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Reaction of upland rice genotypes to the brown spot disease pathogen *Bipolaris oryzae*

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ABSTRACT
Low yields in rice (*Oryza sativa* L.) are attributed to several factors but diseases represent one of the main constraints. The Brown spot disease (caused by *Bipolaris oryza*e (Breda de Haan) Shoem) in most cases causes considerable losses in rice growing areas. These yield loses could be averted through development and deployment of resistant varieties. In this study, a field experiment was conducted at the National Crops Resources Research Institute (NaCRRI) – Namulonge, Uganda during 2013 with the objective of identifying new sources of resistance to brown spot disease. Among the 100 tested rice lines, 18 lines were rated as highly resistant, 52 resistant, 27 moderately resistant and three lines including the checks were susceptible. The results revealed that there was significant variation in brown spot resistance among the genotypes tested. The identified resistant lines will be utilized in rice breeding program in Uganda for development of brown spot resistant genotypes.

Key words: Brown spot, *Bipolaris oryzae, Oryza sativa,* sources of resistance, Uganda

R**É**SUM**É**
Les faibles rendements du riz (*Oryza sativa* L.) sont causés par plusieurs facteurs, mais les maladies constituent l'une des principales contraintes. Dans la plupart des cas, la pathologie des taches brunes (provoquée par Bipolaris oryzae (Breda de Haan) Shoem) entraîne des pertes considérables dans les zones rizicoles. Ces pertes de rendement pourraient être évitées à travers le développement et la promotion des variétés résistantes. Dans la présente étude, une expérimentation a été menée à l'Institut national de recherche sur les cultures (NaCRRI) - Namulonge, en Ouganda en 2013, dans le but d'identifier de nouvelles souches de résistance à la maladie des taches brunes. Parmi les 100 lignées de riz testées, 18 lignées ont été classées comme hautement résistantes, 52 résistantes, 27 modérément résistantes et trois lignées (y compris les contrôles) étaient sensibles. Les résultats ont montré une variation significative de la résistance aux taches brunes entre les génotypes testés. Les lignées résistantes identifiées seront utilisées dans le programme de sélection du riz en Ouganda pour le développement de génotypes résistant aux taches brunes.

Mots clés: Tache brune, *Bipolaris oryzae, Oryza sativa*, souches de résistance, Ouganda

INTRODUCTION

Brown spot disease, caused by the fungus *Bipolaris oryzae* (Breda de Haan) Shoem, is common in both rain-fed and upland rice production systems (Singh and Singh, 2000). Irrigated and lowland rice production systems in Uganda are dominated by local varieties, which are susceptible to many diseases including brown spot (Adur *et al.,* 2011). *Bipolaris oryzae* can spread from plant to plant by airborne spores (Sato *et al*. 2008), and up to 90% losses have been reported during epidemics (Mwalyego *et al.,* 2011). Various measures have been employed worldwide to control the disease. These include application of various agronomic practices, pesticides, biological control and use of resistant varieties. Few resistant cultivars are available for practical use (Biswas *et al.,* 2011). Chemical control has been used and it is effective against the disease, however, it is known to be environmentally unfriendly, expensive and requires expertise that is generally lacking among most resource-poor farmers (Kawube *et al.,* 2005). Host resistance, on the other hand, is considered a more cost-effective and sustainable management option

for the disease. Sources of resistance to brown spot are available in Asia and Africa. These sources can be used for development of resistant varieties for release to farmers (Nneke, 2012). The use of resistant rice genotypes is most effective in the area of development because of the variability of *Bipolaris oryzae* species and differences in varietal susceptibility to the pathogen (Kamal and Mia, 2009). To overcome the problem of pathogen variability, locally available plant materials and pathogen isolates need to be used in breeding for resistance. Rice germplasm should also be screened under low water conditions since the disease is more severe under aerobic conditions (Yaqoob *et al.,* 2011). In the present study, 100 rice genotypes were evaluated against brown spot disease under rain-fed conditions with the purpose of identifying parents for future breeding programs for resistance to the disease.

MATERIAL AND METHODS

Study area. The field trial was conducted at the National Crops Resources Research Institute (NaCRRI) - Namulonge in Uganda, located 27 km north of Kampala at 00 32'' N of the Equator and 320 37'' E. The institute lies in the central region of Uganda, which receives a bimodal cycle of rainfall, averaging 1200 mm per year with peaks between April to May and September to October. The soils are dominated with kaolinite and quartz. It stands at an elevation of 1150 metres above sea level within the Lake Victoria crescent agro-ecological zone.

Germplasm source and experimental design. The field trial was conducted from April to June 2013. One hundred germplasm accessions comprising of interspecific (NERICA) and intraspecific lines from AfricaRice, Centro Internacional de Agricultura Tropical (CIAT), NaCRRI - Namulonge, Tanzania and Madagascar were screened under rain-fed conditions alongside Ugandan landraces. With no prior information on the reaction of these materials to brown spot, they were selected on the basis of yield performance, adaptability, and farmers' preferance. The accessions were evaluated in a field nursery established using an alpha lattice experimental design with two replicates for each entry. Sowing was done by dribbling at a 5 x 20 cm spacing (three seeds per hill) in 5-row plots measuring 1 m in length.

Inoculum source and disease screening. To ensure maximum disease pressure, inoculum was prepared and the plants were artificially infected. In this procedure leaves and panicles with symptoms of brown spot were sampled from different locations, approximately 35 m apart, in an infected rice field at NaCRRI - Namulonge in Uganda. This location is a known as hot spot area for diseases. Disease samples were transferred to the laboratory, where they were crushed in 10 ml of double distilled water, using sterile mortars and pestles until 80% of the leaves and panicles were crushed. The inoculum was applied onto leaves of rice plants at two weeks after planting using the finger-rubbing technique, with contaminated pieces of cotton wool (Mogga *et al.,* 2012). Disease severity was scored at intervals of 15, 30, 45 and 60 days post (after) inoculation (dpi) (Campbell and Madden, 1990) following the standard evaluation system (SES) for rice (IRRI, 2002). In this system, 1 is equivalent to a host response rating of highly resistant (with no visible symptoms) and 9 is highly susceptible (with 76-100% leaf area affected).

Data analyses. The statistical linear model was used to analyze the genotypes as: $Y_{ijk} = Y... + R_j + G_i + B/R_{jk} + e_{ijk}$ where, Y = overall mean, R_j = replication effect of the j^{th}, G_i = effect of the ith genotype, B/R_{jk} = block/reps effect of the jk^{th}, and eijk = the environmental effect of the ijk^{th} observation. Data collected on the disease score were subjected to analysis of variance (ANOVA) using Genstat software, 14^{th} edition (GenStat, 2012) in order to obtain the mean squares and differences in the mean for disease severity. The relative Area Under Disease Progress Curve (rAUDPC) was calculated in order to evaluate germplasm reaction to the disease. The formula for computing rAUDPC was: $rAUDPC = [\Sigma (T_i - 1T_i) * (D_i + 1 + D_i)/2] / T_{Total} * 100$ Where: $T_i = i^{th}$ day after emergence when the estimation of disease was made, D_i = estimate of the percentage of leaf area covered with lesions at Ti and TTotal which are the number of days after emergence at which the final disease assessment was recorded. The mean severity scores across eight weeks and rAUDPC were used to evaluate the reaction of the materials to brown spot disease.

RESULTS

Disease symptoms observed on rice genotypes in the field were typical of brown spot disease (Figure 1). Symptoms included: small, oval or circular and

dark brown leaf spots. Larger lesions had dark brown edges, with pale grayish centers.

Germplasm showed varying responses to brown spot. Of the 100 screened germplasm, more than half (52 lines) had between 1-5% of their leaf area affected by the disease and were considered resistant. In comparison, about half this number (27 lines) was moderately resistant with between 11 and 25% leaf area coverage with symptoms. With less than 1% leaf area affected, 18 lines were rated as highly resistant. Three lines (Pakistan, TXD 306 and NERICA 1), including the check (P4R1), were susceptible (Figure 2). Most of the materials from Africa Rice were in the category resistant to brown spot. Ugandan land races as well as materials from Madagascar and a few from CIAT were moderately resistant. Materials from Tanzania, Pakistan and NaCRRI - Namulonge were generally susceptible (Table 1).

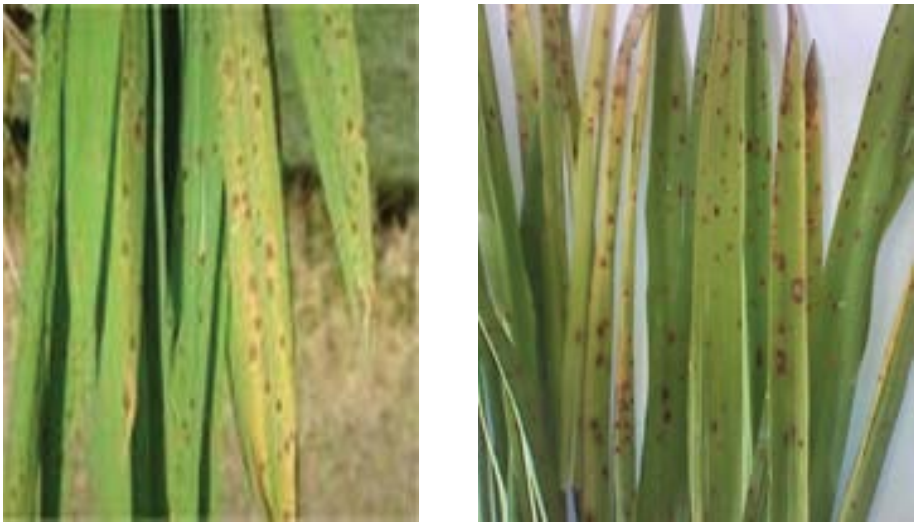

Figure 1: Symptoms of brown spot observed on susceptible rice lines at NaCRRI, Uganda

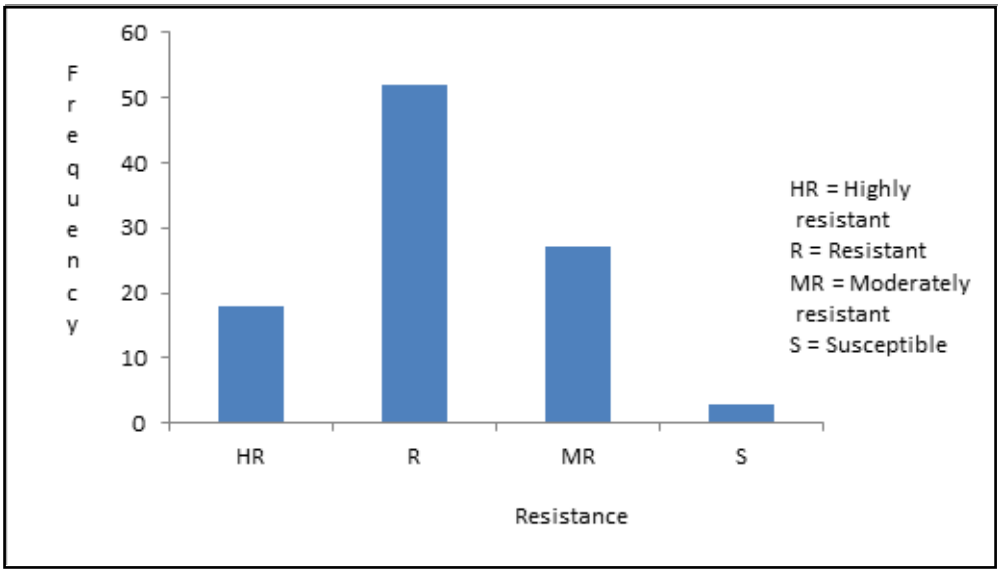

Figure 2: Categorization of screened rice germplasm for resistance to brown spot in NaCRRI, Uganda

Analysis of variance revealed significant differences between replicates in response to disease at 30 and 45 dpi (Table 2). Blocking had a significant effect on variation of disease response at 15 and 60 dpi (at P ≤ 0.1, 0.05 respectively). The response of genotypes to the disease varied significantly from 30 days onwards (at P ≤ 0.001).

Susceptible genotypes had higher mean scores of severity (Figure 3) and rAUDPC (Figure 4) compared to resistant genotypes.

Table 1: A list of resistant germplasm to brown spot disease and their resistance designation

Entry	Source/origin	Designation
E1CV	Africa Rice	HR
E11CV	Africa Rice	HR
E10	Africa Rice	HR
P27H4	NaCRRI- Namulonge	HR
E186	Africa Rice	HR
E51	Africa Rice	HR
P8H13	NaCRRI- Namulonge	HR
E123	Africa Rice	HR
E-3	NaCRRI- Namulonge	HR
E104	Africa Rice	HR
E99	Africa Rice	HR
E16	Africa Rice	HR
E135	Africa Rice	HR
E8CV	NaCRRI- Namulonge	HR
P26H6	NaCRRI- Namulonge	HR
P27H3	NaCRRI- Namulonge	HR
P3R1	NaCRRI- Namulonge	HR
P55H7	NaCRRI- Namulonge	HR

HR = highly resistant

Table 2: Summary ANOVA table for brown spot severity on rice at 15, 30, 45 and 60 days of disease scoring

Source of variation	d.f	Var. 15 days	Var. 30 days	Var. 45 days	Var. 60 days
Total	199				
Rep	1	0.25^{ns}	1.13+	2.0+	0.01^{ns}
Rep. Block	8	0.160^{+}	ns	ns	2.12*
Entries	99	0.09^{ns}	1.97***	1.55***	1.537***
RCB error	99	-	0.458	0.636	-
LEE	72	0.09	-	-	0.915

Var = Variance; d.f = degree of freedom; + = significant at α = 0.1; * = statistically significant at α = 0.05; *** = very highly significant at α = 0.001; ^{ns} = statistically not significant.

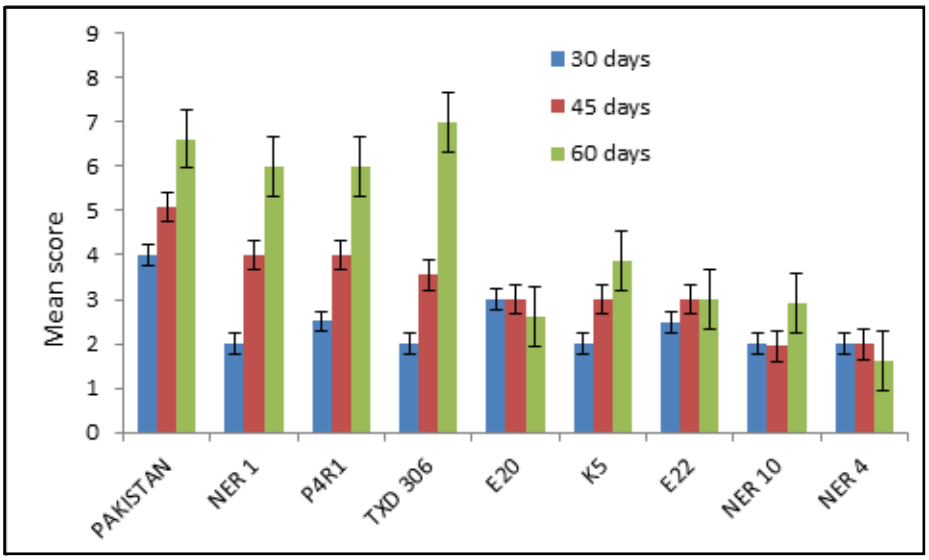

Figure 3: Means for resistance to brown spot in selected accessions at different dates after innoculation

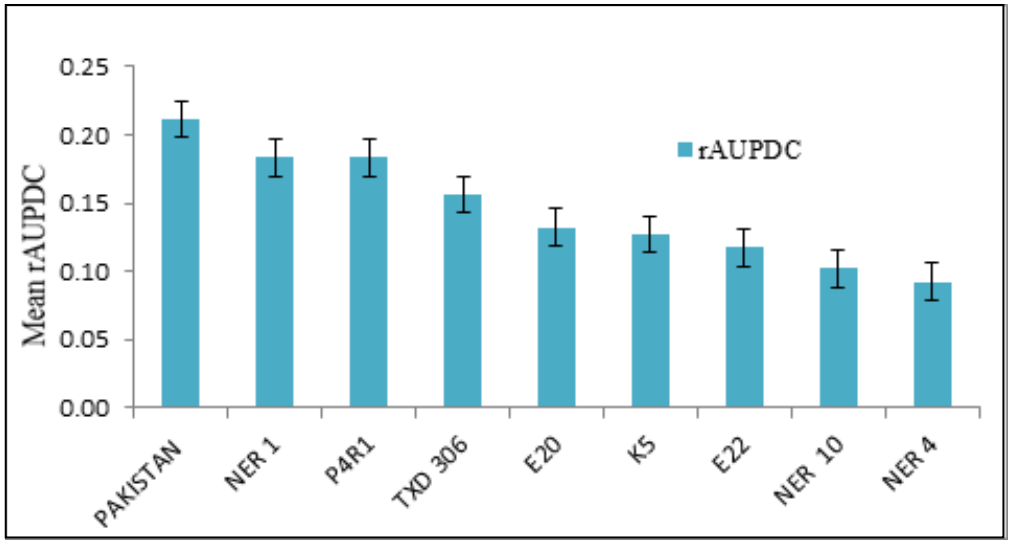

Figure 4: Relative area under disease progress curve means for resistance to brown spot in selected accessions

DISCUSSION

Data from this study showed that sources of resistance against brown spot disease were available amongst some rice lines/varieties tested under field conditions. Race classification of the pathogen, on the basis of reaction on rice varieties, was however not possible. Disease screening using lines from the International Rice Research Institute (IRRI) indicated that rice genotypes differ significantly in their resistance to brown spot disease, from highly resistant to highly susceptible (Yaqoob *et al.,* 2011; Tariq *et al.,* 2012). Earlier, Mosharraf *et al.* (2004) had reported a slightly narrower reaction range from resistant to moderately susceptible. Castano *et al.* (1990) and Hossain and Kulkarni (2001) observed variability in response to various diseases and categorized rice germplasm into groups, from highly susceptible to highly resistant responses to the

various rice diseases. It is not surprising, therefore, that the materials evaluated in this study reacted in a similar manner.

At 60 days post-inoculation most of the germplasm had, however, attained maximum disease scores although they displayed different levels of incidence and severity. Dallagno *et al.* (2012) reported that brown spot severity is strongly influenced by environmental conditions and is independently and additively affected by Silicon and soluble sugar concentrations in leaf tissues. Savary *et al.* (2005) also reported this disease on rice in soils deficient in potassium, manganese, magnesium, silicon, iron, or calcium. Reference to brown spot as "the poor farmer's disease" is thus justified (Zadoks, 2002).

Disease reactions observed in this study suggest wide variation amongst germplasm evaluated against brown spot pathogen populations in the study area. In Uganda, the rice breeding program has introduced materials, some of which have been identified as potential sources of resistance for improving susceptible landraces. While the resistant genotypes identified could be very useful in breeding of resistant varieties, earlier work shows that their use requires an understanding of the nature of inheritance and the gene action controlling resistance (Kornegay *et al.,* 1980; Attere and Fatokun, 1983); this aspect should be investigated in future studies.

CONCLUSION

The study demonstrated that rice lines/varieties markedly differed in levels of resistance to brown spot of rice but could still be used in breeding for resistance to the disease. For their effective use, however, the genetics of resistance should be established. The diversity and pathogenicity of *Bipolaris oryzae* populations in Uganda should also be elucidated in order to guide the rice breeding strategy.

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STATEMENT OF NO CONFLICT OF INTEREST

We the authors of this paper hereby declare that there are no competing interests in this publication.

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