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Pathological Studies of *Pyricularia oryzae* at M'be in Coted'ivoire and Ouedeme in Benin

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ABSTRACT

Study for the genetic diversity of *P. oryzae* populations, under natural inoculation, trapping of virulence races present in ecosystems, was done during the period of 2008 to 2010 in two countries. One location/country was surveyed to highlight that resistance genes: Pi1; Pi11; Pi12; Pi19; Pib; Pi20; Pi5; Pi7; Pia; Pia+Pi19; Pia+Pish; Pif, Pii, Pik, Pik-p, Pish, Pit, Pit, Pita-2, Piz, Piz-t, Piks at M'be and and those at Ouedeme: Pi1, Pi12, Pi19, Pi1b, Pi3, Pi5, Pi7, Pia, Pii, Pik, Pi-p, Pit, Pita, Piks incidence. It was shown that they were overcome by a large proportion of the virulence gene of *P. oryzae* population. However, virulence genes that are capable of overcoming resistance genes: Pi33, Pi5 (t), Pi7, Pi9, Pikh+Pi-1+Pita+Pita, Pik-m ,Pish, Pitta-2, Piz-5, were absent or rare. These genes were effective against the pathogen population studied. This study also showed that Pi5 and Pi7, Pikh +Pi-1+Pita+Pita, genes association which were individually inefficient, has conferred a sustainable resistance to blast that was observed in Moroberekan and Tetep. This work will help rice breeders and plant pathologists to select rice varieties with durable resistance to blast disease.

Key words: Rice blast disease, pathological diversity, *P. oryzae*, resistance genes

INTRODUCTION

Upland rice represents about 44% of the total rice production in West Africa. It covers 57% of cultivated rice surface and more than 70% of farmers are engaged for their subsistence (Jones *et al*., 1997). Rice blast fungus is one the most prevalent and devastating disease of rice in West Africa and in the world (Chen *et al*., 2004). It is caused by a pathogenic fungus which telemorph stage is called Magnaporthe grisea (Pan *et al*., 1998) and the anamorph stage is *Pyricularia oryzae*. Rice cultivation is under a constant pressure of blast of the leaf, node and panicle of the grain causing extensive damage to upland rice, estimated at 21-64% in West Africa (Sere *et al*., 2004). In South Saharan Africa, the use of resistant varieties associated with improved farming techniques is the only method of control available to smallholder farming among which more than half are women. However, if in the fight against *P. oryzae*, the achievements of agricultural research in the field of chemotherapy are significant in developed countries (Sy *et al*., 1994), rice blast caused by the fungus *M. oryzae* was one of the most serious diseases of rice and remains one of the most difficult diseases to manage (Khush and Jena, 2007; Jia and Moldenhauer, 2010).

Rice farmers in South Saharan Africa are not able to purchase fungicides; the use of resistant varieties is the only effective weapon they have. The only control method available (Van der Plank, 1968), however, faces several obstacles, including the variability of the pathogenicity of the causal agent of the disease, *P. oryzae* (Pan *et al*., 1998). Brown (2002) showed that the resistance genes to blast and the rice yield genes are inversely related, the selection of resistant cultivars to blast would be a penalty for the selection of high-yielding cultivars. The objective of this work is to study the genetic diversity of *P. oryzae* populations under natural inoculation at M'Be and Ouedeme in Benin and Côte d'Ivoire.

MATERIALS AND METHODS

Experimental site and Biological materials: These studies were conducted during the period of 2008 to 2010 in the research stations of AfricaRice at M'be in Côte d'Ivoire and at Ouedeme in Benin, in the zone of southern Guinean savanna of West Africa. Soil characteristics of M'be and Ouèdèmè are cosigned in Table 1. The biological materials are composed of 35 strains and varieties mono or oligo gene (Table 2) increased in the absence of pressure blast and seeds are processed by a non-systemic fungicide.

METHODS

Experimental design: The experimental design is a block of Fischer with 4 repetitions of 3 lines of 50 cm separated from each other by 10 cm and a line between the access to facilitate their identification and evaluation.

Table 1: Description of the properties of the soils of Mbé and Ouèdèmè

Soil characteristics	M'bé/Bouaké (RCI)	Totinga/Ouédemè
pH (H_2O_2)	6.1	5.5
SOC (g kg^{-1})	14	12
Total N (g kg^{-1})	1.26	1.0
Extractable P (mg kg^{-1})	1.63	1.7
Sable (g kg^{-1})	730	436
Silt (g kg^{-1})	90	310
Argile (g kg^{-1})	180	310

Table 2: List of biological material

Var code	Cultivar names	Resistance genes	Var code	Cultivar names	Resistance genes
V01	IRBL 1-CL/CO	Pi1	V19	St1	Pif
V02	IRBL 11-ZH	Pi11	V20	IRBLI-F5	Pii
V03	IRBL 12-M	Pi12	V21	IRBLK KA	Pik
V04	IRBL 19-A	Pi19	V22	IRBLkh-K3/CO	Pik-h
V05	C103 TTP	Pi1b	V23	Tetep	Pikh+ Pi-1 +Pita+ Pita
V06	IRBL 20-IR 24	Pi20	V24	IRBLkm- Ts/CO	Pik-m
V07	IRBL 3-CP 4	Pi3	V25	IRBLkp-K60	Pik-p
V08	IR 1529	Pi33	V26	IRBLks-F5	Pik-s
V09	IRBL 5-M/CO	Pi5	V27	IRBLsh-S	Pish
V10	Moroberekan	Pi5(t), Pi7	V28	IRBLT-K59	Pit
V11	IRBL 7-M/CO	Pi7	V29	IRBLTA CP 1	Pita
V12	75-1-127	Pi9	V30	IRBLta2-PI	Pita-2
V13	IRBL 9-W	Pi9	V31	IRBLZ FU	Piz
V14	CO39	Pia	V32	IRBLz5-CA (R)	Piz-5
V15	IRBLA-A	Pia	V33	IRBLZT-T	Piz-t
V16	Aichi Asahi	Pia+Pi19	V34	LIJIANG XINTUAN HEIGU	-
V17	NipponBare	Pia+Pish	V35	Sck\IRBLks-CO/CO	Piks
V18	IRBLB-B	Pib			

The soil was plowed with a disc plow twice to facilitate the upgrade with a harrow. Fertilization composed fertilizer NPK 10-18-18 was applied at 200 kg ha^{-1} before sowing. Urea was given to 21 and 42 days after sowing. Sowing was done by putting 3-4 seeds per hole at 10 cm spacing between the holes and 10 cm between rows. The seedlings were reduced to one plant per hole at 14 days later with eventual replacement of missing plants.

Pathological infection: Growing plants were left for natural infection. Pathological symptoms and severity were recorded according to the IRRI standard evaluation scale for rice (IRRI, 1980). Degree of severity was taken in consideration as shown in Table 3. Screening was done for each variety as well as positive and negative control using the same IRRI standard evaluation scale (IRRI, 1980).

Evaluation of host reaction and statistical analysis: Leaf blast was assessed at the 21, 28, 35, 42, 49, 56, 63 and 70 DAS by using the Standard Evaluation Scale (SES) of IRRI (1980) (Table 3). The notes were converted into sick leaf surface and the data were analyzed by using the software XLSTAT 2010 site by site.

RESULTS

Evaluation of host reaction and analysis: Statistical data analysis by XLTAT 2010 gives the correlation matrices (Table 4 and 5), the distribution graph lines according to their resistance/susceptibility to blast (Fig. 1 and 3), the dendrograms and classes of resistant and susceptible lines (Fig. 2 and 4).

Table 3: Standard evaluation scale of rice blast disease

Scale	Description
0	No typical susceptible lesion observed
1	Small brown specks (bs) of pin-point size without sporulating centre
3	Small roundish to slightly elongated, necrotic grey spots, about 1 to 2 mm in diameter, with a distinct brown margin (bg)
5	Typical susceptible blast lesions 3 mm or longer, infecting less than 10% of the leaf area
7	Typical susceptible blast lesions infecting 11 to 50% of the leaf area
9	More than 75% leaf area affected

Source: IRRI (1980)

Table 4: Matrice of correlation (Pearson (n)) at M'bé

Variables (DAS)	49	56	63	70
49	1.000	0.925	0.786	0.768
56	0.925	1.000	0.905	0.900
63	0.786	0.905	1.000	0.993
70	0.768	0.900	0.993	1.000

Table 5: Matrice of correlation (Pearson (n)) at Ouèdèmè

Variables (DAS)	49	56	63	70
49	1.000	0.876	0.751	0.751
56	0.876	1.000	0.828	0.828
63	0.751	0.828	1.000	1.000
70	0.751	0.828	1.000	1.000

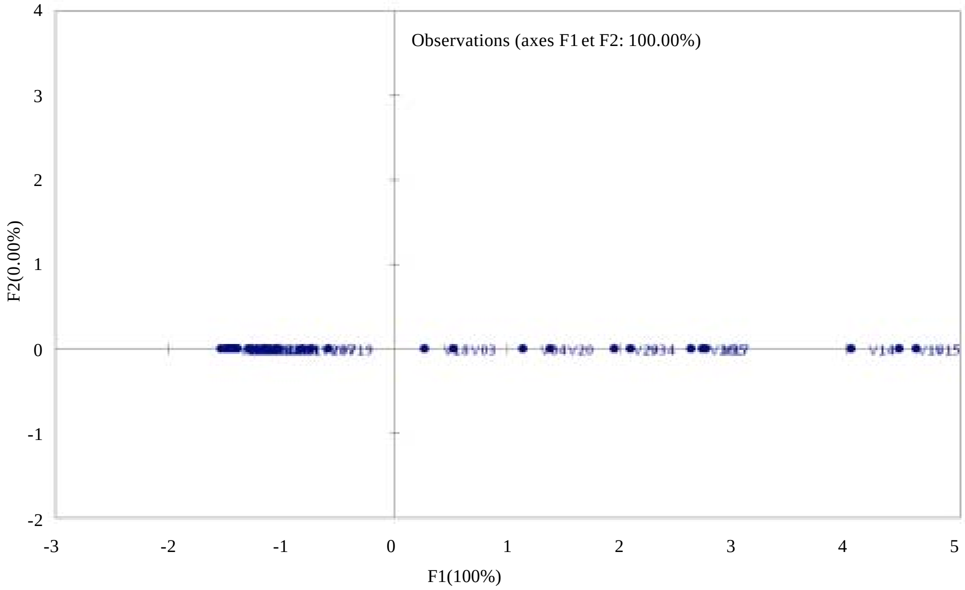

Fig. 1: Graph of distribution lines on the first axis of the Principal Component Analysis (PCA) at M'bé

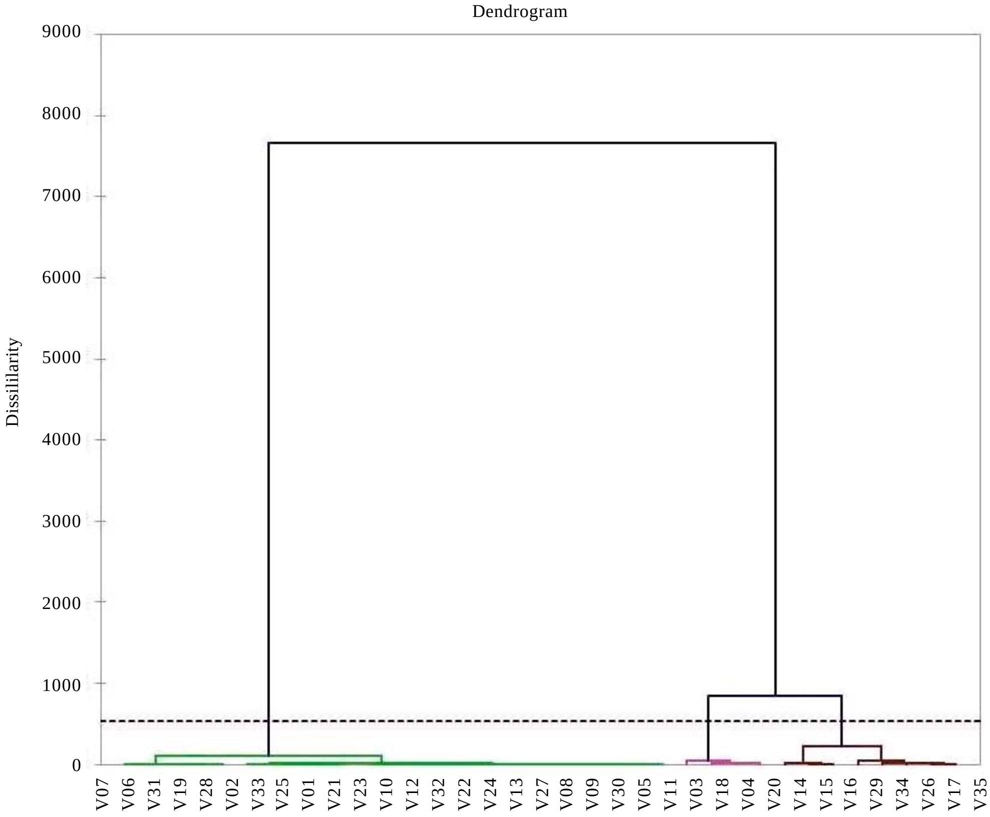

Fig. 2: Dendrogram lines according to their reaction *to P. oryza*e at M'bé

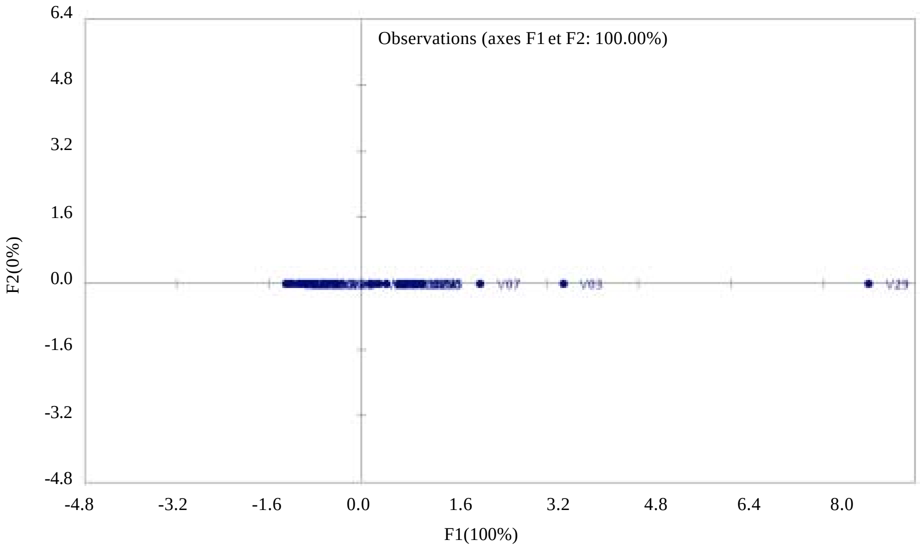

Fig. 3: Graph of distribution lines on the first axis of the Principal Component Analysis (PCA) at uèdèmè

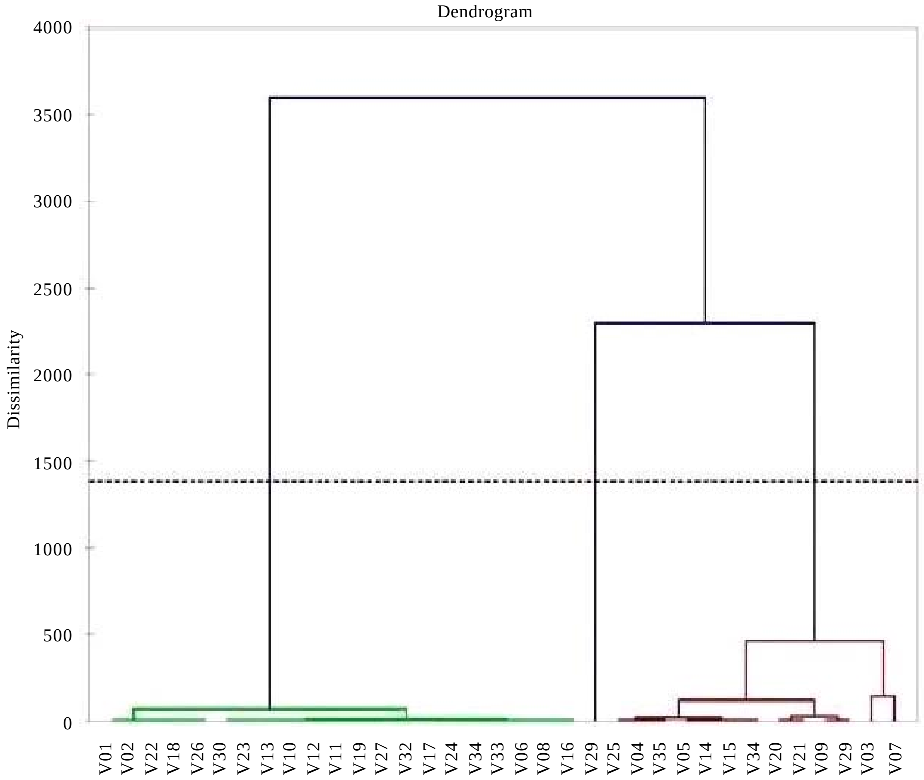

Fig. 4: Dendrogram lines according to their reaction *to P. oryzae* at Ouèdèmè

This correlation is 75.1 to 87.6% in Ouedeme (Benin), while in M'Be (Côte d'Ivoire) it ranges from 76.6 to 99.3%.

The principal component analysis: The Principal Component Analysis (PCA) and the numerical classification results (Fig. 3) showed that the first two principal components explain at 100% the reaction of the varieties and lines to blast fungus. The lines are distributed as follows: The sensitive lines are at the positive side of the axis and the resistant lines are at the negative side of the axis.

Reaction of the different cultivars and lines in relation to blast: The two dendrograms (that of M'Bé (Côte d'Ivoire) and of Ouedeme (Benin)) share out the lines and varieties into two classes. Resistant varieties with Sick Foliar Surface is equal to or less than 1.5% are: At M'be (Côte d'Ivoire): V10, V12, V13, V22, V23, V24, V27, V30, V32 and at Ouèdèmè (Benin): V02, V06, V08, V10, V12, V13, V17, V18, V19, V22, V23, V24, V30, V32. These lines are followed by the susceptible varieties. Thus, at M'be, there are: V01, V02, V03, V04, V05, V06, V07, V09, V11, V14, V15, V16, V17, V18, V19, V20, V21, V25, V26, V28, V29, V31, V33, V34, V35 and at Ouèdèmè, there are: V01 V03 V04 V05 V07 V09 V11 V14 V15 V20 V21 V25 V28 V29 V34 V35.

DISCUSSION

At the both sites, the Principal Component Analysis (PCA) and numerical classification notes collected 49, 56, 63, 70 days after sowing and transformed in Foliar Surface Sick, categorizes the 35 varieties in two distinct classes: the class of the resistant varieties and that of the susceptible ones Fig. 3 and 4. The resistant varieties have been able to react against virulence genes in populationsof *P. Oryzae* at both sites (Table 4 and 5). PCA analysis and numerical classification show that there is great variability between different varieties and lines at the both sites. These genes at the both sites are: Pi5 (t) Pi7 Moroberekan, Pi9 of 75-1-127, Pik-h of IRBLKH-K3, Pikh + Pi-1+Pita+Pita of Tetep, Pik-m of IRBLkm-TS 5, Piz-5 of IRBLZ-CA (R), Pi-9 of IRBL 9-W, Pita-2 of IRBL TA 2-PI, Pi33 of IR 1529 and Pish of IRBLsh-S which are resistant on the two sites. The observation of the behavior of varieties and lines has allowed to show that genes Pikh, Pi-1, Pita, Pi5 (t) are individually Pi7 ineffective whereas their association within Moroberekan (Pi5 (t) Pi7) and Tetep (pikh Pi-1+Pita+Pita) provides high resistance. That means the varieties evaluated exhibited differential but characteristic responses to rice blast fungus as it has been reported earlier (Odjo *et al*., 2011).

The contrasts of the two locations were unexpected at least to the extent and magnitude observed, because the two sites were in the same agro-ecological zones and is expected to share similar macroclimatic conditions. Nevertheless, the differences suggest that site specific microclimatic factors and the variability associated with them are more in blast screening than apparent macroclimatic factors in the choice of site for blast screening. Furthermore, there may be possibility of the presence of different strains of blast fungus at the two sites since the genotypes used in this study were special varieties, each carrying specific known resistant genes. According to Sere *et al*. (2004) it is important that the performance of a variety be viewed in relation to the biotic and abiotic parameters of the environment (or precisely of the site and ecological niches) that have been targeted. Such an effect of several genes in a variety explains the interest of pyramiding resistance genes to confer a high level of resistance to blast. The usefulness of pyramiding resistance genes to blast has been demonstrated experimentally (Hittalmani *et al*., 2000). Such associations also provide the benefit of a durable type of resistance difficult to overcome in time and space by the virulence gene; case of Pi5 (t) and Pi7 Pikh Pi-1 + Pita + Pita and it is established that they are easily circumvented by the pathogen (Conaway-Bormans *et al.*, 2003). This work also confirms the work of Roder *et al.* (1998) in which he showed that resistance genes Pi2 and Pi1 had a spectra of additional resistance and the accumulation of these genes in a variety could confer resistance to broad-spectrum so potentially sustainable.

In the statistical model used for this study, although, there were significant effects for all main factors and interaction effects; it is interesting to note that among the known sources of variation, Genotype by Environment Interaction (GEI) consistently had highest contribution to the total sum of squares for all levels of disease development and severity scores except at 70 days after sowing. For growth traits, however, variety had the highest contribution to total sum of squares. This is an indication that blast disease development and severity scores were greatly conditioned by environmental factors while growth traits were controlled by the genotypic effect of the variety (Van der Plank, 1968). This result is expected because the evaluation was carried out under a natural infection conditions on the field (Jia and Moldenhauer, 2003). Different trends may be observed if evaluation has been done in the screen house conditions or under artificial inoculation on the field. The pattern of disease severity differed at the two locations. At M'Be (Cote d'Ivoire), the difference between resistant and susceptible varieties were not significant until about 70 days after planting while difference became significant by around 78 days at Ouedeme (Benin). This could be explained by the fact that rice was cultivated at M'Be since the 1970s while at Ouèdèmè it just started in 2002.

In conclusion, the study the genetic diversity of *P. oryzae* populations under natural inoculation by using trapping design of virulence races of fungus in ecosystems of M'bé and Ouèdèmè led to the study of the genetic diversity of populations of *P. oryzae*. It has been proven that any pathogen resistance gene is a virulence gene of the host plant. It is clear from this work that the resistance genes of the cultivars are overcome by a significant proportion of virulence genes of the explored experimental sites. Nevertheless, the virulence genes capable of overcoming the resistance genes Pi9 of the isogenic lines 75-1-127, Pi5 (t) of Pi7 Moroberekan, Pikh Pi-1+Pita+Pita of Tetep, Pik-m of IRBLKM TS and Piz-5 of IRBLZ 5-CA are absent or rare in the two sites. These are effective genes from this experiment. These genes could be associated two or three in the same variety to ensure the sustainability of rice resistance to blast. In fact, such associations are effective in the case of Pi5 and Pi7 and it is established that they are difficult to overcome by pathogens.

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REFERENCES

Brown, J.K., 2002. Yield penalties of disease resistance in crops. Curr. Opin. Plant Biol., 5: 339-344.

Chen, X.W., S.G. Li, J.C. Xu, W.X. Zhai and Z.Z. Ling *et al.*, 2004. Identification of two blast resistance genes in a rice variety, digu. J. Phytopathol., 152: 77-85.

Conaway-Bormans, C.A., M.A. Marchetti, C.W. Johnson, A.M. McClung and W.D. Park, 2003. Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker-assisted selection. Theor. Applied Genet., 107: 1014-1020.

Hittalmani, S., A. Parco, T.V. Mew, R.S. Zeigler and N. Huang, 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Applied Genet., 100: 1121-1128.

IRRI, 1980. Standard Evaluation System for Rice. 2nd Edn., The International Rice Research Institute, Los Banos, Philippines, pp: 27.

Jia, Y. and K. Moldenhauer, 2010. Development of monogenic and digenic rice lines for blast resistance genes Pi-ta, Pi-kh/Pi-ks. J. Plant Registrations, 4: 163-166.

Jones, M.P., M. Dingkuhn, G.K. Aluko and M. Semon, 1997. Interspecific *Oryza sativa* L. X O. Glaberrima steud. progenies in upland rice improvement. Euphytica, 94: 237-246.

Khush, G. and K. Jena, 2007. Current status and future prospects of research on blast disease in rice (*Oryza sativa*). Proceedings of the 4th International Rice Blast Conference, October 09-14, 2007, Changsha, China.

Odjo, T., B.C. Ahohuendo, A. Onasanya, K. Akator and Y. Sere, 2011. Analysis of *Magnaporthe oryzae* population structure in Benin. Afr. J. Agric. Res., 6: 6183-6188.

Pan, Q., L. Wang, T. Tanisaka and H. Ikehashi, 1998. Allelism of rice blast resistance genes in two Chinese rice cultivars and identification of two new resistance genes. Plant Pathol., 47: 165-170.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy and M.W. Ganal, 1998. A microsatellite map of wheat. Genetics, 149: 2007-2023.

Sere, Y., A.A. Sy, S.K. Akator, A. Onasanya and K. Zai, 2004. Screening Strategy for Durable Resistance to Rice Blast Disease at WARDA. In: Rice Blast in West Africa: Characterisation of Pathogen Diversity, Key Screening Sites and Host Resistance, Sere, Y., S. Sreenivasaprasad and S.K. Nutsugah (Eds.). Vol. 4. Africa Rice Center (WARDA), Benin, Nigeria, pp: 38-43.

Sy, A.A., Y. Sere, K. Alluri, E.D. Imolein and S. Sreenivasaprasad, 1994. Sources of blast resistance and identification of sites for the selection of blast resistant cultivars. WARDA Annual Report 1993, West Africa Rice Development Association, Bouake (Cote d'Ivoire), pp: 45-47.

Van der Plank, J.E., 1968. Disease Resistance in Plants. Academic Press, New York.