Morphological characteristics, race distribution, and virulence gene analysis of *Pyricularia oryzae* isolates (teleomorph: *Magnaporthe oryzae*)

G Kurrata\(^1\), T Kuswinanti\(^2\), M N Y Izha\(^3\), A Gassa\(^1\) and Melina\(^1\)

\(^1\) Student of Postgraduate School, Hasanuddin University of Makassar, Indonesia
\(^2\) Department of Plant Pest and Diseases, Agriculture Faculty, Hasanuddin University of Makassar, Indonesia
\(^3\) Graduated Student of Postgraduate School, Hasanuddin University of Makassar, Indonesia

E-mail: koeswinanti@yahoo.com

**Abstract.** Rice blast disease caused by fungus *Pyricularia oryzae* (Teleomorph: *Magnaporthe oryzae*) has caused significant losses of rice yields in many world's rice-producing countries. The planting of resistant varieties was initially effective in suppressing blast disease, however, in many cases, as the time goes by, the resistance becomes ineffective due to the emergence of a new race. Study on the distribution of race and genetic diversity of *P. oryzae* isolates can be useful in the development of resistant varieties. The aim of this research was to determine the distribution of *P. oryzae* races and genetic variations of the isolates collected from Pinrang District. Race testing was based on the reaction of a set of an Indonesian differential variety. The severity of blast disease was observed seven days after inoculation using the *Standard Evaluation System for Rice* (IRRI). The pathogen’s genetic diversity was determined using specific primers, coding the fungus virulence genes *Pwl2*, *Erg2* and *Cut1*. The banding patterns of DNA amplification product contained in each primer were scored with a value of 1 (there is a DNA band) and 0 (no DNA bands). A total of 22 isolates of *P. oryzae* obtained from five sub-districts in Pinrang belonged to 10 different races. Race 001 is the dominant race identified in 4 isolate source sites. Molecular analysis of the 10 isolates of *P. oryzae* with different races was found haplotype F-110 (8 isolates) and G-100 (2 isolates).

1. **Introduction**

Asia is the largest rice supplier with the highest consumption of rice in the world. The rate of global rice production has not been able to meet the growing demand of the world population every year [1]. The level of world rice production is strongly influenced by pest and disease factors. *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*), is a pathogen causing rice blast disease that can significantly reduce rice yields in many world's rice plantations [2]. Blast disease control was initially effective by using resistant rice varieties. However, resistant varieties are only effective in the short term due to the emergence of a new race of *P. oryzae* [3]. The emergence of a new race of *P. oryzae* as a result of the continuous genetic changes that pathogens undergo due to various environmental pressures [4]. According to Taheri and Irannejad [5] variation in pathotype and structure of this
pathogen population is strongly influenced by the geographical conditions of a region.

Research on the diversity and distribution of *P. oryzae* races in 2007-2008 from several regions in Indonesia, Bali (Tabanan), Central Kalimantan (Dadahup), West Java (Kuningan), Lampung and South Sumatra (Kayu Agung) were found as many as 18 races namely race 001, 021, 040, 041, 051, 061, 071, 073, 100, 101, 121, 201, 203, 241, 301, 333, 341, 343 [6]. Race variation in a region always changes both diversity and spread. Races 001, 003, 033, and 173 are the races most commonly found every growing season [7]. In order to race to test of isolates of *P. oryzae* at a site in Indonesia, then used a set of differential rice varieties namely Asahan, Cisokan, IR 64, Krueng Aceh, Cisadane, Cisanggarung and Kencana Bali. A total of 64 races have been identified in several regions in Indonesia [8]. Soubabere et al. [9] has identified the *Magnaporthe grisea* currently known as *M. oryzae* (anamorph: *P. oryzae*) using molecular markers as a coder of virulence genes, the Sequence Characterized Amplified Region (SCAR) marker. The SCAR marker simply amplifies the DNA bands that are the target size during the Polymerase Chain Reaction (PCR) process and only produces one DNA band (single band) in the electrophoresis process using agarose gel.

A total of 3 out of 16 SCAR markers developed by Soubabere et al. [9] have been widely used in Indonesia to determine genetic variation or haplotype of *P. oryzae* isolates. (1) *Cut1* is a coder of cutinase enzyme functioning in the degradation process of plant cuticle layers [10], (2) *Erg2* is a gene coding functioning in encoding secondary metabolites in antifungal target fungi on plant cells [11], (3) *Pwl2* is an avirulent gene and is host specific [12]. SCAR markers are widely used to observe changes in population structure in *P. oryzae* [9].

The results of the Reflinur et al., [13] study to determine genetic diversity of 230 isolates of *P. oryzae* (collection of Molecular Biology Laboratory of Indonesian Biotechnology and Genetic Resources Research Center) originated from Bogor, Sukabumi, North Sumatra, Lampung and West Sumatra using markers SCAR (*Cut1*, *Erg2* and *Pwl2*). Produced eight haplotypes, ie A-000, B-001, C-011, D-111, E-010, F-110, G-100, and H-101 with the highest frequency being haplotype D-111 (61.3%) and H-101 (16.1%). Similarly, Rianingsih research [14] to find out the distribution of race and genetic diversity of isolate *P. oryzae* originating from several districts in South Sulawesi (Maros, Bone and Gowa). A total of 20 isolates tested were found in 12 races, in which race 020 was the dominant race found at the three sites. Molecular detection of 10 isolates of *P. oryzae* using specific primers of gene encoding virulence (*Cut1*, *Erg2* and *Pwl2*) obtained 3 haplotypes namely C-011, E-010, and F-110. The predominant C-011 haplotype was found in 8 isolates.

Information on the distribution of race and analysis of genetic diversity of *P. oryzae* fungus in Pinrang District which is one of the major rice producing areas in South Sulawesi is unknown so the characterization of genetic diversity and test pathotype (race) isolates of *P. oryzae* fungus at the site needs to be done for the development of information in the assembly of resistant varieties. This study aims to determine the diversity and distribution of *P. oryzae* races in Pinrang District and characterize the specificity of *P. oryzae* isolates against specific loci related to virulence genes.

2. Methods
Physiologic race testing was conducted at the Laboratory of the class I, Agricultural Quarantine Station of Pare-pare. Polymerase Chain Reaction (PCR) test was conducted at the Laboratory of Microbiology of Hasanuddin University Makassar Hospital. A total of 22 isolates of *P. oryzae* used were private collections isolated from five sub-districts in Pinrang District.

2.1. Race testing of *Pyricularia oryzae* isolates
Race testing of *P. oryzae* follows the method of BB Rice Sukamandi with modification by Prabawa et al., [15], using a set of Indonesian differential varieties consisting of Asahan, Cisokan, IR 64, Krueng Aceh, Cisadane, Cisanggarung and Kencana Bali varieties. Preparation of planting medium includes a mixture of soil and manure (10:1) [16]. Preparation of spore suspension follows the Trisnaningsih and Nasution methods [17] with slightly modified. Each isolate of *P. oryzae* was
replicated on PDA medium (Potato Dextrose Agar) for 5-7 days, then transferred to OMA medium (Oatmeal Agar) for 12 days. Then the surface of the fungal colony is rubbed using a brush image number 10 by first adding 10 ml of sterile aquades containing 0.02% Tween 20, Spore density used for 1 x 10^6 spores/ml. The process of inoculation (spraying) was done when the plants are 18-21 days after planting. Observations were made 7 days after inoculation based on observation scale of a standard evaluation system for rice [18]. Grouping of Pyricularia races was done based on Mogi et al., [19].

2.2. Analysis of Pyricularia oryzae virulence genes

The isolation of genomic DNA isolates of P. oryzae was carried out from the culture of fungal mycelium. DNA extraction followed the gSYNC™ DNA Extraction Kit procedure. DNA pellet was eluted into 100 μl of elution buffer and centrifuged by 14-16,000x g for 30 seconds. The genetic diversity (haplotypes) of P. oryzae isolates were identified using SCAR markers (Erg2, Pwl2 and Cut1), specific markers that encoding virulence genes [9]. Observations were made on the pattern of amplified DNA bands of 1440 bp for the Erg2 gene, 800-900 bp for the Pwl2 gene, and 800-1730 bp for the Cut1 gene. PCR reactions were performed in a total volume of 25 mL containing 5 ng of DNA, 1 pmol/µl forward and reverse primer, 12.5 ml enzyme Taq® Go Green Master Mix, and 5.5 ml H₂O. The PCR program begins with an initial denaturation at 94ºC for 2 minutes. The amplification process is 35 cycles, ie 94ºC for 30 seconds, 54ºC for 30 seconds, 72ºC for 1 minute and last cycle 72ºC for 5 minutes. The electrophoresis of the PCR product was performed on a 1.5% (w / v) agarose gel for 30 minutes to amplify amplicons each primer according to the target size. The amplified DNA bands appear for each primary of each isolate with a value of 1 (there is a DNA band) and 0 (no DNA bands). Haplotype groupings were based on a combination of the three types of genes (Erg2, Pwl2 and Cut1) on the tested isolates.

3. Results and Discussion

3.1. Races grouping of Pyricularia oryzae isolates

Symptoms on inoculated resistant differential rice varieties are limited brown dots and do not develop significantly (figure 1a). In susceptible differential varieties in other hands, the spots are marked with a white to gray sporulation center with brown and tapered borders (figure 1b). Spots on susceptible varieties will coalesce and cover the entire leaf surface (figure 1c). In humid conditions, lesions can enlarge and coalesce, growing together, and kill the entire leaves (figure 1d). According to TeBeest et al., [20], initial symptoms appear as white to gray-green lesions or spots, with dark green borders. Older lesions on the leaves are elliptical or spindle-shaped and whitish to gray centers with red to the brownish or necrotic border. Leaf spots may vary depending on environmental conditions, host susceptibility and age of the plant. In susceptible plants, the center of the gray spots surrounded by dark green to brown then develops rapidly to cover the entire surface of the leaf. Spots appear on resistant plants are brown to dark brown in color.
Figure 1. Symptoms on inoculated rice varieties with different resistance level of P. oryzae. Symptom on resistant variety (a), on susceptible variety (b), on susceptible variety in humid condition (c).

Race determination of P. oryzae was determined based on seven differential varieties reaction (resistant or susceptible). From totally 22 P. oryzae isolates, it found 10 races. Five races were found in Paleteang sub-district, two races in Tiroang sub-district, 3 races in Watang sawitto sub-district, 5 races in Mattiro bulu sub-district and 5 races in Suppa Sub-district. Race 001 was found in 4 locations. Race 003, 013 and 033 were found in 3 locations. Race 041 is found in 2 locations, whereas races 020, 023, 061, 073 and 101 are found only in 1 location (table 1).

Race determination of P. oryzae isolates using a set of Indonesian differential rice varieties found a total of 10 races of 22 isolates tested. The number of races from each location ranged from 2 to 5 races. Race 001 is the most dominant race (5 isolates) found in 4 locations. According to Yuliani and Maryana [21], the composition of P. oryzae race in a region may be different from other regions, so that a variety may be susceptible to certain locations and elsewhere resistant. Virulence of P. oryzae is strongly influenced by various environmental factors. Information concerning the distribution of P. oryzae dominant race in an endemic area of the blast can be used as a reference in the blast control using resistant varieties adapted to P. oryzae race at that location.

Asahan is the highest endurance resistant variety to all P. oryzae isolates tested, while Kencana Bali variety is only resistant to 1 isolate from a total of 22 isolates tested. Mogi et al., [19] described, that Asahan varieties have the highest endurance level of all differential rice varieties of Indonesia, also stated that Kencana Bali varieties have the lowest resistance to blast disease. The total of 10 races that have been identified in five sub-districts in Pinrang District are races of 001, 003, 013, 020, 023, 033, 041, 061, 073 and 101 respectively. Amir et al., [7] variations of P. oryzae races are always different every growing season. Races 001, 003, 033 and 173 are the dominant races that are always found every growing season. Mogi et al., [19] found that race 001 is the dominant race in some regions of Indonesia. Furthermore, Yuliani and Maryana [21] reported, that information on the diversity and distribution of the P. oryzae races can be used as a reference in the release of resistant varieties tailored to the dominant race at that location.

Table 1. Differential rice varieties reaction to 22 isolates of Pyricularia oryzae.

| Isolates code | Race | AS | CN | IR | KA | CE | CG | KB |
|---------------|------|----|----|----|----|----|----|----|
| PoP11         | 003  | R  | R  | R  | R  | R  | S  | S  |
| PoP12         | 020  | R  | R  | R  | S  | R  | R  | R  |
| PoP13         | 023  | R  | R  | R  | S  | R  | S  | S  |
| PoP14         | 001  | R  | R  | R  | R  | R  | R  | S  |
| PoP15         | 033  | R  | R  | R  | S  | S  | S  | S  |
| PoP16         | 033  | R  | R  | R  | S  | S  | S  | S  |
3.2. Virulence gene analysis of Pyricularia oryzae

A total of 10 isolates analyzed for the presence of virulence genes (Pwl2, Erg2, Cut1) obtained 2 haplotypes F-110 (8 isolates) and G-100 (2 isolates). The F-110 haplotype was amplified on a Pwl2 primer with a DNA band size of 800-900 bp and Erg2 with a DNA band size of 1440 bp. Haplotype G-100 was only amplified on primer Pwl2 (table 2). Out of 10 P. oryzae isolates representing each race was further analyzed for the presence of virulence genes (Pwl2, Erg2, Cut1). Based on the presence and absence of band coding for each virulence gene, it obtained only 2 haplotypes there are F-110 (8 isolates) and G-100 (2 isolates).

| Number | Isolates code | Pwl2 800-900 bp | Erg2 1440 bp | Cut1 800-1730 bp | Haplotype |
|--------|---------------|-----------------|--------------|-----------------|-----------|
| 1      | PoPl2        | 1               | 1            | 0               | F-110     |
| 2      | PoPl3        | 1               | 0            | 0               | G-100     |
| 3      | PoPl6        | 1               | 1            | 0               | F-110     |
| 4      | PoWs2        | 1               | 1            | 0               | F-110     |
| 5      | PoWs4        | 1               | 1            | 0               | F-110     |
| 6      | PoMb1        | 1               | 1            | 0               | F-110     |
| 7      | PoMb4        | 1               | 1            | 0               | F-110     |
| 8      | PoMb5        | 1               | 1            | 0               | F-110     |
| 9      | PoSp4        | 1               | 1            | 0               | F-110     |
| 10     | PoSp5        | 1               | 0            | 0               | G-100     |

1 = band is present     0 = band is absent
The F-110 haplotype is found in four locations, whereas the G-100 haplotype is found only in two locations. There are no haplotypes A-000, B-001, C-011, D-111, E, 010 and H-101. Lack of variation of haplotype obtained can be caused by sources of isolation taking that have environmental factors that are less diverse eg humidity and temperature as well as varieties grown. Tenjo and Hamer [22] reported, that the genetic variation of P. oryzae is largely determined by environmental conditions such as humidity and temperature, whereas Chen et al., [23] stated, that variations of agroecology will cause large differences in genomic DNA fingerprint of the fungus with MGR586 marker.

Electrophoresis results based on the specific primers of the virulence genes showed that the Pwl2 primer was amplified in all isolates with DNA banding size of 900 bp. The Erg2 primer was amplified on 8 isolates with a DNA band size of 1440 bp. In Cut1 primer, 9 isolates were amplified beyond the target size ranging from 800-1730 bp and 1 isolate not amplified isolate (data not shown).

Pwl2 primer was amplified in ten isolates tested (100%), the Erg2 primer was amplified on eight isolates tested (80%), while Cut1 primer was not amplified in all isolates tested. Cut1 is a coder of cutinase enzyme functioning in the degradation process of plant cuticle layers [10], Pwl2 is a host-specific avirulent gene [12]. Erg2 is a secondary metabolite encoding gene for blast fungi as an antifungal target in plant cells [11].

Some isolates that are not amplified in Erg2 and Cut1 primers can be caused by mutations that are due to the activity of some transposon elements (moving elements). Genetic variation of plant pathogen fungi is influenced by the presence of mutations and recombination. Mutations cause a diversity of populations as well as pathogenic virulence properties [24]. Mutation of P. oryzae fungi can be caused by the abundance of transposon elements of over 30 species eg MAGGY, grh, Pot2, Pot3, MGLR-3 and others [25].

Molecular analysis to detect haplotype diversity of P. oryzae isolates showed no haplotype variation from two locations (Watang sawitto and Mattiro bulu sub-district). Only haplotype F-110 was found. Different races may not necessarily different in genetic variation and otherwise. This is consistent with the research of Chuwa et al., [26], seven isolates of P. oryzae from three different races showed no genetic variation. Reflinur et al., [13], molecular characterization of 230 isolates of P. oryzae consisting of 5 races using a specific marker of virulence gene-coding yielded eight haplotypes. Li et al., [27], P. oryzae isolates are genetically stable but have pathogenic variations. Leung et al., [28], host plant selection is an evolutionary pressure that plays a major role in the formation of genetic variations of pathogens. Leung et al., [29], DNA fingerprint analysis of P. oryzae isolates showed that on land planted with one variety (monogenic), P. oryzae isolates has less genetic variation when compared to P. oryzae isolates obtained from land planted with several varieties (polygenic).

4. Conclusions
Based on the reaction of a set of Indonesian differential rice varieties, 10 races were found out of a total of 22 isolates of P. oryzae tested. Ras 001 is the most dominant race and found in 4 sub-districts (Paleteang, Tiroang, Watang sawitto and Mattiro bulu). Molecular analysis of 10 isolates based on the specific primers of the virulence gene encodes for Pwl2, Erg2, Cut1, obtained only 2 haplotypes of F-110 (8 isolates) and G-100 (2 isolates).

Acknowledgment
Thanks to the Agriculture Quarantine Station Class I of Parepare City, and the Education Hospital Hasanuddin University of Makassar for providing research facilities during this research.

References
[1] Khush G S and Jena K K 2009 Current status and future prospects for research on blast resistance in rice (Oryza Sativa L.) Advances in Genetics, Genomics, and Control of Rice Blast Disease ed G Wang and B Val lent (Germany: Springer Science Business Media B.V) pp 1-10.
[2] Mathioni S M, Patel N, Riddick B, Sweigard J A, Czymmek K J, Caplan J L, Kunjeti S G,
Kunjeti S, Raman V, Hillman B I, Kobayashi D Y and Donofrio N M 2013 Transcriptomics of the rice blast fungus *Magnaporthe oryzae* in response to the bacterial antagonist *Lysobacter enzymogenes* reveals candidate fungal defense response genes *PloS. one* 8 1-16.

[3] Utami D W, Aswidinnoor H, Moeljopawiro S, Hanarida I and Reflinur 2006 Inheritance of blast resistance (*P. grisea* Sacc) on interspecific crossing between IR 64 and *Oryza rufipogon* Sacc. *Hayati J.* 13 107-112.

[4] Kang S and Lee Y H 2000 Population structure and race variation of the rice blast fungus *Plant Pathol. J.* 16 1-8.

[5] Taheri P and Irannejad A 2014 Genetic structure of various *Magnaporthe oryzae* populations in Iran and Uruguay *Proc. of the XV National Congress (Purwokerto)* (Indonesia: Agency for Agricultural Research and Development) pp 531-563.

[6] Santoso and Nasution A 2009 Pengendalian penyakit blas dan penyakit cendawan lainnya *Inovasi Teknologi Produksi Padi* 2nd book (Indonesia: Agency for Agricultural Research and Development) pp 148-151.

[7] Amir M, Nasution A and Santoso 2000 Inventarisasi ras *Pyricularia grisea* di Sukabumi, Jawa Barat musim tanam 1995-1998 *Proc. of the XV National Congress (Purwokerto)* (Indonesia: PFI) pp 148-151.

[8] Sudir, Nasution A, Santoso and Nuryanto B 2014 Penyakit blas *Pyricularia grisea* pada tanaman padi dan strategi pengendaliannya *J. Iptek Tanaman Pangan* 9(2): 85-96.

[9] Soubabere O, Jorge V, Notteghem J L, Lebrun M H and Tharreau D 2001 Sequence characterized amplified region markers for the rice blast fungus, *Magnaporthe grisea* *Molecular Ecology Notes* 1: 19–21.

[10] Sweigard J A, Chumley F G and Valent B 1992 Disruption of *Magnaporthe grisea* cutinase gene *Mol. Gene Genet.* 232(2): 183-190.

[11] Keon J P, James C S, Court S, Baden-Daintree C, Bailey A M, Burden R S, Bard M and Hargreaves J A 1994 Isolation of Erg2 gene, encoding sterol delta 8 to delta isomerase, from the rice blast fungus *Magnaporthe grisea* and its expression in the maize smut pathogen *Ustilago maydis* *Curr. Genet.* 25(6): 531-537.

[12] Valent B and Chumley F G 1994 Avirulence genes and mechanisms of genetic instability in rice blast fungus *Rice Blast Disease* Ed. R S Zeigler, S A Leong, and P S Teng (Wallingford: CAB International-IRRI, Manila, Philipinnes) pp 111-134.

[13] Reflinur, Bustamam M, Widyawatuti U and Aswidinnoor H 2005 Keragaman genetik cendawan *pyricularia oryzae* berdasarkan primer spesifik gen virulensi *J. Biotek. Pertanian* 10(2): 55-60.

[14] Rianingsih 2017 *Studi Keragaman Ras Isolat Pyricularia oryzae* Cavara Penyebab Penyakit Blas pada Tanaman Padi dari Beberapa Kabupaten di Sulawesi Selatan (Makassar) Thesis (Indonesia: Graduate School of Hasanuddin University)

[15] Prabawa P S, Yulianah I and Basuki N 2015 Uji ketahanan 10 genotip padi merah (*Oryza sativa* L.) terhadap penyakit blas daun (*Pyricularia oryzae* Cav.) *J. Produksi Tanaman* 3(6): 496–502.

[16] Lestari A U, Widyawatuti and Enggarini W 2016 Uji virulensi 100 isolat cendawan blas (*Pyricularia oryzae* Cavara) terhadap satu set varietas padi diferensial Indonesia *J. Agrotek Indonesia* 1(1): 37-46.

[17] Trisaningsih and Nasution A 2016 Respon ketahanan berbagai galur padi rawa terhadap wereng cokelat, penyakit blas, dan hawar daun bakteri. *Proc. of the National Seminar of Indonesian Biodiversity Society* (Indonesia: Indonesian Biodiversity Society) 2(1): 85-92.

[18] International Rice Research Institute (IRRI) 2013 *Standard Evaluation System for Rice 5th ed* (Philippines: IRRI, Manila)

[19] Mogi S, Suroto, Sugandhi Z and Baskoro S W 1992 Keadaan studi penyakit padi di Jatisari Penyakit padi *Laporan Akhir Tulisan Ilmiah Penyakit Blas Kerjasama Teknis Indonesia-Jepang* (Indonesia: Directorate General of Food Protection)

[20] TeBeest D O, Guerber C and Ditmore M 2007 Rice blast *The Plant Health Instructor.*
[21] Yuliani D and Maryana Y E 2014 Integrasi teknologi pengendalian penyakit blas pada tanaman padi di lahan sub-optimal Proc. of National Seminar of Suboptimal Land (Palembang) (Indonesia: The Center for Suboptimal Land Development Research of Sriwijaya University) pp 835 – 845.
[22] Tenjo F A and Hamer J E 2002 Pathogenic development in Magnaporthe grisea Molecular Biology of Fungal Development ed J W Goethe (Germany: Universitat Frankfurt) pp 399-418.
[23] Chen D, Zeigler R S, Leung H and Nelson R J 1995 Population structure of Pyricularia grisea at two screening sites in the Philippines Phytopathology 85: 1011-1020.
[24] Burdon J J and Silk J 1997 Sources and patterns of diversity in plant-pathogenic fungi Phytopathology 87(7):664-669.
[25] Kito H, Sato J, Takahashi Y, Fukiya S, Sone T, and Tomita F 2002 Occan, a new transposon-like sequence in Magnaporthe grisea Abstracts 3rd International Rice Blast Conference (Ibaraki) (Japan: Tsukuba International Congress Center-Epochal Tsukuba Science City) Abstract no. 29 p 15.
[26] Chuwa C J, Mabagala R B and Reuben M S O W 2015 Pathogenic variation and molecular characterization of Pyricularia oryzae, causal agent of rice blast disease in Tanzania. Int. J. of Science and Research (IJSR) Volume 4 Issue 11.
[27] Li Y, Liang S, Yan X, Wang H, Li D, Soanes D M, Talbot N J, Wang Z, and Wang Z 2010 Characterization of MoLDB1 required for vegetative growth, infection-related morphogenesis and pathogenicity in the rice blast fungus Magnaporthe oryzae Molecular Plant-Microbe Interactions 23(10): 1260-1274.
[28] Leung H, Nelson R J, and Leach J E 1993 Population structure of plant pathogenic fungi and bacteria Plant Pathol. 10:157-205.
[29] Leung H, Fan J X, Zhu Y, Chen H, Revilla-Monila I, Pangga I, Cruz C V and Mew T W 2003 Using genetic diversity to achieve sustainable rice diseases management Plant Disease 87(10): 1156-1169.