta and Baier, 1998; Urashima et al., 1993). Virulence diversity of *P. sorghi* Schw. in maize has been mainly attributed to parasexual recombination and mutation processes.

Morphologically, it is difficult to distinguish between various isolates of *P. sorghi* Schw. (common rust). Therefore, advance identification techniques are necessary to develop in order to distinguish both corn rust fungi species. The advantage of the molecular phylogenetic analysis methods have been preceded successful application on the rust fungi. The authors have studied the variation of nucleotide sequence in DNA of rust, fungi although they are obligate parasites that are difficult to culture and maintain on synthetic medium. The molecular phylogenetic analysis is now possible performed by Bruns et al. (1990), and Lee and Taylor (1990) who extracted DNA from a single spore of dry herbarium specimens and amplified target DNA by polymerase chain reaction (PCR). Virtudazo et al. (2001) modified DNA extraction methods from Suyama et al. (1996) and extracted genomic DNA from spores obtained from single uredium or telium and then amplified target DNA by PCR (Pfunder et al., 2001; Roy et al., 1998; Zambino and Szobo, 1993; Weber et al., 2003; Vogler and Bruns, 1998).

Research work with respect to the molecular variation of this pathogen in India is limited and needs further investigation. Majority of cultivars show similar type of disease reaction and presence of pathogenic variation in *P. sorghi* Schw. is not clear. Molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) marker have been successfully used as tools for studying the phylogenetic relationships and diversity of rust fungi (Villareal et al., 2002; Hovmoller et al., 2002; Becerra et al., 2007; Manuela et al., 2005; Dracatos et al., 2006; Yu et al., 2006). RAPD marker is a simple and rapid technique. Only small amounts of DNA template are required and the results are clearly scorable demonstrated. Therefore, in the present investigation a thought was given to detect molecular variation in the pathogen through molecular tools such as RAPD.

MATERIALS AND METHODS

Maize leaf samples showing typical symptoms of rust (*P. sorghi* Schw.) were collected from 15 different locations *viz.*, Tamil Nadu (TLN), Maharashtra (MAH), Sikkim (SIK), Andhra Pradesh (ANP), West Bengal (WBN), Bihar (BIH), Madhya Pradesh (MDP), Uttar Pradesh (UTP), Haryana (HAR), Jammu & Kashmir (JMK), Haveri (HAV), Bijapur (BIJ), Belgaum (BEL), Bengaloru (BEN) and Dharwad (DWD) during *Kharif* 2009-2010. Morphological study on the characteristics of rust pustules on the infected leaves and stems demonstrated the diversity on the symptomatology including colors, shapes and the distribution of the pustules (Figure 1). The shapes of uredium on leaf and stalk were circular to elongate. The urediospores were mostly ovoid-oblong with cinnamon-brown color. Morphological-based identification determined that all of the rust specimens were *P. sorghi*. (Figure 2).Two hundred and fifty gram (250 g) of leaf sample was collected from each location/cultivar. The samples were placed in polythene bag with appropriate labeling. They were preserved in deep freezer at -20°C and used for further studies.

DNA isolation

The DNA was isolated according to the method given by Lorys et al. (2002). 20 mg of uredospores were placed in 2.2 ml centrifuge

Figure 1. Symptom diversity of common rust incited by *P. sorghi* on corn leaves.

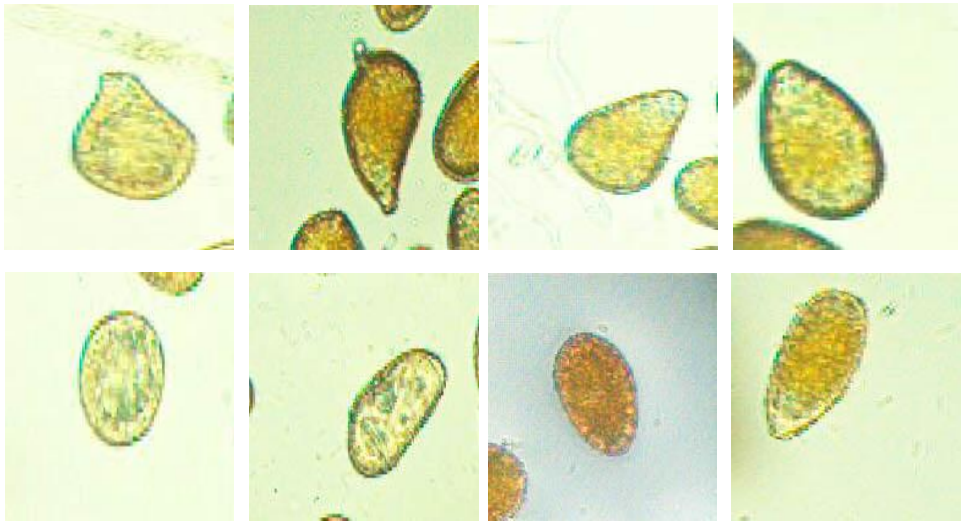

Figure 2. Morphological characters of urediospore of *P. sorghi*.

tube with 7 autoclaved glass beads (diameter 3.5 mm) with 200 µl of extraction buffer and vortexed for 5 min. The vortexed product was transferred to fresh 2.2 ml centrifuge tube. 300 µl of extraction buffer was again added. Crushing efficiency was observed under microscope at this stage. 5 µl of proteinase K was added and kept for incubation at 65° C for 90 min. A gentle inversion was done at every 10 min interval. An equal volume of Chloroform:Phenol:Isoamyl alcohol was added (500 µl) and gently inverted for 5 min and centrifuged at 16000 rpm for 20 min at 4°C. The supernatant was transferred to a new 2.2 ml tube. An equal volume of cold isopropanol was added (500 µl) and gently inverted and kept for incubation at -20°C for 1 h. The mixture was then centrifuged at 16000 rpm for 20 min at 4°C. The pellet was washed with 200 µl of 70% ethanol with a short spin of 13000 rpm. The ethanol was poured and the tube was kept for air drying till ethanol smell vanished. The dried pellet was dissolved in 40 µl of TE buffer. The solution was then treated with 1 µl of RNase and incubated for 1 h at 37°C. The DNA was quantified on agarose gel and stored under -20°C for further use.

PCR conditions

The isolated DNA was kept for PCR. Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing the following components: the total volume of each reaction mixture was 20 µl. The cocktail for the PCR amplification was found to be optimum: template DNA (25 ng/µl) 1.0 µl, 10x assay buffer with 15

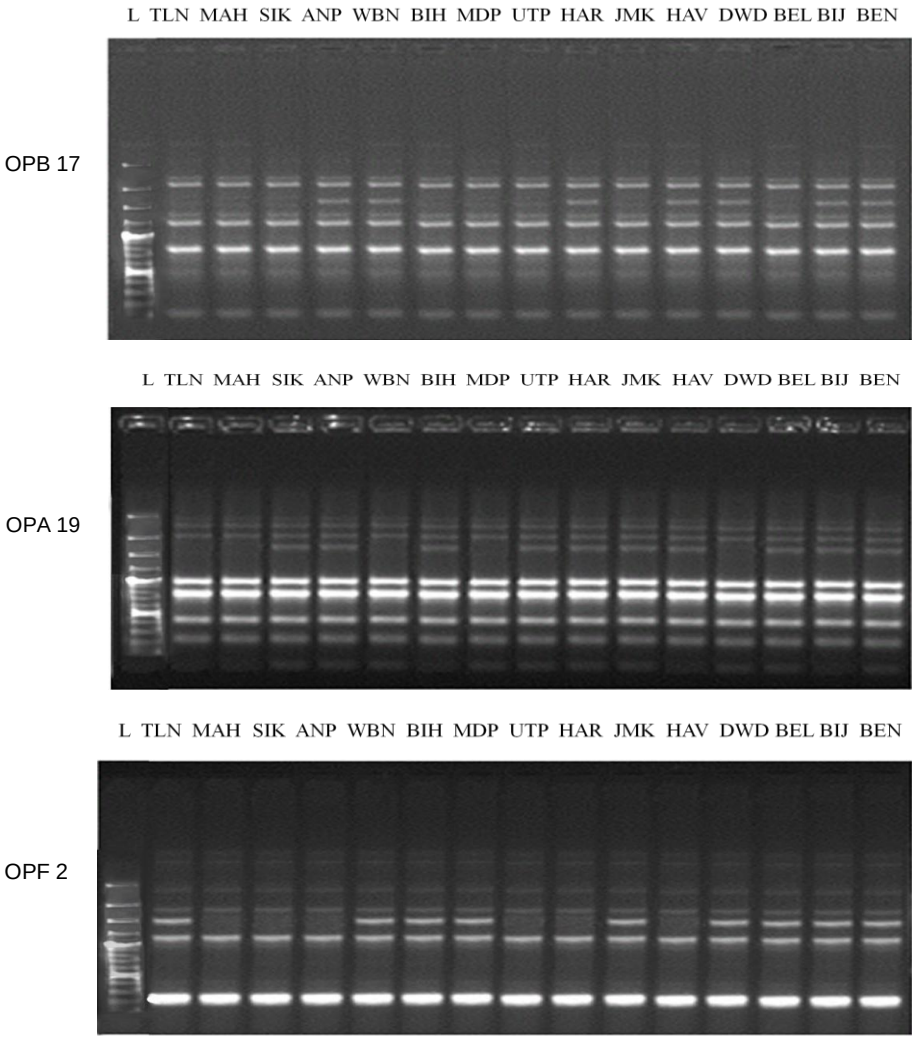

Figure 3. Genetic variability of isolates of *P. sorghi* from different locations.

mM $MgCl_2$ 2.00 µl, dNTP's mix (2.5 mM) each 2.00 µl, primer (5 PM/µl) 2.00 µl, Taq DNA polymerase (3 units/µl) 0.3 µl and sterile distilled water 12.7 µl. Except template the master mix was distributed to PCR tubes (19 µl/tube) and later 1 µl of template DNA from the respective isolates was added making the final volume of 20 µl. The conditions followed for PCR are as follows: initial denaturation was given at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension of 72°C for 2 min. The final extension was given at 72°C for 10 min. The amplified product was run on 0.8% agarose gel and documented.

Ten (10) random primers were used for the present investigation. OPA-19 (5'-CAAACGTCGG-3'), OPB-08 (5'-GTCCACACGG-3'), OPB-15 (5'-GGAGGGTGGT-3'), OPB-17 (5'-AGGGAACGAG-3'), OPC-08 (5'-TGGACCGGTG-3'), OPD-07 (5'-TTGGCACGGG-3'), OPD-13 (5'-GGGGTGACGA-3'), OPD-18 (5'-GAGAGCCAAC-3'), OPF-02 (5'-GAGGATCCCT-3') and OPG-05 (5'-CTGAGACGGA-3')

RESULTS AND DISCUSSION

It is difficult to distinguish these species using traditional morphological differences. The suitability of RAPD was used to detect the variations among the isolates of *P. sorghi* Schw. OPA, OPB, OPC, OPD, OPG and OPF series primers were used to determine genetic distance between isolates and to construct a dendrogram.

Out of 10 primers used, 3 primers *viz*., OPA-19 (11.11%), OPB-17 (20%) and OPF-2 (14%) showed polymorphism information on banding pattern for all the primers was used to determine genetic distance between isolates (Figure 3) and to construct a dendrogram. The similarity coefficient values were calculated based on 'presence' and 'absence' of the bands. Pair wise genetic similarities between the different isolates of *P. sorghi* were estimated by Dice similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group method with arithmetic mean (UPGMA) using sequential agglomerative hierarchal nested (SAHN) cluster analysis of NTSYS-PC programme version 2.02 (Exeter Software, New York, USA described by Rohlf (1998).

RAPD data distinguished the 15 isolates into major

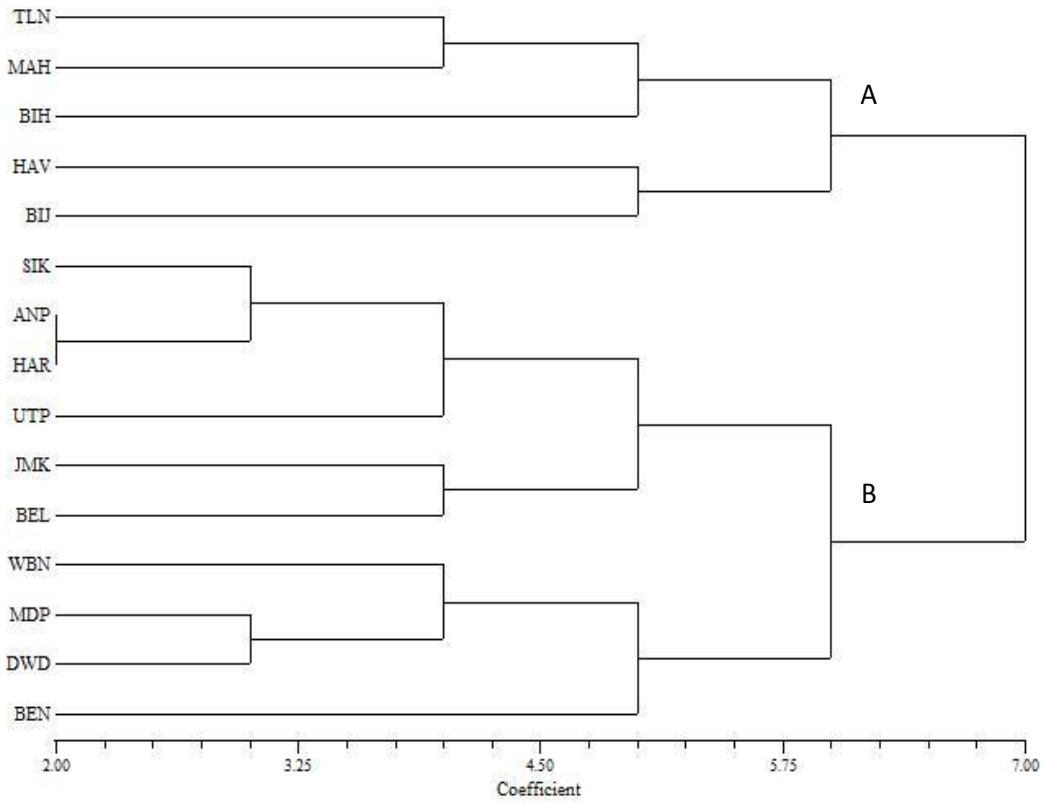

Figure 4. Phenetic dendrogram obtained from 15 isolates of *P. sorghi* with UPGMA, based on the binary matrix of polymorphic bands, using the UPGMA algorithm and Dice's similarity coefficient (NTSYS program). Isolates are indicated at the terminal branches. The line below the dendrogram represents the similarity index.

Clusters A and B (Figure 4). The similarity coefficient values revealed that, the least similarity (81%) was among Tamil Nadu-Andhra Pradesh, Haryana-Tamil Nadu and Madhya Pradesh-Haveri isolates. The maximum similarity (100%) was found among Andhra Pradesh-Haryana, Belgaum-Jammu and Kashmir isolates. The dendrogram cluster analysis (Figure 4) revealed two major groups *viz*., A and B. Tamil Nadu, Maharashtra, Bihar, Haveri and Bijapur isolates formed Group A, while remaining isolates formed Group B. Several sub groups and sub-sub groups were found within these groups.

Bihar isolate formed entirely separate cluster within Group A, whereas Tamil Nadu-Maharashtra and Bijapur-Haveri isolates formed separate clusters within Group A. In Group B, Sikkim, Uttar Pradesh, West Bengal and Bengaluru isolates formed entirely separate clusters, respectively; whereas Andhra Pradesh-Haryana, Jammu and Kashmir-Belgaum and Madhya Pradesh-Dharwad isolates formed separate clusters. This shows that geographical dissimilarities have no effect on the genetic makeup of the pathogen revealing little genetic variability. So the results obtained from the cluster analysis revealed that sub-cluster groups composed of isolates belonging to same geographical locations with some variability. Hence, molecular variability parameters are more meaningful and perfect when compared to morphological characters. Hence, the molecular variability expressed through RAPD-DNA markers does not exhibit much variation, since out of 15 primers, only 3 primers showed 100% polymorphism.

In the present study, rust samples from three different geographical regions in India were collected. The dendrogram study revealed that the geographic origin of strains does not play crucial role in lineage formation, as in each lineage (group), there were mixed populations of the three geographical regions. Similar results have been shown by Ngueko et al. (2004) in their study on isolates of *Magnaporthe grisea* from different nurseries of Hunan province in China. The phylogenetic grouping based on our RAPD data did not appear to be harmonious with geographical locations. The topology of the dendrogram suggests that most isolates are about 25 to 40% different from each other, indicating that both local and geographical polymorphisms exist. Genetic mechanisms that could explain such diversity include simple mutations, meiotic recombination and mitotic (para sexual) recombination (Yamasaki and Niizeki, 1965; Zeigler, 1998). On the basis of the present study, it is concluded that the Indian population of common rust

fungus may be genetically heterogeneous and the interrelationships amongst the different isolates can be easily, precisely and reliably explained by RAPD-PCR technology.

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REFERENCES

Anonymous (2010). 54th Annual Progress Report, All India Co-ordinated Maize Research Project, Directorate of Maize Research, New Delhi. p. 15.

Anonymous (2011). International Grains Council, Grain Market Report. p. 2.

Becerra V, Paredes M, Madariaga R, Bariana HS, Mellado M and Rojo C (2007). High Genetic Diversity in Chilean Population of Wheat Yellow Rust (*Puccinia striiformis* f.sp. *tritici* West.) Assessed by RAPD and AFLP. Australian J. Agric. Res. 58(6):525-531.

Bruno AC, Urashima AS (2001). Inter-relação sexual de *Magnaporthe grisea* do trigo e de outros hospedeiros. Fitopatol Bras. 26:21-26.

Bruns TD, Fogel R, Taylor JW (1990). Amplification and Sequencing of DNA from Fungal Herbarium Specimens. Mycologia 82:175-184.

Correa-Victoria FJ, Zeigler RS (1993). Pathogenic variability in *Pyricularia grisea* at a rice blast "hot spot" site. Plant Dis. 77:1029-1034.

Dey Utpal, Harlapur S I, Dhutraj DN, Suryawanshi AP, Badgujar SL, Jagtap GP, Kuldhar DP (2012). Spatiotemporal yield loss assessment in corn due to common rust caused by *Puccinia sorghi* Schw. Afr. J. Agric. Res. 7(37):5265-5269.

Dracatos PM, Dumsday JL, Olle RS, Cogan NOI, Dobrowolski MP, Fujimori M, Roderick H, Stewart AV, Smith KF, Forster JW (2006). Development and Characterization of EST-SSR Markers for the Crown Rust Pathogen of Ryegrass (*Puccinia coronate* f.sp. *lolii*). Genome 49:572-583.

Hovmoller MS, Justesen AF and Brown JKM (2002). Clonality and Long-Distance Migration of *Puccinia striiformis* f.sp. *tritici* in North-West Europe. Plant Pathol. 51: 24-32.

Lorys MM, Villareal A, Christian Lannou, Claude de Vallavieille-Pope, Claire Neema (2002). Genetic variability in *Puccinia striiformis* f. sp. *tritici* populations Sampled on a local scale during Natural Epidemics, Appl. Environ. Microbiol. 68(12):6138-6145.

Lee SB, TaylorJW (1990). Isolation of DNA from fungal mycelia and single spores.Pp. 282-287 in M. A. INNIS, D. H. GELFAND, J. J. SNINSKY, and T. J. WHITE. PCR protocols: a guide to methods and applications. Academic Press, San Diego.

Manuela M, Gouveia C, Ribeiro A, Varzea VMP, Rodrigues CJ (2005). Genetic diversity in *Hemileia vastatrix* based on RAPD markers", Mycologia 97(2):396-404.

Mehta YR, Baier A (1998). Variação patogênica entre isolados de *Magnaporthe grisea* atacando triticale e trigo no Estado do Paraná. Summa Phytopathologica 24:119-125.

Ngueko RB, Shen Y, Wang H-K, Lin FC, Xu T (2004). Genetic diversity analysis of *Magnaporthe grisea* from some blast nurseries of Hunan province using Random Amplified Polymorphic DNA.

Pfunder M, Schurch S, Roy BA (2001). Sequence Variation and Geographic Distribution of Pseudoflower-Forming Rust Fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol. Res. 105:57-66.

Rohlf FJ (1998). NTSYS-PC *Numerical Taxonomy and Multivariate Analysis* version 2. 0 Applied Biostatistics, Inc., New York.

Roy BA, Vogler DR, Bruns TD, Szaro TM (1998). Cryptic Species in the *Puccinia monoica* Complex. Mycologia 90:846-853.

Sharma RC, Payak MM, Shankerlingam S, Laxminarayana C (1982). A comparision of two methods of estimating yield losses in maize caused by common rust. Indian Phytopathol. 35:18-20.

Suyama Y, Kawamuro K, Kinoshita I, Yoshimura K, Tsumura Y, Takahara H (1996). *DNA sequence from a fossil pollen of Abies spp. from Pleistocene peat. Genes Genetic Syst. 71:145-149.*

Urashima AS, Igarashi S, Kato H (1993). Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Dis. 77:1211-1216.

Villareal LMA, Lannou C, de Vallvielle C, Neema C (2002). Genetic Variability in *Puccinia striiformis* f.sp. *tritici* Population Sampled on a Local Scale During Natural Epidemics. Appl. Environ. Microbiol. 68(12):6138-6145.

Virtudazo EV, Nakamura H, Kakishima M (2001). Phylogenetic Analysis of Sugarcane Rusts Based on Sequences of ITS, 5.8S rDNA and D1/D2 Regions of LSU rDNA. J. Gen. Plant. Pathol. 67:28-36.

Vogler DR. and Bruns TD (1998). Phylogenetic Relationships among the Pine Stem Rust Fungi (*Cronartium* and *Peridermium* spp.). Mycologia 90:244-257.

Weber RWS, Webster J. Engel G (2003). Phylogenetic Analysis of *Puccinia distinct* and *P. lagenophorae,* Two Closely Related Rust Fungi Causing Epidemics on Asteraceae in Europe. Mycol. Res. 107:15-24.

Yamasaki Y, Niizeki H (1965). Studies on variation of the rice blast fungus *Pyricularia oryzae* Cav. I. Karyological and genetical studies on variation. Bull. Natl. Inst. Agric. Sci. 13:231-274.

Yu ZD, Zang XY and Cao ZM (2006). ISSR Marker and ITS Sequence Study of *Melampsora larici-populina*. Agric. Sci. China 5(11):847-854.

Zambino PJ, Szobo LJ (1993). Phylogenetic Relationships of Selected Cereal and Grass Rusts Based on rDNA Sequence Analysis. Mycologia 85:401-414.

Zeigler RS (1998). Recombination in *Magnaporthe grisea.* Ann. Rev. Phytopathol. 36:249-276.